Pro Gly Thr Cys Xaa' Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2487)
Pro Gly Thr Cys Xaa' Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2488)
Pro Gly Thr Cys Xaa' Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2489)
Pro Gly Thr Cys Xaa' Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2490)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2491)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2492)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2493)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2494)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2495)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2496)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2497)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2498)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2499)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2500)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2501)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2502)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2503)
Pro Gly Thr Cys Xaa' Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2504)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2505)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2506)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2507)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2508)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2509)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2510)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2511)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2512)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2513)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2514)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2515)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2516)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2517)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2518)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2519)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2520)

FIG. 2
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Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2521)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2522)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2523)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2524)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2525)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2526)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2527)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2528)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2529)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2530)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2531)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2532)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2533)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2534)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2535)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2536)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2537)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2538)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2539)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2540)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2541)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2542)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2543)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2544)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2545)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2546)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2547)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2548)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2549)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2550)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2551)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2552)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2553)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2554)

FIG. 2
(sheet 49 of 114)

Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys	(SEQID NO: 2555)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys	(SEQID NO: 2556)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 2557)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 2558)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 2559)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 2560)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 2561)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 2562)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys	(SEQID NO: 2563)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys	(SEQID NO: 2564)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys	(SEQID NO: 2565)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys	(SEQID NO: 2566)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa'	(SEQID NO: 2567)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 2568)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys	(SEQID NO: 2569)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys	(SEQID NO: 2570)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys	(SEQID NO: 2571)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa'	(SEQID NO: 2572)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 2573)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys	(SEQID NO: 2574)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys	(SEQID NO: 2575)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa'	(SEQID NO: 2576)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 2577)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys	(SEQID NO: 2578)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa'	(SEQID NO: 2579)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 2580)
Pro Gly Thr Cys Xaa' Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa'	(SEQID NO: 2581)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2582)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2583)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2584)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2585)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2586)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2587)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys	(SEQID NO: 2588)

FIG. 2
(sheet 50 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2589)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2590)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2591)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2592)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2593)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2594)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2595)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2596)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2597)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2598)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2599)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2600)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2601)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2602)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2603)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2604)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2605)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2606)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2607)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2608)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2609)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2610)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2611)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2612)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2613)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2614)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2615)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2616)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2617)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2618)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2619)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2620)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2621)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2622)

FIG. 2
(sheet 51 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2623)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2624)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2625)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2626)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2627)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2628)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2629)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2630)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2631)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2632)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2633)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2634)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2635)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2636)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2637)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2638)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2639)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2640)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2641)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2642)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2643)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2644)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2645)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2646)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2647)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2648)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2649)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2650)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2651)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2652)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2653)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2654)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2655)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2656)

FIG. 2
(sheet 52 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2657)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2658)
Pro Gly Thr Cys Xaa' Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2659)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2660)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2661)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2662)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2663)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2664)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2665)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2666)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2667)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2668)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2669)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2670)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2671)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2672)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2673)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2674)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2675)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2676)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2677)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2678)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2679)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2680)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2681)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2682)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2683)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2684)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2685)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2686)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2687)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2688)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2689)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2690)

FIG. 2
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Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2691)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2692)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2693)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2694)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2695)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2696)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2697)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2698)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2699)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2700)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2701)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2702)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2703)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2704)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2705)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2706)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2707)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2708)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2709)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2710)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2711)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2712)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2713)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2714)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2715)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2716)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2717)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2718)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2719)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2720)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2721)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2722)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2723)
Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2724)

FIG. 2
(sheet 54 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2725)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2726)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2727)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2728)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2729)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2730)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2731)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2732)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2733)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2734)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2735)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2736)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2737)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2738)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2739)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2740)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2741)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2742)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2743)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2744)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2745)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2746)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2747)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2748)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2749)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2750)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2751)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2752)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2753)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2754)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2755)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2756)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2757)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2758)

FIG. 2
(sheet 55 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2759)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2760)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2761)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2762)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2763)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2764)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2765)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2766)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2767)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2768)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2769)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2770)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2771)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2772)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2773)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2774)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2775)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2776)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2777)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2778)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2779)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2780)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2781)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2782)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2783)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2784)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2785)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2786)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2787)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2788)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2789)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2790)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2791)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2792)

FIG. 2
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Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2793)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2794)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2795)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2796)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2797)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2798)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2799)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2800)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2801)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2802)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2803)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2804)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2805)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2806)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2807)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2808)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2809)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2810)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2811)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2812)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2813)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2814)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2815)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2816)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2817)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2818)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2819)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2820)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2821)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2822)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2823)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2824)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2825)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2826)

FIG. 2
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Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2827)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2828)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2829)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2830)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2831)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2832)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2833)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2834)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2835)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2836)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2837)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2838)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2839)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2840)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2841)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2842)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2843)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2844)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2845)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2846)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2847)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2848)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2849)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2850)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2851)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2852)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2853)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2854)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2855)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2856)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2857)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2858)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2859)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2860)

FIG. 2
(sheet 58 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2861)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2862)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2863)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2864)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2865)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2866)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2867)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2868)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2869)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2870)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2871)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2872)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2873)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2874)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2875)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2876)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2877)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2878)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2879)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2880)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2881)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2882)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2883)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2884)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2885)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2886)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2887)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2888)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2889)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2890)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2891)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 2892)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2893)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2894)

FIG. 2
(sheet 59 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2895)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2896)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 2897)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2898)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2899)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2900)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2901)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 2902)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2903)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 2904)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 2905)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 2906)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 2907)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 2908)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 2909)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 2910)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2911)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 2912)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 2913)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 2914)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 2915)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 2916)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 2917)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 2918)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 2919)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 2920)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 2921)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 2922)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 2923)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 2924)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 2925)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2926)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 2927)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 2928)

FIG. 2
(sheet 60 of 114)

Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys	(SEQID NO: 2929)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa'	(SEQID NO: 2930)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys	(SEQID NO: 2931)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys	(SEQID NO: 2932)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa'	(SEQID NO: 2933)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa'	(SEQID NO: 2934)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa'	(SEQID NO: 2935)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 2936)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys	(SEQID NO: 2937)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys	(SEQID NO: 2938)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa'	(SEQID NO: 2939)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa'	(SEQID NO: 2940)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa'	(SEQID NO: 2941)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 2942)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa'	(SEQID NO: 2943)
Pro Gly Thr Cys Xaa' Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa'	(SEQID NO: 2944)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2945)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2946)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2947)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2948)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2949)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2950)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2951)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2952)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys	(SEQID NO: 2953)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys	(SEQID NO: 2954)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 2955)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 2956)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 2957)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 2958)
Pro Gly Thr Cys Gly Xaa' Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 2959)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2960)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2961)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 2962)

FIG. 2
(sheet 61 of 114)

Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2963)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2964)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2965)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2966)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2967)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2968)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2969)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2970)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2971)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2972)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2973)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2974)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2975)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2976)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2977)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2978)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2979)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2980)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2981)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2982)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2983)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2984)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2985)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2985)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2987)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2988)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2989)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2990)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 2991)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 2992)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2993)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 2994)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 2995)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 2996)

FIG. 2
(sheet 62 of 114)

Pro Gly Thr Cys Gly Xaa' Xaa' Glu Xle Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 2997)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 2998)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 2999)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3000)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3001)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3002)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3003)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3004)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3005)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3006)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3007)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3008)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3009)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3010)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3011)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3012)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3013)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3014)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3015)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3016)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3017)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3018)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3019)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3020)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3021)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3022)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3023)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3024)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3025)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3026)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3027)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3028)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3029)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3030)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3031)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3032)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3033)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3034)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3035)
Pro Gly Thr Cys Gly Xaa' Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3036)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3037)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3038)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3039)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3040)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3041)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3042)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3043)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3044)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3045)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3046)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3047)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3048)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3049)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3050)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3051)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3052)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3053)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3054)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3055)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3056)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3057)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3058)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3059)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3060)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3061)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3062)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3063)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3064)

FIG. 2
(sheet 64 of 114)

Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3065)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3066)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3067)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3068)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3069)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3070)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3071)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3072)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3073)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3074)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3075)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3076)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3077)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3078)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3079)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3080)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3081)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3082)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3083)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3084)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3085)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3086)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3087)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3088)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3089)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3090)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3091)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3092)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3093)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3094)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3095)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3096)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3097)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3098)

FIG. 2
(sheet 65 of 114)

Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3098)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3099)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3101)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3102)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3103)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3104)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3105)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3106)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3107)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3108)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3109)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3110)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3111)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3112)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3113)
Pro Gly Thr Cys Gly Xaa' Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3114)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3115)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3116)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3117)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3118)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3119)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3120)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3121)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3122)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3123)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3124)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3125)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3126)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3127)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3128)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3129)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3130)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3131)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3132)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3133)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3134)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3135)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3136)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3137)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3138)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3139)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3140)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3141)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3142)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3143)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3144)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3145)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3146)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3147)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3148)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3149)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3150)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3151)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3152)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3153)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3154)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3155)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3156)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3157)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3158)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3159)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3160)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3161)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3162)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3163)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3164)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3165)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3166)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 3167)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys	(SEQID NO: 3168)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys	(SEQID NO: 3169)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys	(SEQID NO: 3170)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa'	(SEQID NO: 3171)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 3172)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys	(SEQID NO: 3173)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys	(SEQID NO: 3174)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa'	(SEQID NO: 3175)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 3176)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys	(SEQID NO: 3177)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa'	(SEQID NO: 3178)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 3179)
Pro Gly Thr Cys Gly Xaa' Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa'	(SEQID NO: 3180)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3181)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3182)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3183)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3184)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys	(SEQID NO: 3185)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys	(SEQID NO: 3186)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 3187)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 3188)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 3189)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 3190)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 3191)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3192)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys	(SEQID NO: 3193)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys	(SEQID NO: 3194)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys	(SEQID NO: 3195)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 3196)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 3197)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 3198)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 3199)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 3200)

FIG. 2
(sheet 68 of 114)

Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3201)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3202)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3203)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3204)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3205)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3206)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3207)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3208)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3209)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3210)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3211)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3212)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3213)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3214)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3215)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3216)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3217)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3218)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3219)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3220)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3221)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3222)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3223)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3224)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3225)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3226)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3227)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3228)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3229)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3230)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3231)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3232)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3233)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3234)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3235)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3236)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3237)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3238)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3239)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3240)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3241)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3242)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3243)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3244)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3245)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3246)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3247)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3248)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3249)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3250)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3251)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3252)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3253)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3254)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3255)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3256)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3257)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3258)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3259)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3260)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3261)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3262)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3263)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3264)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3265)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3266)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3267)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3268)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3268)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3270)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3271)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3272)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3273)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3274)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3275)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3276)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3277)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3278)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3279)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3280)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3281)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3282)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3283)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3284)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3285)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3286)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3287)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3288)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3289)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3290)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3291)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3292)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3293)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3294)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3295)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3296)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3297)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3298)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3299)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3300)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3301)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3302)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3303)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3304)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3305)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3306)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3307)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3308)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3309)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3310)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3311)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3312)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3313)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3314)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3315)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3316)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3317)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3318)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3319)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3320)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3321)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3322)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3323)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3324)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3325)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3326)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3327)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3328)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3329)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3330)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3331)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3332)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3333)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3334)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3335)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3336)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3337)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3338)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3339)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3340)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3341)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3342)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3343)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3344)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3345)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3346)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3347)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3348)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3349)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3350)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3351)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3352)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3353)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3354)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3355)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3356)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3357)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3358)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3359)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3360)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3361)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3362)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3363)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3364)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3365)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3366)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3367)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 3368)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 3369)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 3370)

FIG. 2
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Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 3371)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3372)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 3373)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 3374)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 3375)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3376)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 3377)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 3378)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3379)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 3380)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3381)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3382)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 3383)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 3384)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 3385)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3386)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 3387)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 3388)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3389)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 3390)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3391)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3392)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 3393)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 3394)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3395)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 3396)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3397)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3398)
Pro Gly Thr Cys Gly Xaa' Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 3399)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3400)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3401)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3402)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3403)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3404)

FIG. 2
(sheet 74 of 114)

Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3405)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3406)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3407)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3408)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3409)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3410)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3411)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3412)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3413)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3414)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3415)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3416)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3417)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3418)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3419)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3420)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3421)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3422)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3423)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3424)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3425)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3426)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3427)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3428)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3429)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3430)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3431)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3432)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3433)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3434)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3435)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3436)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3437)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3438)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3439)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3440)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3441)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3442)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3443)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3444)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3445)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3446)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3447)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3448)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3449)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3450)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3451)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3452)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3453)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3454)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3455)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3456)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3457)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3458)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3459)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3460)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3461)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3462)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3463)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3464)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3465)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3466)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3467)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3468)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3469)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3470)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3471)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3472)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3473)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3474)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3475)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3476)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3477)
Pro Gly Thr Cys Gly Glu Xaa' Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3478)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3479)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3480)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3481)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3482)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3483)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3484)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3485)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3486)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3487)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3488)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3489)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3490)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3491)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3492)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3493)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3494)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3495)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3496)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3497)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3498)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3499)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3500)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3501)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3502)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3503)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3504)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3505)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3506)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3507)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3508)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3509)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3510)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3511)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3512)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3513)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3514)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3515)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3516)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3517)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3518)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3519)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3520)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3521)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3522)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3523)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3524)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3525)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3526)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3527)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3528)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3529)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3530)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3531)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3532)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3533)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3534)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3535)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3536)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3537)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3538)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3539)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3540)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3541)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3542)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3543)
Pro Gly Thr Cys Gly Glu Xaa' Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3544)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3545)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3546)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3547)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3548)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3549)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3550)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3551)
Xaa Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3552)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3553)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3554)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3555)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3556)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3557)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3558)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3559)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3560)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3561)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3562)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3563)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3564)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3565)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3566)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3567)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3568)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3569)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3570)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3571)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3572)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3573)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3574)

FIG. 2
(sheet 79 of 114)

Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3575)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3576)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3577)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3578)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3579)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3580)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3581)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3582)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3583)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3584)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3585)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3586)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3587)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3588)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3589)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3590)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3591)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3592)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3593)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3594)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3595)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3596)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3597)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3598)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3599)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3600)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3601)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3602)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3603)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3604)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3605)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3606)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3607)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3608)

FIG. 2
(sheet 80 of 114)

Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3609)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3610)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3611)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3612)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3613)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3614)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3615)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3616)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3617)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3618)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3619)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3620)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3621)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3622)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3623)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3624)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3625)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3626)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3627)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3628)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3629)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3630)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3631)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3632)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3633)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3634)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3635)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3636)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3637)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3638)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3639)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3640)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3641)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3642)

FIG. 2
(sheet 81 of 114)

Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3643)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3644)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3645)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3646)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3647)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3648)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3649)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3650)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3651)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3652)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3653)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3654)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3655)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3656)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3657)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3658)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3659)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3660)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3661)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3662)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3663)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3664)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3665)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3666)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3667)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3668)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3669)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3670)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3671)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3672)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3673)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3674)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3675)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3676)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3677)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3678)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3679)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3680)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3681)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3682)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3683)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3684)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3685)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3686)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3687)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3688)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3689)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3690)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3691)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3692)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3693)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3694)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3695)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3696)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3697)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3698)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3699)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3700)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3701)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3702)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3703)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3704)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3705)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3706).
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3707)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3708)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3709)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3710)

FIG. 2
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Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3711)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3712)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3713)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3714)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3715)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3716)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3717)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3718)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3719)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3720)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3721)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3722)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3723)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3724)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3725)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3726)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3727)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3728)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3729)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3730)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3731)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 3732)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 3733)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 3734)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 3735)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3736)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 3737)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 3738)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 3739)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3740)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 3741)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 3742)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3743)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 3744)

FIG. 2
(sheet 84 of 114)

Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3745)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3746)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 3747)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 3748)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 3749)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3750)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 3751)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 3752)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3753)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 3754)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3755)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3756)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 3757)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 3758)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3759)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 3760)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3761)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3762)
Pro Gly Thr Cys Gly Glu Xaa' Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 3763)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3764)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3765)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3766)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3767)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3768)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3769)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3770)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3771)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3772)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3773)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3774)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3775)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3776)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3777)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3778)

FIG. 2
(sheet 85 of 114)

Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3779)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3780)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3781)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3782)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3783)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3784)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3785)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3786)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3787)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3788)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3789)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3790)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3791)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3792)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3793)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3794)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3795)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3796)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3797)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3798)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3799)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3800)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3801)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3802)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3803)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3804)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3805)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3806)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3807)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3808)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3809)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3810)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3811)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3812)

FIG. 2
(sheet 86 of 114)

Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3813)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3814)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3815)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3816)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3817)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3818)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3819)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3820)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3821)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3822)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3823)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3824)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3825)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3826)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3827)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3828)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3829)
Pro Gly Thr Cys Gly Glu Ile Xaa' Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3830)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3831)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3832)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3833)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3834)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3835)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3836)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3837)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3838)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3839)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3840)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3841)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3842)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3843)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3844)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3845)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3846)

FIG. 2
(sheet 87 of 114)

Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3847)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3848)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3849)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3850)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3851)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3852)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3853)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3854)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3855)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3856)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3857)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3858)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3859)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3860)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3861)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3862)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3863)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3864)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3865)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3866)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3867)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3868)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3869)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3870)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3871)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3872)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3873)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3874)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3875)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3876)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3877)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3878)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3879)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3880)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3881)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3882)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3883)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3884)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3885)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3886)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3887)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 3888)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3889)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3890)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3891)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3892)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3893)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3894)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3895)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3896)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3897)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3898)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3899)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3900)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3901)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3902)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3903)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3904)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3905)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3906)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3907)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3908)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3909)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3910)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3911)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3912)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3913)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3914)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3915)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3916)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3917)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3918)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3919)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3920)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3921)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3922)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3923)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3924)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3925)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3926)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3927)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3928)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3929)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3930)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3931)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3932)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 3933)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3934)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3935)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3936)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3937)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3938)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3939)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3940)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3941)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3942)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3943)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3944)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3945)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3946)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3947)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3948)

FIG. 2
(sheet 90 of 114)

Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3949)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3950)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3951)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3952)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3953)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3954)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3955)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3956)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3957)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3958)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3959)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3960)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3961)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3962)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3963)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3964)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3965)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3966)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3967)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3968)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 3969)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3970)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3971)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3972)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3973)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3974)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3975)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3976)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3977)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3978)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 3979)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 3980)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 3981)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 3982)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 3983)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 3984)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 3985)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 3986)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 3987)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 3988)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 3989)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 3990)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 3991)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 3992)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 3993)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 3994)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 3995)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3996)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 3997)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 3998)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 3999)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4000)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4001)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4002)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4003)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4004)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4005)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4006)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4007)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4008)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4009)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4010)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4011)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4012)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4013)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4014)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4015)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4016)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4017)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4018)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4019)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4020)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4021)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4022)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4023)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4024)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4025)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4026)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4027)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4028)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4029)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4030)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4031)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4032)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4033)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4034)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4035)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4036)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4037)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4038)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4039)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4040)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4041)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4042)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4043)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4044)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4045)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4046)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4047)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4048)
Pro Gly Thr Cys Gly Glu Ile Xaa' Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4049)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4050)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4051)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4052)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4053)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4054)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4055)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4056)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4057)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4058)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4059)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4060)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4061)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4062)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4063)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4064)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4065)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4066)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4067)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4068)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4069)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4070)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4071)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4072)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4073)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4074)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4075)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4076)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4077)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4078)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4079)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4080)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4081)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4082)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4083)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4084)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4085)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4086)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4087)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4088)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4089)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4090)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4091)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4092)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4093)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4094)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4095)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4096)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4097)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4098)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4099)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4100)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4101)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4102)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4103)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4104)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4105)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4106)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4107)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4108)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4109)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4110)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4111)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4112)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4113)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4114)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4115)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4116)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4117)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4118)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4119)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4120)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4121)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4122)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4123)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4124)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4125)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4126)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4127)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4128)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4129)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4130)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4131)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4132)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4133)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4134)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4135)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4136)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4137)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4138)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4139)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4140)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4141)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4142)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4143)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4144)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4145)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4146)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4147)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4148)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4149)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4150)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4151)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4152)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4153)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4154)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4155)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4156)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4157)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4158)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4159)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4160)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4161)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4162)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4163)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4164)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4165)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4166)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4167)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4168)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4169)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4170)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4171)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4172)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4173)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4174)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4175)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4176)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4177)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4178)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4179)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4180)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4181)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4182)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4183)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4184)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4185)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4186)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4187)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4188)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4189)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4190)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4191)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4192)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4193)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4194)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4195)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4196)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4197)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4198)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4199)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4200)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4201)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4202)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4203)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4204)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4205)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4206)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4207)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4208)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4209)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4210)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4211)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4212)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4213)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4214)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4215)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4216)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4217)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4218)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4219)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4220)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4221)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4222)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4223)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4224)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4225)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4226)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4227)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4228)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4229)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4230)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4231)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4232)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4233)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4234)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4235)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4236)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4237)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4238)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4239)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4240)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4241)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4242)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4243)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4244)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4245)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4246)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4247)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4248)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4249)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4255)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4251)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4252)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4253)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4254)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4255)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4256)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4257)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4258)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4259)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4260)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4261)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4262)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4263)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4264)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4265)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4266)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4267)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4268)
Pro Gly Thr Cys Gly Glu Ile Cys Xaa' Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4269)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4270)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4271)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4272)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys (SEQID NO: 4273)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4274)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4275)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4276)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4277)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4278)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4279)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4280)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4281)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4282)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4283)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4284)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4285)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4286)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4287)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4288)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4289)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4290)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4291)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4292)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4293)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4294)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4295)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4296)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4297)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4298)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4299)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4300)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4301)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4302)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4303)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4304)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4305)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4306)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4307)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4308)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4309)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4310)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4311)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4312)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4313)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4314)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4315)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4316)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4317)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4318)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4319)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4320)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4321)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4322)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4333)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4334)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4335)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4336)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4337)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4338)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4339)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4340)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4341)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4342)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4343)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4344)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4345)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4346)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4347)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4348)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4349)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4350)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4351)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4352)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4353)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4354)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4355)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4356)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4357)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4358)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4359)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4360)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4361)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4362)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4363)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4364)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4365)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4366)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4367)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4368)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4369)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4370)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4371)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4372)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4373)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4374)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4375)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4376)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4377)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4378)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4379)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4380)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4381)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4382)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4383)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4384)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4385)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4386)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4387)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4388)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4389)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4390)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4391)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4392)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4393)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4394)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4395)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4396)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4397)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4398)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4399)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4400)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4401)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4402)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4403)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4404)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4405)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4406)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4407)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4408)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4409)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4410)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4411)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4412)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4413)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4414)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4415)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4416)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4417)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4418)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4419)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4420)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4421)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4422)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4423)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4424)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4425)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4426)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4427)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4428)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4429)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4430)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4431)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4432)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4433)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Xaa' Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4434)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4435)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4436)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4437)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys (SEQID NO: 4438)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4439)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4440)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4441)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4442)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4443)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4444)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4445)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4446)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4447)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4448)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4449)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4450)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4451)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4452)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4453)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4454)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4455)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4456)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4457)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4458)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4459)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4460)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4461)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4462)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4463)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4464)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4465)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4466)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4467)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4468)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4469)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4470)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4471)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4472)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4473)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4474)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4475)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4476)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4477)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4478)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4479)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4480)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4481)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4482)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4483)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4484)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4485)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4486)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4487)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4488)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4489)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4490)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4491)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4492)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4493)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4494)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4495)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4496)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4497)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4498)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4499)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4500)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4501)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4502)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4503)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4504)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4505)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4506)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4507)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4508)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4509)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4510)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4511)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4512)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4513)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4514)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4515)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4516)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4517)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4518)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4519)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4520)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4521)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4522)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4523)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4524)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4525)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4526)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4527)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4528)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4529)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4530)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4531)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4532)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4533)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4534)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4535)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4536)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4537)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4538)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4539)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4540)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4541)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4542)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4543)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4544)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4545)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4546)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4547)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4548)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4549)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4550)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4551)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4552)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4553)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Xaa' Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4554)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4555)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4556)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4557)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys (SEQID NO: 4558)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4559)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4560)

FIG. 2
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Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 4561)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 4562)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Xaa' Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 4563)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 4564)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys	(SEQID NO: 4565)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys	(SEQID NO: 4566)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys	(SEQID NO: 4567)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys	(SEQID NO: 4568)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Xaa' Cys Thr Gly Cys Xaa'	(SEQID NO: 4569)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys	(SEQID NO: 4570)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys	(SEQID NO: 4571)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys	(SEQID NO: 4572)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys	(SEQID NO: 4573)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Xaa' Thr Gly Cys Xaa'	(SEQID NO: 4574)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys	(SEQID NO: 4575)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys	(SEQID NO: 4576)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys	(SEQID NO: 4577)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Xaa' Gly Cys Xaa'	(SEQID NO: 4578)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys	(SEQID NO: 4579)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys	(SEQID NO: 4580)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Xaa' Cys Xaa'	(SEQID NO: 4581)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa'	(SEQID NO: 4582)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Xaa' Ala Cys Thr Gly Cys Xaa' Xaa'	(SEQID NO: 4583)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys	(SEQID NO: 4584)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys	(SEQID NO: 4585)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys	(SEQID NO: 4586)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys	(SEQID NO: 4587)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys	(SEQID NO: 4588)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys	(SEQID NO: 4589)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Xaa' Cys Thr Gly Cys Xaa'	(SEQID NO: 4590)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys	(SEQID NO: 4591)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys	(SEQID NO: 4592)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys	(SEQID NO: 4593)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys	(SEQID NO: 4594)

FIG. 2
(sheet 109 of 114)

Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4595)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4596)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4597)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4598)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4599)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4600)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4601)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4602)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4603)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4604)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4605)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4606)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4607)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4608)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4609)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4610)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4611)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4612)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4613)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4614)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4615)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4616)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4617)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4618)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4619)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4620)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4621)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4622)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4623)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4624)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4625)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4626)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4627)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4628)

FIG. 2
(sheet 110 of 114)

Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4629)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4630)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4631)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4632)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4633)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4634)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4635)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4636)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4637)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Xaa' Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4638)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys (SEQID NO: 4639)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4640)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4641)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Xaa' Cys Thr Gly Cys (SEQID NO: 4642)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4643)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4644)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4645)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4646)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4647)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4648)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4649)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4650)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4651)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4652)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4653)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4654)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4655)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4656)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4657)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4658)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4659)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4660)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys (SEQID NO: 4661)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4662)

FIG. 2
(sheet 111 of 114)

Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4663)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4664)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4665)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4666)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4667)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4668)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4669)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4670)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4671)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4672)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4673)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4674)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4675)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys (SEQID NO: 4676)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4677)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4678)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4679)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4680)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4681)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4682)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4683)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4684)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4685)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys (SEQID NO: 4686)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4687)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4688)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4689)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4690)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4691)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' (SEQID NO: 4692)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4693)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Xaa' Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4694)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys (SEQID NO: 4695)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys (SEQID NO: 4696)

FIG. 2
(sheet 112 of 114)

Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4697)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Xaa' Thr Gly Cys (SEQID NO: 4698)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4699)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4700)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4701)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys (SEQID NO: 4702)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4703)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4704)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4705)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys (SEQID NO: 4706)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4707)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4708)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' (SEQID NO: 4709)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4710)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys (SEQID NO: 4711)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys (SEQID NO: 4712)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4713)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4714)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4715)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys (SEQID NO: 4716)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4717)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4718)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' (SEQID NO: 4719)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4720)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys (SEQID NO: 4721)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys (SEQID NO: 4722)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4723)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4724)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' (SEQID NO: 4725)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4726)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' (SEQID NO: 4727)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' (SEQID NO: 4728)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Xaa' Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4729)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys (SEQID NO: 4730)

FIG. 2
(sheet 113 of 114)

Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys (SEQID NO: 4731)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4732)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Xaa' Gly Cys (SEQID NO: 4733)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4734)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4735)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys (SEQID NO: 4736)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4737)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4738)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' (SEQID NO: 4739)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4740)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys (SEQID NO: 4741)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys (SEQID NO: 4742)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4743)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4744)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' (SEQID NO: 4745)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4746)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' (SEQID NO: 4747)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' (SEQID NO: 4748)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Xaa' Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4749)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys (SEQID NO: 4750)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys (SEQID NO: 4751)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys (SEQID NO: 4752)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Xaa' Cys (SEQID NO: 4753)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Xaa' Cys Xaa' (SEQID NO: 4754)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' (SEQID NO: 4755)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Xaa' Cys Xaa' Xaa' (SEQID NO: 4756)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' (SEQID NO: 4757)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' (SEQID NO: 4758)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Xaa' Cys Xaa' Xaa' Xaa' (SEQID NO: 4759)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' (SEQID NO: 4760)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' (SEQID NO: 4761)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' (SEQID NO: 4762)
Pro Gly Thr Cys Gly Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys Xaa' Xaa' Xaa' Xaa' (SEQID NO: 4763)

FIG. 2
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(SEQ ID NO: 4764)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4765)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4766)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4767)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4768)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4769)	Cys Glu Tyr Cys Ala Asn Pro Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4770)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4771)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4772)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4773)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4774)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4775)	Cys Glu Tyr Cys Ala Asn Pro Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4776)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4777)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4778)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4779)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4780)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4781)	Cys Glu Tyr Cys Ala Asn Gly Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4782)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4783)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4784)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4785)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4786)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4787)	Cys Glu Tyr Cys Ala Asn Gly Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4788)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4789)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4790)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4791)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4792)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4793)	Cys Glu Tyr Cys Arg Asn Pro Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4794)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4795)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4796)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4797)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4798)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4799)	Cys Glu Tyr Cys Arg Asn Pro Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4800)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4801)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4802)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4803)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4804)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4805)	Cys Glu Tyr Cys Arg Asn Gly Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4806)	Cys Glu Tyr Cys Arg Asn Gly Thr Cys Thr Gly Cys Tyr

FIG. 3
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(SEQ ID NO: 4807) Cys Glu Tyr Cys Arg Asn Gly Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4808) Cys Glu Tyr Cys Arg Asn Gly Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4809) Cys Glu Tyr Cys Arg Asn Gly Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4810) Cys Glu Tyr Cys Arg Asn Gly Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4811) Cys Glu Tyr Cys Arg Asn Gly Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4812) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4813) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4814) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4815) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4816) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4817) Cys Glu Tyr Cys Asn Asn Pro Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4818) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4819) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4820) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4821) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4822) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4823) Cys Glu Tyr Cys Asn Asn Pro Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4824) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4825) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4826) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4827) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4828) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4829) Cys Glu Tyr Cys Asn Asn Gly Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4830) Cys Glu Tyr Cys Asn Asn Gly Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4831) Cys Glu Tyr Cys Asn Asn Gly Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4832) Cys Glu Tyr Cys Asn Asn Gly Thr Cys Val Gly Cys Tyr
(SEQ ID NO: 4833) Cys Glu Tyr Cys Asn Asn Gly Thr Cys Val Ala Cys Tyr
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(SEQ ID NO: 4835) Cys Glu Tyr Cys Asn Asn Gly Thr Cys Gly Ala Cys Tyr
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(SEQ ID NO: 4839) Cys Glu Tyr Cys Asp Asn Pro Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4840) Cys Glu Tyr Cys Asp Asn Pro Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4841) Cys Glu Tyr Cys Asp Asn Pro Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4842) Cys Glu Tyr Cys Asp Asn Pro Thr Cys Thr Gly Cys Tyr
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(SEQ ID NO: 4845) Cys Glu Tyr Cys Asp Asn Pro Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4846) Cys Glu Tyr Cys Asp Asn Pro Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4847) Cys Glu Tyr Cys Asp Asn Pro Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4848) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4849) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Thr Ala Cys Tyr

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(SEQ ID NO: 4850) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4851) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4852) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4853) Cys Glu Tyr Cys Asp Asn Gly Ala Cys Gly Ala Cys Tyr
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(SEQ ID NO: 4856) Cys Glu Tyr Cys Asp Asn Gly Thr Cys Val Gly Cys Tyr
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(SEQ ID NO: 4859) Cys Glu Tyr Cys Asp Asn Gly Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4860) Cys Glu Tyr Cys Gln Asn Pro Ala Cys Thr Gly Cys Tyr
(SEQ ID NO: 4861) Cys Glu Tyr Cys Gln Asn Pro Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4862) Cys Glu Tyr Cys Gln Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4863) Cys Glu Tyr Cys Gln Asn Pro Ala Cys Val Ala Cys Tyr
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(SEQ ID NO: 4867) Cys Glu Tyr Cys Gln Asn Pro Thr Cys Thr Ala Cys Tyr
(SEQ ID NO: 4868) Cys Glu Tyr Cys Gln Asn Pro Thr Cys Val Gly Cys Tyr
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(SEQ ID NO: 4892) Cys Glu Tyr Cys Glu Asn Pro Thr Cys Val Gly Cys Tyr

FIG. 3
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(SEQ ID NO: 4893) Cys Glu Tyr Cys Glu Asn Pro Thr Cys Val Ala Cys Tyr
(SEQ ID NO: 4894) Cys Glu Tyr Cys Glu Asn Pro Thr Cys Gly Gly Cys Tyr
(SEQ ID NO: 4895) Cys Glu Tyr Cys Glu Asn Pro Thr Cys Gly Ala Cys Tyr
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(SEQ ID NO: 4897) Cys Glu Tyr Cys Glu Asn Gly Ala Cys Thr Ala Cys Tyr
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(SEQ ID NO: 4902) Cys Glu Tyr Cys Glu Asn Gly Thr Cys Thr Gly Cys Tyr
(SEQ ID NO: 4903) Cys Glu Tyr Cys Glu Asn Gly Thr Cys Thr Ala Cys Tyr
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(SEQ ID NO: 4907) Cys Glu Tyr Cys Glu Asn Gly Thr Cys Gly Ala Cys Tyr
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(SEQ ID NO: 4909) Cys Glu Tyr Cys Gly Asn Pro Ala Cys Thr Ala Cys Tyr
(SEQ ID NO: 4910) Cys Glu Tyr Cys Gly Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4911) Cys Glu Tyr Cys Gly Asn Pro Ala Cys Val Ala Cys Tyr
(SEQ ID NO: 4912) Cys Glu Tyr Cys Gly Asn Pro Ala Cys Gly Gly Cys Tyr
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(SEQ ID NO: 4925) Cys Glu Tyr Cys Gly Asn Gly Ala Cys Gly Ala Cys Tyr
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(SEQ ID NO: 4931) Cys Glu Tyr Cys Gly Asn Gly Thr Cys Gly Ala Cys Tyr
(SEQ ID NO: 4932) Cys Glu Tyr Cys His Asn Pro Ala Cys Thr Gly Cys Tyr
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(SEQ ID NO: 4934) Cys Glu Tyr Cys His Asn Pro Ala Cys Val Gly Cys Tyr
(SEQ ID NO: 4935) Cys Glu Tyr Cys His Asn Pro Ala Cys Val Ala Cys Tyr

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(SEQ ID NO: 4936) Cys Glu Tyr Cys His Asn Pro Ala Cys Gly Gly Cys Tyr
(SEQ ID NO: 4937) Cys Glu Tyr Cys His Asn Pro Ala Cys Gly Ala Cys Tyr
(SEQ ID NO: 4938) Cys Glu Tyr Cys His Asn Pro Thr Cys Thr Gly Cys Tyr
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(SEQ ID NO: 4941) Cys Glu Tyr Cys His Asn Pro Thr Cys Val Ala Cys Tyr
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(SEQ ID NO: 4953) Cys Glu Tyr Cys His Asn Gly Thr Cys Val Ala Cys Tyr
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FIG. 3
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Electronic Acknowledgement Receipt

EFS ID:	21544835
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	58249
Filer:	Anne Elizabeth Fleckenstein
Filer Authorized By:	
Attorney Docket Number:	40737-509001US
Receipt Date:	19-FEB-2015
Filing Date:	15-MAR-2012
Time Stamp:	19:04:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Merck_Manual.pdf	153958 5d39c22b3720d23ab349f32bb0086348304 57f71	no	1

Warnings:	
Information:	

2	Non Patent Literature	Medline_Plus.pdf	709057 137626f785f088ff90a07522f39ce06ce48126de	no	6
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3	Non Patent Literature	Li_and_Chiang_J_Lipids_2012_1_9_2011.pdf	1209737 6b26e7df2012885a2e99543cd632cf862f58e3d7	no	10
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8	Foreign Reference	W02007022531_Part5.pdf	8879247 6fb39887dea5d600b4068ffaf8e4a2671a3e1c68	no	127
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9	Foreign Reference	WO2007101158_Part1.pdf	6328672 279227778465790b0aef98b1de6fd728413b1fa29	no	105
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			Total Files Size (in bytes):	74538840	

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

(12) **Offenlegungsschrift**

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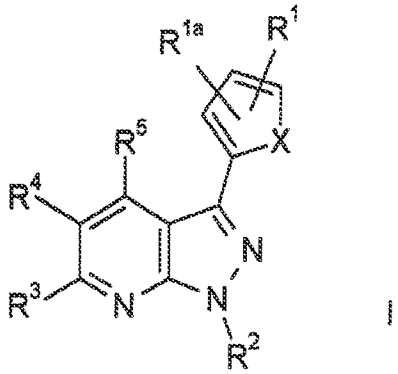
Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

(54) Substituierte Pyrazolo[3,4-b]pyridine, ihre Herstellung und Verwendung in Arzneimitteln

(57) Die vorliegende Erfindung betrifft substituierte Pyrazolo[3,4-b]pyridine der Formel I,



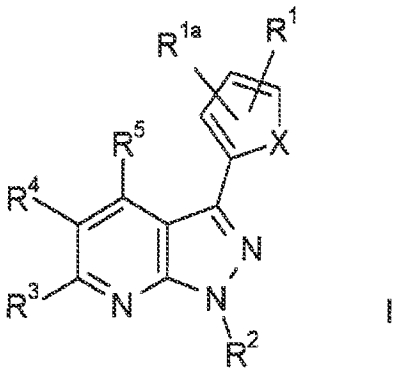
I

in der X, R^1, R^{1a}, R^2, R^3, R^4 und R^5 die in den Ansprüchen angegebenen Bedeutungen haben, die wertvolle Arzneimittelwirkstoffe zur Therapie und Prophylaxe von Krankheiten sind, zum Beispiel von Herz-Kreislauferkrankungen wie Bluthochdruck, Angina pectoris, Herzinsuffizienz, Thrombosen oder Atherosklerose. Die Verbindungen der Formel I haben die Fähigkeit zur Modulation der körpereigenen Produktion von cyclischem Guanosinmonophosphat (cGMP) und eignen sich generell zur Therapie und Prophylaxe von Krankheitszuständen, die mit einem gestörten cGMP-Haushalt verbunden sind. Die Erfindung betrifft weiterhin Verfahren zur Herstellung von Verbindungen der Formel I, ihre Verwendung zur Therapie und Prophylaxe der bezeichneten Krankheitszustände und zur Herstellung von Arzneimitteln dafür sowie pharmazeutische Präparate, die Verbindungen der Formel I enthalten.

DE 197 44 027 A 1

Beschreibung

Die vorliegende Erfindung betrifft substituierte Pyrazolo[3,4-b]pyridine der Formel I,



I

in der X, R^1, R^{1a}, R^2, R^3, R^4 und R^5 die unten angegebenen Bedeutungen haben, die wertvolle Arzneimittelwirkstoffe zur Therapie und Prophylaxe von Krankheiten sind, zum Beispiel von Herz-Kreislauferkrankungen wie Bluthochdruck, Angina pectoris, Herzinsuffizienz, Thrombosen oder Atherosklerose. Die Verbindungen der Formel I haben die Fähigkeit zur Modulation der körpereigenen Produktion von cyclischem Guanosinmonophosphat (cGMP) und eignen sich generell zur Therapie und Prophylaxe von Krankheitszuständen, die mit einem gestörten cGMP-Haushalt verbunden sind. Die Erfindung betrifft weiterhin Verfahren zur Herstellung von Verbindungen der Formel I, ihre Verwendung zur Therapie und Prophylaxe der bezeichneten Krankheitszustände und zur Herstellung von Arzneimitteln dafür sowie pharmazeutische Präparate, die Verbindungen der Formel I enthalten.

cGMP ist ein wichtiger intrazellulärer Botenstoff, der über die Modulation cGMP-abhängiger Proteinkinasen, Phosphodiesterasen und Ionenkanälen eine Vielzahl verschiedener Effekte auslöst. Beispiele sind die Glattmuskelrelaxation, die Inhibition der Thrombozytenaktivierung und die Hemmung von Glattmuskelzellproliferation und Leukozytenadhäsion. cGMP wird durch partikuläre und lösliche Guanylatcyclasen (GC) als Antwort auf eine Reihe extra- und intrazellulärer Stimuli produziert. Im Falle der partikulären Guanylatcyclasen erfolgt die Stimulation im wesentlichen durch peptidische Signalstoffe, wie dem atrialen natriuretischen Peptid oder dem cerebralen natriuretischen Peptid. Die löslichen Guanylatcyclasen (sGC), bei denen es sich um cytosolische, heterodimere Hämproteine handelt, werden dagegen im wesentlichen durch eine Familie niedermolekularer, enzymatisch gebildeter Faktoren reguliert. Wichtigstes Stimulans ist das Stickstoffmonoxid (NO) oder eine nahe verwandte Spezies. Die Bedeutung anderer Faktoren wie Kohlenmonoxid oder dem Hydroxyiradikal ist noch weitgehend ungeklärt. Als Aktivierungsmechanismus der Aktivierung durch NO wird die Anbindung von NO an das Häm unter Ausbildung eines pentakoordinierten Häm-Nitrosyl-Komplexes diskutiert. Die damit verbundene Freisetzung des im Basal-Zustand an das Eisen gebundenen Histidins überführt das Enzym in die aktivierte Konformation.

Aktive lösliche Guanylatcyclasen setzen sich aus je einer α- und einer β-Untereinheit zusammen. Von den Untereinheiten wurden mehrere Subtypen beschrieben, die sich untereinander bezüglich Sequenz, gewebespezifischer Verteilung und Expression in verschiedenen Entwicklungsstadien unterscheiden. Die Subtypen α_1 und β_1 werden hauptsächlich in Gehirn und Lunge exprimiert, während β_2 vor allem in Leber und Niere gefunden wird. In humanem fötalen Gehirn konnte der Subtyp α2 nachgewiesen werden. Die als α3 und β_3 bezeichneten Untereinheiten wurden aus menschlichem Gehirn isoliert und sind homolog zu α1 und β_1. Neuere Arbeiten weisen auf eine α_{2i}-Untereinheit hin, die ein Insert in der katalytischen Domäne enthält. Alle Untereinheiten zeigen große Homologien im Bereich der katalytischen Domäne. Die Enzyme enthalten vermutlich ein Häm pro Heterodimer, das über β_1-Cys-78 und/oder β_1-His-105 gebunden ist und Teil des regulatorischen Zentrums ist.

Unter pathologischen Bedingungen kann die Bildung Guanylatcyclase-aktivierender Faktoren vermindert sein oder es kann durch das vermehrte Auftreten freier Radikale ein verstärkter Abbau derselben erfolgen. Die daraus resultierende verminderte Aktivierung der sGC führt über die Abschwächung der jeweiligen cGMp-vermittelten Zellantwort beispielsweise zum Anstieg des Blutdrucks, zur Plättchenaktivierung oder zu vermehrter Zellproliferation und Zelladhäsion. Als Folge kommt es zur Ausbildung von endothelialer Dysfunktion, Atherosklerose, Bluthochdruck, stabiler und instabiler Angina pectoris, Thrombosen, Myocardinfarkt, Schlaganfällen oder erektiler Dysfunktion. Die pharmakologische Stimulation der sGC bietet eine Möglichkeit zur Normalisierung der cGMp-Produktion und erlaubt damit die Behandlung bzw. Prävention derartiger Krankheiten.

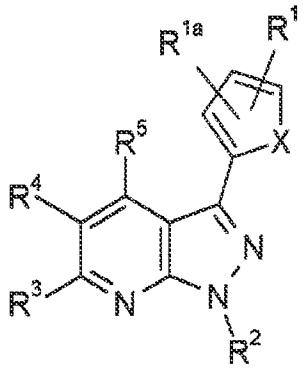
Zur pharmakologischen Stimulation der sGC wurden bisher fast ausschließlich Verbindungen verwendet, deren Wirkung auf einer intermediären NO-Freisetzung beruht, beispielsweise organische Nitrate. Der Nachteil dieser Behandlungsweise liegt in der Toleranzentwicklung und Wirkungsabschwächung und der deshalb erforderlich werdenden höheren Dosierung.

Verschiedene nicht über eine NO-Freisetzung wirkende sGC-Stimulatoren wurden von Vesely in einer größeren Zahl von Arbeiten beschrieben. Die Verbindungen, bei denen es sich zumeist um Hormone, Pflanzenhormone, Vitamine oder zum Beispiel Naturstoffe wie Echsengifte handelt, zeigen jedoch durchweg nur schwache Effekte auf die cGMP-Bildung in Zellysaten (D. L. Vesely, Eur. J. Clin. Invest. 15 (1985) 258; D. L. Vesely, Biochem. Biophys. Res. Comm. 88 (1979)1244). Eine Stimulation Häm-freier Guanylatcyclase durch Protoporphyrin IX wurde durch Ignarro et al. (Adv. Pharmacol. 26 (1994) 35) nachgewiesen. Pettibone et al. (Eur. J. Pharmacol. 116 (1985) 307) beschrieben für Diphenyliodoniumhexafluorophoshat eine blutdrucksenkende Wirkung und führten diese auf eine Stimulation der sGC zurück.

Isoliquiritiginin, das an isolierten Rattenaorten eine relaxierende Wirkung zeigt, aktiviert laut Yu et al. (Brit. J. Pharmacol. 114 (1995)1587) ebenfalls die sGC. Ko et 21. (Blood 84 (1994) 4226), Yu et al. (Biochem. J. 306 (1995) 787) und Teng et al. (Brit. J. Pharmacol. 116 (1995)1973) wiesen eine sGC-stimulierende Aktivität von 1-Benzyl-3-(5-hydroxymethyl-2-furyl)-indazol nach und demonstrierten eine antiproliferative und thrombozytenhemmende Wirkung. Verschiedene Indazole werden in EP-A-667 345 als Inhibitoren der Thrombozytenaggregation beschrieben.

Im Pyridinring unsubstituierte Pyrazolo[3,4-b]pyridine, die in der 1-Position einen Arylrest oder einen Heteroarylrest und in der 3-Position einen Arylrest oder einen Heteroarylrest tragen, sind als Antitumor-Agentien und Immunstimulanzien in der japanischen Patentanmeldung 01-190681 beschrieben. Überraschend wurde nun gefunden, daß demgegenüber die erfindungsgemäßen Pyrazolo[3,4-b]-pyridin-Derivate der Formel I eine völlig verschiedene Wirkung aufweisen und eine Guanylatcyclase-Aktivierung bewirken, aufgrund derer sie zur Therapie und Prophylaxe von Krankheiten geeignet sind, die mit einem niedrigen cGMp-Spiegel verbunden sind.

Die vorhegende Erfindung betrifft somit Verbindungen der Formel I



I

in der X für O, S, NH oder N(CH_3) steht;
R^1 und R^{1a} unabhängig voneinander für Wasserstoff, Halogen, CO-R^{10} oder (C_1-C_8)-Alkyl stehen, wobei Alkylgruppen gesättigt oder ungesättigt sein können und unsubstituiert oder durch einen oder mehrere gleiche oder verschiedene Reste R^{11} substituiert sein können;
R^2 für (C_1-C_6)-Alkyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl steht;
R^3 und R^5 unabhängig voneinander für Wasserstoff, (C_1-C_6)-Alkyl, unsubstituiertes oder substituiertes Phenyl, in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl, Hydroxy, (C_1-C_6)-Alkoxy oder in der Phenylgruppe unsubstituiertes oder substituiertes Benzyloxy stehen;
R^4 für Wasserstoff, (C_1-C_6)-Alkyl, unsubstituiertes oder substituiertes Phenyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl steht;
R^{10} für Wasserstoff, (C_1-C_6)-Alkyl oder unsubstituiertes oder substituiertes Phenyl steht;
R^{11} für $R^{12}O$, $R^{12}R^{13}N$, Halogen oder unsubstituiertes oder substituiertes Phenyl steht;
R^{12} für Wasserstoff, (C_1-C_6)-Alkyl, in der Phenylgruppe unsubstituiertes oder substituiertes Benzyl, H-CO oder (C_1-C_6)-Alkyl-CO steht;
R^{13} für Wasserstoff oder (C_1-C_6)-Alkyl steht;
oder R^{12} und R^{13} zusammen mit dem Stickstoffatom, an das sie gebunden sind, einen gesättigten 5-Ring- oder 6-Ring-Heterocyclus bilden, der als zusätzliches Ring-Heteroatom noch ein Sauerstoffatom, Schwefelatom oder ein durch eine Methylgruppe substituiertes Stickstoffatom enthalten kann;
in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

Alkylreste können geradkettig oder verzweigt sein. Dies gilt auch, wenn sie substituiert sind, zum Beispiel durch einen Phenylrest oder durch Hydroxy, oder wenn sie in anderen Gruppen enthalten sind, zum Beispiel in Alkoxygruppen. Beispiele für Alkylgruppen sind Methyl, Ethyl, n-Propyl, Isopropyl, n-Butyl, Isobutyl, sec-Butyl, tert-Butyl, n-Pentyl, Isopentyl, Neopentyl, n-Hexyl, 3,3-Dimethylbutyl, n-Heptyl, n-Octyl. Unter dem Begriff Alkyl sind hier auch ungesättigte Alkylreste zu verstehen, insbesondere Alkylreste, die eine oder zwei Doppelbindungen oder eine oder zwei Dreifachbindungen oder eine Doppelbindung und eine Dreifachbindung enthalten. Beispiele für solche Reste sind der Vinylrest, der 2-Propenylrest (Allylrest), der 2-Butenylrest, der 3-Methyl-2-butenylrest, der Ethinylrest, der 2-Propinylrest (Propargylrest) oder der 3-Butinylrest.

Phenylreste können unsubstituiert sein oder einfach oder mehrfach, zum Beispiel zweifach oder dreifach, durch gleiche oder verschiedene Substituenten substituiert sein, wobei sich die Substituenten in beliebigen Positionen befinden können. Bevorzugt sind substituierte Phenylreste einfach oder zweifach substituiert. Monosubstituierte Phenylreste können in der 2-Position, der 3-Position oder der 4-Position substituiert sein, disubstituierte Phenylreste in der 2,3-Position, der 2,4-Position, der 2,5-Position, der 2,6-Position, der 3,4-Position oder der 3,5-Position. In trisubstituierten Phenylresten können sich die Substituenten beispielsweise in 2,3,4-Position, 2,3,5-Position, 2,3,6-Position, 2,4,5-Position, 2,4,6-Position oder 3,4,5-Position befinden. Als Substituenten an substituierten Phenylringen kommen insbesondere in Betracht (C_1-C_4)-Alkyl, Halogen, (C_1-C_4)-Alkoxy, Benzyloxy, Amino, ((C_1-C_4)-Alkyl)-carbonylamino, Mono- und Di-((C_1-C_4)-alkyl)-amino, Nitro, Cyano, Trifluormethyl, Hydroxycarbonyl, Aminocarbonyl (= Carbamoyl) und ((C_1-C_4)-Alkoxy)-carbonyl. Die Erläuterungen zu Phenylresten gelten auch für solche Phenylreste, die in Phenylalkylresten oder Benzylresten enthalten sind. Bevorzugte Phenylalkylreste sind Phenylethylreste und insbesondere der Benzylrest.

Beispiele für Heterocyclen, die R^{12} und R^{13} zusammen mit dem sie tragenden Stickstoffatom bilden können, sind Pyr-

rolidin, Piperidin, Piperazin, Morpholin und Thiomorpholin. Diese Heterocyclen können auch zum Beispiel durch eine oder mehrere, zum Beispiel ein, zwei, drei oder vier, Alkylgruppen, insbesondere Methylgruppen, substituiert sein und im Falle des Thiomorpholins am Schwefel zum Sulfoxid oder Sulfon oxidiert sein.

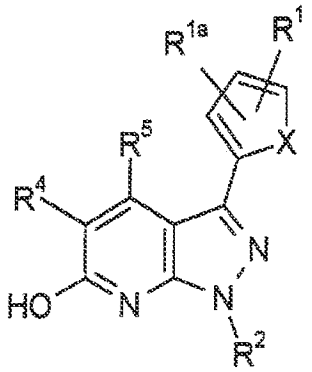
Halogen bedeutet Fluor, Chlor, Brom oder Iod, vorzugsweise Fluor oder Chlor.

Sind Alkylreste durch Reste R^{11} substituiert, so können sie zum Beispiel durch einen, zwei, drei, vier oder mehr gleiche oder verschiedene Reste R^{11} substituiert sein. Bevorzugt enthalten solche substituierten Reste einen, zwei oder drei, besonders bevorzugt einen oder zwei gleiche oder verschiedene Reste R^{11}. In den substituierten Alkylresten können sich die Substituenten in beliebigen Positionen befinden.

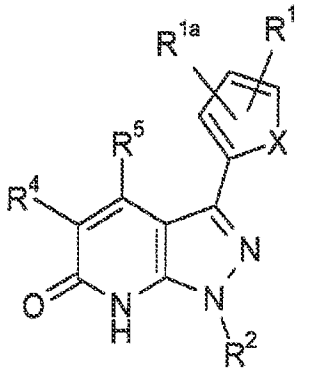
Die beiden Substituenten R^1 und R^{1a} können sich in beliebigen Positionen des Heterocyclus befinden, also in den Positionen 3, 4 und 5 des Furanrings, Thiophenrings oder Pyrrolrings.

Die Verbindungen der Formel I können bei entsprechender Substitution in stereoisomeren Formen vorliegen. Enthalten die Verbindungen der Formel I ein oder mehrere Asymmetriezentren, so können diese unabhängig voneinander die S-Konfiguration oder die R-Konfiguration aufweisen. Zur Erfindung gehören alle möglichen Stereoisomeren, zum Beispiel Enantiomere und Diastereomere, und Mischungen von zwei oder mehr stereoisomeren Formen, zum Beispiel Mischungen von Enantiomeren und/oder Diastereomeren, in allen Verhältnissen. Enantiomere sind also in enantiomerenreiner Form, sowohl als links- als auch als rechtsdrehende Antipoden, in Form von Racematen und in Form von Mischungen der beiden Enantiomeren in allen Verhältnissen Gegenstand der Erfindung. Bei Vorliegen einer cis/trans-Isomerie sind sowohl die cis-Form als auch die trans-Form und Gemische dieser Formen Gegenstand der Erfindung. Die Herstellung von einzelnen Stereoisomeren kann gewünschtenfalls durch Auftrennung eines Gemisches nach üblichen Methoden, zum Beispiel durch Chromatographie oder Kristallisation, durch Verwendung von stereochemisch einheitlichen Ausgangssubstanzen bei der Synthese oder durch stereoselektive Synthese erfolgen. Gegebenenfalls kann vor einer Trennung von Stereoisomeren eine Derivatisierung erfolgen. Beispielsweise kann eine Verbindung der Formel I, die zum Beispiel in der Gruppe R^1 ein chirales Kohlenstoffatom und eine Hydroxygruppe enthält, zunächst an der Hydroxygruppe in den Ester einer optisch aktiven Säure überführt werden und die erhaltenen diastereomeren Ester können dann nach üblichen Methoden, zum Beispiel durch Kristallisation oder Chromatographie, getrennt werden und die Ester anschließend wieder verseift werden. Die Trennung eines Stereoisomerengemisches kann auf der Stufe der Verbindungen der Formel I erfolgen oder auf der Stufe eines Zwischenprodukts im Verlaufe der Synthese.

Bei Vorliegen von beweglichen Wasserstoffatomen umfaßt die vorliegende Erfindung auch alle tautomeren Formen der Verbindungen der Formel I, zum Beispiel Lactam/Lactim-Tautomere. Als Beispiel für derartige Tautomere seien die Formen Ia und Ib von Verbindungen der Formel I genannt, in der R^3 für Hydroxy steht. Entsprechend sind tautomere Formen beispielsweise möglich, wenn R^5 in der Formel I für Hydroxy steht.



Ia



Ib

Enthalten die Verbindungen der Formel I eine oder mehrere saure oder basische Gruppen, so sind auch die entsprechenden physiologisch oder toxikologisch verträglichen Salze Gegenstand der Erfindung, insbesondere die pharmazeutisch verwendbaren Salze. So können die Verbindungen der Formel I, die eine oder mehrere saure Gruppen enthalten, zum Beispiel Hydroxygruppen im Pyridinring oder COOH-Substituenten in Phenylringen, an diesen Gruppen beispielsweise als Alkalimetallsalze, Erdalkalimetallsalze oder als Ammoniumsalze vorliegen und erfindungsgemäß verwendet werden. Beispiele für solche Salze sind Natriumsalze, Kaliumsalze, Calciumsalze, Magnesiumsalze oder Salze mit Ammoniak oder organischen Aminen wie beispielsweise Ethylamin, Ethanolamin, Triethanolamin oder Aminosäuren. Verbindungen der Formel I, die eine oder mehrere basische, das heißt protonierbare, Gruppen enthalten, können in Form ihrer Säureadditionssalze mit anorganischen oder organischen Säuren vorliegen und erfindungsgemäß verwendet werden, zum Beispiel als Salze mit Chlorwasserstoff, Bromwasserstoff, Phosphorsäure, Schwefelsäure, Salpetersäure, Methansulfonsäure, p-Toluolsulfonsäure, Naphthalindisulfonsäuren, Oxalsäure, Essigsäure, Weinsäure, Milchsäure, Salicylsäure, Benzoesäure, Ameisensäure, Propionsäure, Pivalinsäure, Diethylessigsäure, Malonsäure, Bernsteinsäure, Pimelinsäure, Fumarsäure, Maleinsäure, Äpfelsäure, Sulfaminsäure, Phenylpropionsäure, Gluconsäure Ascorbinsäure, Isonicotinsäure, Zitronensäure, Adipinsäure usw. Enthalten die Verbindungen der Formel I gleichzeitig saure und basische Gruppen im Molekül, so gehören neben den geschilderten Salzformen auch innere Salze oder Betaine zu der Erfindung. Salze können aus den Verbindungen der Formel I nach üblichen, dem Fachmann bekannten Verfahren erhalten werden, beispielsweise durch Vereinigung mit einer organischen oder anorganischen Säure oder Base in einem Lösungsmittel oder Dispergiermittel, oder auch durch Anionenaustausch oder Kationenaustausch aus anderen Salzen. Die vorliegende Erfindung umfaßt auch alle Salze der Verbindungen der Formel I, die sich wegen geringer physiologischer Verträglichkeit nicht direkt für eine Verwendung in Arzneimitteln eignen aber zum Beispiel als Zwischenprodukte für chemische Reaktionen oder für die Herstellung physiologisch verträglicher Salze in Betracht kommen.

Die vorliegende Erfindung umfaßt weiterhin alle Solvate von Verbindungen der Formel I, zum Beispiel Hydrate oder Addukte mit Alkoholen, sowie Derivate der Verbindungen der Formel I, zum Beispiel Ester, Pro-Drugs und Metabolite, die wie die Verbindungen der Formel I wirken.

In der Formel I steht X bevorzugt für O oder S, besonders bevorzugt für O.

R^1 steht bevorzugt für Wasserstoff, Formyl, Halogen, die Gruppe $R^{20}CH(OH)$ oder die Gruppe $((C_1\text{-}C_6)\text{-Alkyl})_2N\text{-}CH_2$, wobei R^{20} für Wasserstoff, $(C_1\text{-}C_6)$-Alkyl, das gesättigt ist oder eine Dreifachbindung enthält und das unsubstituiert ist oder durch eine Gruppe $((C_1\text{-}C_6)\text{-Alkyl})_2N$ substituiert ist, oder für unsubstituiertes oder substituiertes Phenyl steht, und wobei in einem Rest $((C_1\text{-}C_6)\text{-Alkyl})_2N$ die Alkylreste gleich oder verschieden sein können. Besonders bevorzugt steht R^1 für Wasserstoff, Formyl oder die Gruppe $R^{21}CH(OH)$, wobei R^{21} für Wasserstoff, $(C_1\text{-}C_4)$-Alkyl oder unsubstituiertes Phenyl steht. Bevorzugt befindet sich der Rest R^1 in der 5-Position der Heterocyclus, also des Furanrings, Thiophenrings oder Pyrrolrings.

R^{1a} steht bevorzugt für Wasserstoff oder $(C_1\text{-}C_4)$-Alkyl, besonders bevorzugt für Wasserstoff.

R^2 steht bevorzugt für $(C_1\text{-}C_6)$-Alkyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-$(C_1\text{-}C_2)$-alkyl, besonders bevorzugt für $(C_1\text{-}C_6)$-Alkyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Benzyl. Ganz besonders bevorzugt steht R^2 für unsubstituiertes Benzyl oder tert-Butyl.

R^3 und R^5 stehen bevorzugt unabhängig voneinander für Wasserstoff, $(C_1\text{-}C_6)$-Alkyl, unsubstituiertes oder substituiertes Phenyl oder Hydroxy, besonders bevorzugt für Wasserstoff, $(C_1\text{-}C_4)$-Alkyl oder Hydroxy, insbesondere Wasserstoff, Methyl oder Hydroxy und ganz besonders bevorzugt für $(C_1\text{-}C_4)$-Akyl oder Hydroxy, insbesondere für Methyl oder Hydroxy.

R^4 steht bevorzugt für Wasserstoff oder $(C_1\text{-}C_6)$-Alkyl, besonders bevorzugt für Wasserstoff oder $(C_1\text{-}C_4)$-Alkyl, ganz besonders bevorzugt für Wasserstoff.

Bevorzugt ist es auch, wenn einer oder mehrere der Reste R^3, R^4 und R^5 eine andere Bedeutung als Wasserstoff hat.

Bevorzugte Verbindungen der Formel I sind solche, in denen einer oder mehrere der darin enthaltenen Reste bevorzugte Bedeutungen haben, wobei alle Kombinationen von bevorzugten Substituentendefinitionen umfaßt werden. Auch von allen bevorzugten Verbindungen der Formel I umfaßt die vorliegende Erfindung alle ihre stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

Eine Gruppe von bevorzugten Verbindungen der Formel I bilden solche Verbindungen, in denen in der Formel I

X für O oder S steht;

R^1 für Wasserstoff, Formyl, Halogen, die Gruppe $R^{20}CH(OH)$ oder die Gruppe $((C_1\text{-}C_6)\text{-Alkyl})_2N\text{-}CH_2$ steht, wobei R^{20} für Wasserstoff, $(C_1\text{-}C_6)$-Alkyl, das gesättigt ist oder eine Dreifachbindung enthält und das unsubstituiert ist oder durch eine Gruppe $((C_1\text{-}C_6)\text{-Alkyl})_2N$ substituiert ist, oder für unsubstituiertes oder substituiertes Phenyl steht, und R^1 sich in der 5-Position befindet;

R^{1a} für Wasserstoff steht;

R^2 für $(C_1\text{-}C_6)$-Alkyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-$(C_1\text{-}C_2)$-alkyl steht;

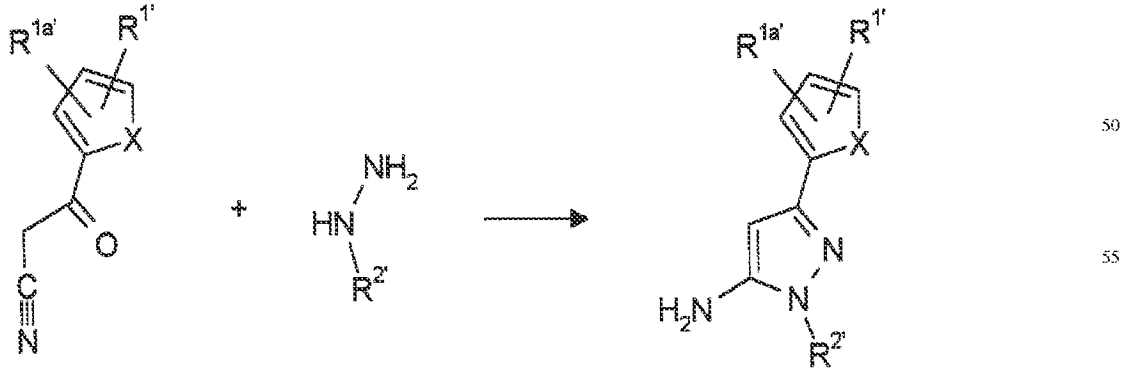
R^3 und R^5 unabhängig voneinander für Wasserstoff, $(C_1\text{-}C_6)$-Alkyl, unsubstituiertes oder substituiertes Phenyl oder Hydroxy stehen;

R^4 für Wasserstoff oder $(C_1\text{-}C_6)$-Alkyl steht;

in allen ihren stereoisomeren Formen und Gemische davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

Die erfindungsgemäßen Verbindungen der Formel I können beispielsweise analog dem in J. Het. Chem. 12 (1975) 1303 und J. Het. Chem. 15 (1978) 319 beschriebenen Verfahren hergestellt werden, das im folgenden erläutert ist und das ebenfalls Gegenstand der vorliegenden Erfindung ist.

Ausgangssubstanzen für den ersten Schritt der Herstellung sind die β-Ketonitrile der Formel II, die mit den Hydrazinen der Formel III oder deren Salzen zu den Aminopyrazolen der Formel IV umgesetzt werden können.



II III IV

In den Formeln II, III und IV haben X, R^1, $R^{1a'}$ und $R^{2'}$, die oben für X, R^1, R^{1a} und R^2 angegebenen Bedeutungen und zusätzlich können in den Resten $R^{1'}$, $R^{1a'}$ und $R^{2'}$ funktionelle Gruppen in geschützter Form oder in Form von Vorstufen vorliegen. Geeignete Schutzgruppen bzw. günstige Vorstufen für funktionelle Gruppen in diesen Resten sind dem Fachmann bekannt. Beispielsweise kann eine Carbonylgruppe in diesen Resten zunächst in geschützter Form vorliegen, zum Beispiel in Form eines Acetals oder Ketals, oder es kann eine Aminogruppe in acylierter Form vorliegen, oder es kann

ein Wasserstoffatom als Vorstufe für eine Gruppe vorliegen, die in einer später durchgeführten elektrophilen Substitutionsreaktion eingeführt wird, oder es können Abwandlungen von erfindungsgemäßen funktionellen Gruppen in andere erfindungsgemäße Gruppen durchgeführt werden.

Geeignete Verbindungen der Formeln II und III für die Herstellung der erfindungsgemäßen Verbindungen der Formel I sind kommerziell erhältlich oder können nach oder analog zu Verfahren erhalten werden, die in der Literatur beschrieben sind, zum Beispiel in den Standardwerken Houben-Weyl, Methoden der Organischen Chemie, Thieme-Verlag, Stuttgart, oder Organic Reactions, John Wiley & Sons, New York. Die heterocyclischen Acylacetonitrile der Formel II können zum Beispiel durch Acylierung von Acetonitril oder von Cyanessigester mit heterocyclischen Estern oder Acylchloriden in Gegenwart einer Base wie Lithiumdiisopropylamid und, im Falle der Cyanessigester, anschließende Abspaltung der Estergruppe erhalten werden. Die Hydrazine der Formel III können zum Beispiel durch Alkylierung von geeigneten Hydrazinderivaten oder durch Reduktion von Hydrazonen erhalten werden.

Die Umsetzungen der β-Ketonitrile der Formel II mit den Hydrazinen der Formel III oder deren Salzen werden bevorzugt in einem Lösungsmittel oder Dispergiermittel vorgenommen. Geeignete Lösungsmittel sind zum Beispiel Wasser, Alkohole wie Methanol, Ethanol, n-Propanol, Isopropanol oder Butanole, Ether wie Diethylether, Dipropylether, Dibutylether, tert-Butylmethylether, Tetrahydrofuran oder Dioxan, Monoether und Diether des Ethylenglykols und des Di- und Triethylenglykols wie Ethylenglykolmonomethylether, Ethylenglykolmonoethylether, Ethylenglykoldimethylether, Ethylenglykolmonobutylether, Diethylenglykolmonomethylether oder Diethylenglykoldimethylether, Ester wie Essigsäureethylester oder Essigsäurebutylester, Amide wie Dimethylformamid, Dimethylacetamid, N-Methylpyrrolidon oder Hexamethylphosphorsäuretriamid Nitrile wie Acetonitril, Säuren wie Essigsäure, Sulfoxide und Sulfone wie Dimethylsulfoxid oder Sulfolan, Kohlenwasserstoffe und chlorierte Kohlenwasserstoffe wie Benzinfraktionen, Benzol, Toluol, Xylol, Chlorbenzol, Dichlorbenzol, Methylenchlorid oder Chloroform. Es können auch Mischungen von zwei oder mehr Lösungsmitteln eingesetzt werden, beispielsweise Mischungen aus Wasser und Alkoholen oder Mischungen aus Wasser und Säuren. Bevorzugte Lösungsmittel sind Alkohole wie Methanol und Ethanol.

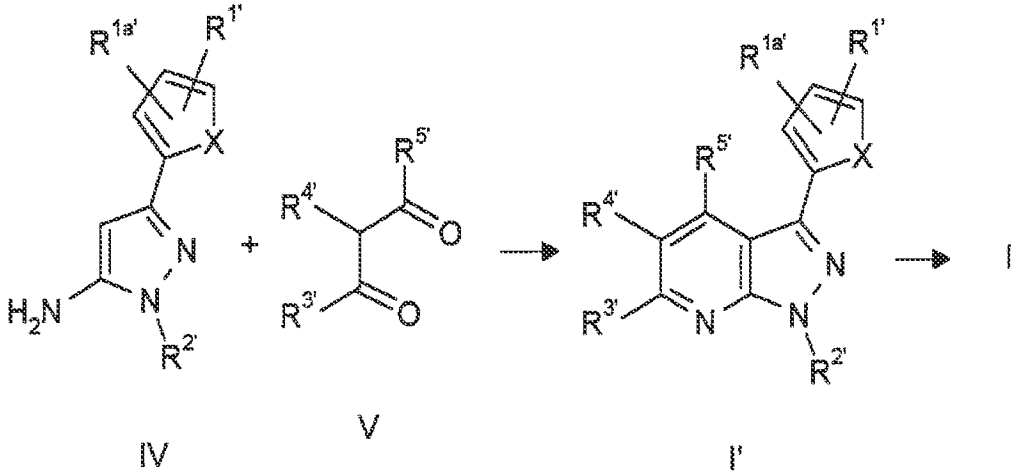
Die Umsetzung der Verbindungen der Formeln II und III kann in einem weiten Temperaturbereich durchgeführt werden. Im allgemeinen wird sie bei Temperaturen von 0°C bis 150°C, bevorzugt bei Temperaturen von 20°C bis 120°C durchgeführt. Besonders bevorzugt ist es, sie unter Rückfluß bei der Siedetemperatur des verwendeten Lösungsmittels oder Lösungsmittelgemisches durchzuführen, zum Beispiel bei der Siedetemperatur des Methanols oder Ethanols. Die Reaktionsdauer richtet sich nach dem Einzelfall und hängt zum Beispiel von der Reaktivität der Reaktionspartner und den Reaktionsbedingungen ab. Im allgemeinen ist, wenn die Umsetzung in Methanol oder Ethanol bei Siedetemperatur durchgeführt wird, die Umsetzung nach 1 bis 10 Stunden beendet. Die Aufarbeitung des Reaktionsgemisches kann nach Standardverfahren erfolgen und das Produkt gewünschtenfalls nach üblichen Reinigungsmethoden, zum Beispiel durch Umkristallisation, Destillation, Sublimation oder durch Chromatographie gereinigt werden.

Als Reaktion einer Carbonylverbindung mit einer Aminoverbindung kann die Umsetzung der Verbindungen der Formeln II und III sowohl durch den Zusatz von Basen als auch den Zusatz von Säuren beschleunigt werden. Geeignete Säuren sind zum Beispiel organische Carbonsäuren und Sulfonsäuren wie Essigsäure, Trifluoressigsäure, Methansulfonsäure oder p-Toluolsulfonsäure, anorganische Säuren wie Chlorwasserstoff, Schwefelsäure oder Phosphorsäure, saure Salze wie Ammoniumsalze oder Hydrogenphosphate, oder saure Ionenaustauscher. Geeignete Basen sind zum Beispiel Hydroxide, Carbonate, Hydrogencarbonate, Acetate oder Alkoholate von Alkalimetallen und Erdalkalimetallen, zum Beispiel Natriumhydroxid, Natriumcarbonat, Natriumhydrogencarbonat, Natriumacetat, Natriummethylat, Natriumethylat, Kaliumcarbonat, Kalium-tert-butylat, basische Ionenaustauscher oder Amine wie Triethylamin oder Pyridin.

Werden freie Hydrazine der Formel III eingesetzt, so ist es vielfach besonders vorteilhaft, die Umsetzung mit den Verbindungen der Formel II unter saurer Katalyse durchzuführen. Als Katalysatoren kommen die beispielhaft genannten Säuren in Betracht. Es kann auch günstig sein, einen bestimmten pH-Wert einzustellen oder in Gegenwart eines Puffersystems zu arbeiten. Bevorzugt ist es, die Umsetzung von Verbindungen der Formel II mit freien Hydrazinen der Formel III in Gegenwart von Essigsäure durchzuführen. Die Art und Menge eines zugesetzten sauren Katalysators richten sich nach dem Einzelfall und hängen zum Beispiel von der Reaktivität der Reaktionspartner, dem Lösungsmittel oder der vorgesehenen Temperatur ab. Wird zum Beispiel eine Säure wie Essigsäure verwendet, so kann diese je nach der eingesetzten Menge sowohl als Lösungsmittel als auch als Katalysator fungieren. Wird an Stelle eines freien Hydrazins ein Säureadditionssalz eines Hydrazins eingesetzt, zum Beispiel ein R^2-substituiertes Hydraziniumchlorid oder Hydraziniumsulfat, so wird damit bereits eine saure Verbindung in das Reaktionsgemisch eingebracht, die katalytisch wirken kann. Bei Verwendung eines Hydraziniumsalzes ist es vielfach besonders günstig, einen Teil der durch dieses eingebrachten Säure durch Zusatz einer gewissen Menge einer Base abzufangen, zum Beispiel durch Zusatz von Natriumacetat oder einer anderen abstumpfenden Substanz zum Reaktionsgemisch.

Im Einzelfall kann je nach den Reaktionsbedingungen und den Reaktivitäten der Reaktionspartner die NH_2-Gruppe des Hydrazins der Formel III statt mit der Carbonylgruppe in der Verbindung der Formel II auch mit der Nitrilgruppe reagieren. In diesem Falle kann die Umsetzung auch zu dem unerwünschten Isomeren des Aminopyrazols der Formel IV führen, in dem sich die Aminogruppe in der 3-Position und der Heterocyclus in der 5-Position befindet. Der Reaktionsverlauf läßt sich gegebenenfalls durch die Wahl der Reaktionsbedingungen steuern. Liefert die Umsetzung der Verbindungen der Formeln II und III Gemische der isomeren Pyrazole, so können diese nach üblichen Verfahren in die Komponenten aufgetrennt werden, zum Beispiel durch Umkristallisation, Destillation, Sublimation oder Chromatographie.

Im zweiten Schritt der Herstellung der Verbindungen der Formel I werden dann die Aminopyrazole der Formel IV mit 1,3-Dicarbonylverbindungen der Formel V zu den Pyrazolopyridinen der Formel I' umgesetzt.



IV V I'

Entsprechend den obigen Erläuterungen zu den Formeln II, III und IV haben auch in den Formeln V und I' die Reste X, $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$ und $R^{5'}$ die oben für X, R^1, R^{1a}, R^2, R^3, R^4 und R^5 angegebenen Bedeutungen und zusätzlich können in den Resten $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$ und $R^{5'}$ wiederum funktionelle Gruppen in geschützter Form oder in Form von Vorstufen vorliegen. Aus den Verbindungen der Formel I' können dann gegebenenfalls Verbindungen der Formel I erhalten werden, indem man in einem oder mehreren anschließenden Reaktionsschritten die in geschützter Form bzw. in Form von Vorstufen vorliegenden Gruppen in die gewünschten, in den obigen Definitionen von R^1, R^{1a}, R^2, R^3, R^4 und R^5 genannten funktionellen Gruppen überführt oder erfindungsgemäße Gruppen modifiziert.

Sollen Verbindungen der Formel I hergestellt werden, in denen die Reste R^3 und R^5 in der Formel I bzw. die Reste $R^{3'}$ und $R^{5'}$ in der Formel I' beide für Reste aus der Reihe Wasserstoff, Alkyl, Phenyl und Phenylalkyl stehen, so handelt es sich bei den Verbindungen der Formel V um β-Dialdehyde, β-Ketoaldehyde oder β-Diketone. Sollen Verbindungen hergestellt werden, in denen einer der Reste R^3 und R^5 in der Formel I bzw. der Reste $R^{3'}$ und $R^{5'}$ in der Formel I' für Hydroxy oder einen in einem späteren Reaktionsschritt aus einer Hydroxygruppe erhältlichen Alkoxyrest oder Benzyloxyrest steht, so handelt es sich bei den Verbindungen der Formel V um β-Aldehydsäurederivate oder β-Ketosäurederivate. Als Säurederivate kommen hier insbesondere die Ester in Betracht, zum Beispiel die (C_1-C_4)-Alkylester wie die Methylester oder die Ethylester. Sollen Verbindungen hergestellt werden, in denen die Reste R^3 und R^5 in der Formel I bzw. der Reste $R^{3'}$ und $R^{5'}$ in der Formel I' beide für Hydroxy oder daraus in einem späteren Schritt erhältliche Alkoxyreste oder Benzyloxyreste stehen, so handelt es sich bei den Verbindungen der Formel V um Malonsäurederivate. Auch als Malonsäurederivate kommen wiederum insbesondere die (C_1-C_4)-Alkylester wie die Methylester oder die Ethylester in Betracht. Alle diese Verbindungen der Formel V sind in großer Zahl kommerziell erhältlich oder können nach oder analog zu Verfahren erhalten werden, die in der Literatur, zum Beispiel den oben genannten Standardwerken, ausführlich beschrieben sind. Aldehydgruppen und Ketongruppen können in den Verbindungen der Formel V auch in Form von Acetalen oder Ketalen vorliegen, beispielsweise in Form der geminalen Dimethoxyverbindungen, der geminalen Diethoxyverbindungen oder der Dioxolane, aus denen die Aldehydgruppen oder Ketongruppen erst während der Umsetzung freigesetzt werden, zum Beispiel durch eine Säure.

Auch die Umsetzungen von Verbindungen der Formel IV mit den Dicarbonylverbindungen der Formel V werden bevorzugt in einem Lösungsmittel oder Dispergiermittel vorgenommen. Geeignete Lösungsmittel sind auch hier zum Beispiel Wasser, Alkohole, Ether, Monoether und Diether des Ethylenglykols und des Di- und Triethylenglykols, Amide, Säuren, Sulfoxide und Sulfone Kohlenwasserstoffe und chlorierte Kohlenwasserstoffe. Die oben genannten Beispiele für diese Lösungsmittel gelten auch hier. Je nach dem Einzelfall kann es hier aber auch angebracht sein, bei höheren Temperaturen als im ersten Schritt zu arbeiten, so daß neben den oben beispielhaft genannten Lösungsmitteln auch entsprechende höhersiedende Lösungsmittel in Betracht kommen, zum höhersiedende Alkohole wie Decanol oder höhersiedende Ether wie Diphenylether. Es können auch wiederum Mischungen von zwei oder mehr Lösungsmitteln eingesetzt werden. Ein bevorzugte Lösungsmittel ist Eisessig (Essigäure). Die Umsetzung der Verbindungen der Formeln IV und V wird im allgemeinen bei Temperaturen von 0°C bis 200°C, bevorzugt bei Temperaturen von 50°C bis 200°C durchgeführt. Besonders bevorzugt ist, sie unter Rückfluß bei der Siedetemperatur des verwendeten Lösungsmittels durchzuführen, sie kann aber beispielsweise auch unter Druck in einem Autoklaven durchgeführt werden. Die Reaktionsdauer richtet sich wiederum nach dem Einzelfall und hängt zum Beispiel von der Reaktivität der Reaktionspartner und den Reaktionsbedingungen ab. Im allgemeinen ist die Umsetzung nach 1 bis 12 Stunden beendet. Die Aufarbeitung des Reaktionsgemisches kann nach Standardverfahren erfolgen und das Produkt gewünschtenfalls nach üblichen Reinigungsmethoden gereinigt werden.

Auch die Umsetzung der Verbindungen der Formeln IV und V kann kann sowohl den Zusatz von Basen als auch den Zusatz von Säuren beschleunigt werden. In vielen Fällen besonders günstig und daher bevorzugt ist die Verwendung von sauren Katalysatoren. Die obigen Ausführungen zur Katalyse und die oben genannten Beispiele für Basen und Säuren gelten auch hier. Ein besonders bevorzugter Katalysator für die Umsetzung der Verbindungen der Formeln IV und V ist Essigsäure, die wiederum besonders günstig sowohl als Lösungsmittel als auch als Katalysator eingesetzt werden kann.

Auch bei den Umsetzungen der Verbindungen der Formeln IV und V können im Einzelfall kann je nach den Reaktionsbedingungen und den Reaktivitäten der Reaktionspartner Isomerengemische entstehen: Ist die Verbindung der Formel V nicht symmetrisch, kann neben der Verbindung der Formel I' das entsprechende Pyrazolopyridin entstehen, in dem

die Reste $R^{3'}$ und $R^{5'}$ vertauscht sind. Liefert die Umsetzung der Verbindungen der Formeln IV und V ein Gemisch der beiden isomeren Verbindungen, so können diese nach üblichen Verfahren in die Komponenten aufgetrennt werden, zum Beispiel durch Umkristallisation, Destillation, Sublimation oder Chromatographie.

In den Verbindungen der Formeln I' können, wie schon oben gesagt, die Reste $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$ und $R^{5'}$ die in den Definition von R^1, R^{1a}, R^2, R^3, R^4 und R^5 angegebenen Bedeutungen haben, so daß die nach dem erläuterten Syntheseverfahren erhaltenen Reaktionsprodukte der Formel I' bereits erfindungsgemäße Verbindungen der Formel I darstellen. Es können aber in den nach dem erläuterten Syntheseverfahren erhaltenen Verbindungen der Formel I' auch noch in vielfältiger Weise strukturelle Abwandlungen vorgenommen werden. Wie schon gesagt, kann es sich dabei um die Freisetzung von funktionellen Gruppen handeln, die während der Synthese in geschützter Form vorlagen. Es können aber auch in erfindungsgemäße Verbindungen der Formel I' nach üblichen chemischen Methoden zusätzliche funktionelle Gruppen eingeführt werden oder in erfindungsgemäßen Verbindungen vorhandene Strukturelemente oder funktionelle Gruppe nach üblichen Methoden in andere Strukturelemente oder funktionelle Gruppen abgewandelt werden. Diese Methoden sind dem Fachmann wohlbekannt und zum Beispiel in den bereits genannten Standardwerken ausführlich beschrieben, also zum Beispiel in Houben-Weyl, Methoden der Organischen Chemie, Thieme-Verlag, Stuttgart, oder Organic Reactions, John Wiley & Sons, New York, oder in Jerry March, Advanced Organic Chemistry, 4. Aufl., John Wiley & Sons, New York. Eine gegebenenfalls notwendige Anpassung von Reaktionsbedingungen an die Reaktivität der Verbindungen der Formel I' bereitet dem Fachmann keine Probleme. Im folgenden sind einige in Betracht kommende Reaktionstypen beispielhaft genannt.

Ein besonders wertvoller Reaktionstyp zur Einführung von Resten R^1 oder R^{1a} bei der Herstellung von Verbindungen der Formel I ist eine elektrophile aromatische Substitution an dem in der 3-Position des Pyrazolopyridins gebundenen Furanring, Thiophenring oder Pyrrolring, die in einem weiteren Reaktionsschritt nach der Umsetzung der Verbindungen der Formeln IV und V durchgeführt wird. Dadurch können funktionelle Gruppe in das Molekül eingeführt werden, die anschließend vielfältig abgewandelt werden können. Ein besonders günstiger und bevorzugter Weg zur Herstellung einer Vielzahl erfindungsgemäßer Verbindungen geht von Verbindungen der Formel II aus, in der einer der Reste $R^{1'}$ und $R^{1a'}$ für Wasserstoff oder Alkyl steht und der andere für Wasserstoff steht, und insbesondere beide Reste $R^{1'}$ und $R^{1a'}$ für Wasserstoff stehen. Dieser Weg führt nach dem oben beschriebenen Verfahren zunächst zu Verbindungen der Formel I', in der einer der Reste $R^{1'}$ und $R^{1a'}$ für Wasserstoff oder Alkyl steht und der andere für Wasserstoff steht, und insbesondere beide Reste $R^{1'}$ und $R^{1a'}$ für Wasserstoff stehen. In diese Verbindungen der Formel I', also erst nach dem Aufbau des Pyrazolopyridin-Systems, werden auf diesem bevorzugten Weg dann durch elektrophile aromatische Substitutionen in den Furanring, Thiophenring oder Pyrrolring für R^1 und/oder R^{1a} stehende funktionelle Gruppen eingeführt, die anschließend noch abgewandelt werden können. Die somit in den Formeln II, IV und I' zunächst für Wasserstoff stehenden Reste $R^{1'}$ und/oder $R^{1a'}$ können dabei als die oben erwähnten Vorstufen der später eingeführten Reste R^1 und/oder R^{1a} angesehen werden. Dieser Weg führt zu einer Gruppe erfindungsgemäßer Verbindungen, in der mindestens einer der Reste R^1 und R^{1a} eine andere Bedeutung als Wasserstoff hat. Als elektrophile aromatische Substitutionen kommen zum Beispiel Formylierungen wie die Vilsmeier-Formylierung, Acylierungen, Halogenierungen, beispielsweise mit N-Halogensuccininiden, Chlormethylierungen oder Aminoalkylierungen wie die Mannich-Reaktion, beispielsweise mit Formaldehyd und einem sekundären Amin, in Betracht. Wie derartige Reaktionen durchzuführen sind, ist dem Fachmann wohlbekannt, ist ausführlich in den genannten Standardwerken beschrieben und geht aus den Ausführungsbeispielen hervor.

Carbonylgruppen in Acylgruppen oder insbesondere in Aldehydgruppen, die zum Beispiel durch eine elektrophile aromatische Substitution in das Molekül eingeführt worden sein können, können nach Standardverfahren zu Alkoholgruppen reduziert werden, zum Beispiel durch Reduktion mit komplexen Hydriden wie Natriumborhydrid oder Lithiumborhydrid in Alkoholen oder Ethern. Weiterhin können metallorganische Verbindungen, zum Beispiel Grignardverbindungen oder lithiumorganische Verbindungen, an Carbonylgruppen addiert werden. Beispielsweise kann so durch Reduktion bzw. durch Umsetzung mit metallorganischen Verbindungen eine Aldehydgruppe CHO in die Gruppe $R^{20}CH(OH)$ überführt werden. Reduktionen und Umsetzungen mit metallorganischen Verbindungen können auch stereoselektiv erfolgen. Carbonylverbindungen oder Hydroxyverbindungen oder zum Beispiel Sulfonsäureester wie Mesylate oder Tosylate von Hydroxyverbindungen können auch bis zur Stufe des Kohlenwasserstoffs reduziert werden. Eine Alkoholgruppe CH(OH) kann auch wiederum zu einer Carbonylgruppe CO oxidiert werden, wie sie zum Beispiel in einer für R^1 stehenden Gruppe $R^{10}CO$ vorliegt, beispielsweise mit Hilfe von Dimethylsulfoxid und einem aktivierenden Agens nach Standardverfahren oder durch Oppenauer-Oxidation.

Hydroxyalkylverbindungen können zum Beispiel mit anorganischen Säurehalogeniden wie Thionylchlorid oder Thionylbromid in die Halogenverbindungen überführt werden, an denen wiederum Austauschreaktionen mit Alkoholen unter Bildung von Ethern, mit Halogeniden unter Halogenaustausch oder mit Aminen durchgeführt werden können. Zur Durchführung derartiger Austauschreaktionen können Hydroxyalkylverbindungen zum Beispiel auch zunächst durch Umsetzung mit Sulfonsäurechloriden wie Tosylchlorid oder Mesylchlorid aktiviert werden. Aminoverbindungen können auch direkt aus Carbonylverbindungen erhalten werden.

Hydroxyalkylverbindungen können mit Carbonsäuren in Gegenwart eines die Säure aktivierenden Agens oder mit reaktiven Carbonsäurederivaten wie Säureanhydriden, gemischten Säureanhydriden oder Säurechloriden in Ester überführt werden. Entsprechend können Aminoalkylverbindungen auf diese Weise zu Amiden acyliert werden. Hydroxygruppen können weiterhin zum Beispiel mit Alkylhalogeniden und Benzylhalogeniden oder den Sulfonsäureestern von Alkylalkoholen und Benzylalkoholen verethert werden. Letzteres gilt auch für Hydroxygruppen, die für die Reste R^3 und/oder R^5 im Pyridinring stehen.

Weiterhin können können vielfältige Abwandlungen von Substituenten an Phenylringen durchgeführt werden.

Bei allen diesen Reaktionen handelt es sich um Standardreaktionen, die dem Fachmann wohlvertraut sind und zu denen sich nähere Angaben zum Beispiel in den genannten Standardwerken finden.

Die erfindungsgemäßen Verbindungen der Formel I bewirken über die Aktivierung der löslichen Guanylat-Cyclase (sGC) eine Erhöhung der cGMP-Konzentration und sind deshalb wertvolle Agenzien zur Therapie und Prophylaxe von Krankheiten, die mit einem niedrigen oder erniedrigten cGMP-Spiegel verbunden sind oder durch einen solchen verur-

sacht werden oder zu deren Therapie oder Prophylaxe eine Erhöhung des vorhandenen cGMP-Spiegels angestrebt wird. Die Aktivierung der sGC durch die Verbindungen der Formel I kann zum Beispiel in dem unten beschriebenen Aktivitätsassay untersucht werden.

Krankheiten und pathologische Zustände, die mit einem niedrigen cGMP-Spiegel verbunden sind oder bei denen eine Erhöhung des cGMP-Spiegels angestrebt wird und zu deren Therapie und Prophylaxe Verbindungen der Formel I eingesetzt werden können, sind zum Beispiel Herz-Kreislauf-Erkrankungen wie endotheliale Dysfunktion, diastolische Dysfunktion, Atherosklerose, Bluthochdruck, stabile und instabile Angina pectoris, Thrombosen, Restenosen, Myocardinfarkt, Schlaganfälle, Herzinsuffizienz oder Pulmonalhypertonie, oder zum Beispiel erektile Dysfunktion, Asthma bronchiale, chronische Niereninsuffizienz und Diabetes. Verbindungen der Formel I können darüber hinaus eingesetzt werden bei der Therapie der Leberzirrhose sowie aufgrund ihrer zum Teil synergistischen Wirkung mit der retrograden Messenger-Substanz NO zur Verbesserung einer eingeschränkten Lernfähigkeit oder Gedächtnisleistung.

Die Verbindungen der Formel I und ihre physiologisch verträglichen Salze können somit am Tier, bevorzugt am Säugetier, und insbesondere am Menschen als Arzneimittel für sich allein, in Mischungen untereinander oder in Form von pharmazeutischen Zubereitungen verwendet werden. Gegenstand der vorliegenden Erfindung sind daher auch die Verbindungen der Formel I und ihre physiologisch verträglichen Salze zur Anwendung als Arzneimittel, ihre Verwendung zur Normalisierung eines gestörten cGMP-Haushalts und insbesondere ihre Verwendung in der Therapie und Prophylaxe der oben genannten Krankheitsbilder, sowie ihre Verwendung zur Herstellung von Medikamenten dafür. Weiterhin sind Gegenstand der vorliegenden Erfindung pharmazeutische Präparate, die als aktiven Bestandteil eine wirksame Dosis mindestens einer Verbindung der Formel I und/oder eines physiologisch verträglichen Salzes davon neben üblichen pharmazeutisch einwandfreien Trägerstoffen und Zusatzstoffen enthalten.

Die Arzneimittel können oral, zum Beispiel in Form von Pillen, Tabletten, Filmtabletten, Dragees, Granulaten, Hart- und Weichgelatinekapseln, wäßrigen, alkoholischen oder öligen Lösungen, Sirupen, Emulsionen oder Suspensionen, oder rektal, zum Beispiel in Form von Suppositorien, verabreicht werden. Die Verabreichung kann aber auch parenteral erfolgen, zum Beispiel subkutan, intramuskulär oder intravenös in Form von Injektionslösungen oder Infusionslösungen. Weitere in Betracht kommende Applikationsformen sind zum Beispiel die perkutane oder topische Applikation, zum Beispiel in Form von Salben, Tinkturen, Sprays oder transdermalen therapeutischen Systemen, oder die inhalative Applikation in Form von Nasalsprays oder Aerosolmischungen, oder zum Beispiel Mikrokapseln, Implantate oder Rods. Die bevorzugte Applikationsform hängt zum Beispiel von der zu behandelnden Krankheit und ihrer Stärke ab.

Die pharmazeutischen Präparate enthalten normalerweise 0,5 bis 90 Gewichtsprozent der Verbindungen der Formel I und/oder ihrer physiologisch verträglichen Salze. Die Herstellung der pharmazeutischen Präparate kann in an sich bekannter Weise erfolgen. Dazu werden ein oder mehrere Verbindungen der Formel I und/oder ihre physiologisch verträglichen Salze zusammen mit einem oder mehreren festen oder flüssigen galenischen Trägerstoffen und/oder Hilfsstoffen und, wenn gewünscht, in Kombination mit anderen Arzneimittelwirkstoffen mit therapeutischer oder prophylaktischer Wirkung in eine geeignete Verabreichungsform bzw. Dosierungsform gebracht, die dann als Arzneimittel in der Humanmedizin oder Veterinärmedizin verwendet werden kann.

Für die Herstellung beispielsweise von Pillen, Tabletten, Dragees und Hartgelatinekapseln kann man Lactose, Stärke, zum Beispiel Maisstärke, oder Stärkederivate, Talk, Stearinsäure oder deren Salze, etc. verwenden. Trägerstoffe für Weichgelatinekapseln und Suppositorien sind zum Beispiel Fette, Wachse, halbfeste und flüssige Polyole, natürliche oder gehärtete Öle etc. Als Trägerstoffe für die Herstellung von Lösungen, zum Beispiel Injektionslösungen, oder von Emulsionen oder Sirupen eignen sich beispielsweise Wasser, physiologische Kochsalzlösung, Alkohole wie Ethanol, Glycerin, Polyole, Saccharose, Invertzucker, Glucose, Mannit, pflanzliche Öle etc. Die Verbindungen der Formel I und ihre physiologisch verträglichen Salze können auch lyophilisiert werden und die erhaltenen Lyophilisate zum Beispiel zur Herstellung von Injektions- oder Infusionspräparaten verwendet werden. Als Trägerstoffe für Mikrokapseln, Implantate oder Rods eignen sich zum Beispiel Mischpolymerisate aus Glykolsäure und Milchsäure.

Die pharmazeutischen Präparate können neben den Wirkstoffen und Trägerstoffen noch übliche Zusatzstoffe enthalten, zum Beispiel Füllstoffe, Spreng-, Binde-, Gleit-, Netz-, Stabilisierungs-, Emulgier-, Dispergier-, Konservierungs-, Süß-, Färbe-, Geschmacks- oder Aromatisierungs-, Dickungs-, Verdünnungsmittel, Puffersubstanzen, ferner Lösungsmittel oder Lösungsvermittler oder Mittel zur Erzielung eines Depoteffekts, Salze zur Veränderung des osmotischen Drucks, Überzugsmittel oder Antioxidantien.

Die Dosierung des zu verabreichenden Wirkstoffs der Formel I und/oder eines physiologisch verträglichen Salze davon hängt vom Einzelfall ab und ist wie üblich für eine optimale Wirkung den individuellen Gegebenheiten anzupassen. So hängt sie ab von der Art und Stärke der zu behandelnden Krankheit sowie von Geschlecht, Alter, Gewicht und individueller Ansprechbarkeit des zu behandelnden Menschen oder Tieres, von der Wirkstärke und Wirkdauer der eingesetzten Verbindungen, davon, ob akut oder chronisch therapiert wird oder Prophylaxe betrieben wird, oder davon, ob neben Verbindungen der Formel I weitere Wirkstoffe verabreicht werden. Im allgemeinen ist eine Tagesdosis von etwa 0,01 bis 100 mg/kg, vorzugsweise 0,1 bis 10 mg/kg, insbesondere 0,3 bis 5 mg/kg (jeweils mg pro kg Körpergewicht) bei Verabreichung an einen ca. 75 kg schweren Erwachsenen zur Erzielung wirksamer Ergebnisse angemessen. Die Tagesdosis kann in einer Einzeldosis verabreicht werden oder, insbesondere bei der Applikation größerer Mengen, in mehrere, zum Beispiel zwei, drei oder vier Einzeldosen aufgeteilt werden. Gegebenenfalls kann es, je nach individuellem Verhalten erforderlich werden, von der angegebenen Tagesdosis nach oben oder nach unten abzuweichen. Pharmazeutische Präparate enthalten normalerweise 0,2 bis 500 mg, vorzugsweise 1 bis 200 mg Wirkstoff der Formel I und/oder dessen physiologisch verträgliche Salze pro Dosis.

Die Verbindungen der Formel I aktivieren die lösliche Guanylatcyclase. Aufgrund dieser Eigenschaft können sie außer als Arzneimittelwirkstoffe in der Humanmedizin und Veterinärmedizin auch als wissenschaftliches Tool oder als Hilfsmittel für biochemische Untersuchungen eingesetzt werden, bei denen eine derartige Beeinflussung der Guanylatcyclase beabsichtigt ist, sowie für diagnostische Zwecke, zum Beispiel in der in vitro-Diagnostik von Zell- oder Gewebsproben. Ferner können die Verbindungen der Formel I und ihre Salze, wie bereits oben erwähnt, als Zwischenprodukte zur Herstellung weiterer Arzneimittelwirkstoffe dienen.

Die nachfolgenden Beispiele erläutern die Erfindung, ohne sie einzuschränken.

Beispiel 1

5-Amino-1-benzyl-3-(2-furyl)-pyrazol

Die Mischung aus 2,7 g (2-Furoyl)-acetonitril, 3,9 g Benzylhydrazin-dihydrochlorid, 3,3 g Natriumacetat und 50 ml Ethanol wurde 6 Stunden unter Rühren am Rückfluß erhitzt. Die Reaktionsmischung wurde abgekühlt und der unlösliche Anteil abgesaugt. Das Filtrat wurde im Vakuum eingeengt, wobei ein fester Rückstand verblieb, der aus Isopropanol umkristallisiert wurde und ein farbloses Produkt lieferte. Ausbeute: 2,4 g.
Schmp.: 148°C

Beispiel 2

1-Benzyl-3-(2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

7,2 g 5-Amino-1-benzyl-3-(2-furyl)-pyrazol und 3,4 ml Acetylaceton wurden in 30 ml Eisessig gelöst und diese Mischung wurde 6 Stunden zum Sieden erhitzt. Die flüchtigen Anteile wurden im Vakuum abdestilliert und dieser Vorgang nach Zugabe von 30 ml Toluol wiederholt. Der verbleibende Rückstand wurde aus Isopropanol umkristallisiert und lieferte ein feinkristallines, farbloses Produkt. Ausbeute: 6,3 g.
Schmp.: 83°C

Beispiel 3

1-Benzyl-3-(5-formyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

2,5 g Phosphoroxychlorid wurden in 25 ml wasserfreiem Dimethylformamid (DMF) unter Eiskühlung gelöst und 45 Minuten bei Raumtemperatur stehen gelassen. Zu dieser Mischung wurden unter Umrühren 4,1 g 1-Benzyl-3-(2-furyl)-4,6-dimethylpyrazolo[3,4-b]pyridin gegeben und die Temperatur wurde auf 55°C gesteigert, wobei erst eine klare Lösung entstand, dann sich aber allmählich ein dicker Niederschlag bildete, so daß die Mischung mit weiteren 30 ml DMF verdünnt werden mußte. Nach 3 Stunden wurde die Mischung auf Raumtemperatur abgekühlt und mit 60 ml Wasser verdünnt. Nach Abkühlen im Eisbad wurde der gebildete Niederschlag abgesaugt, mit Wasser gewaschen und im Vakuum getrocknet. Ausbeute: 4,0 g.
Schmp.: 133°C

Beispiel 4

1-Benzyl-3-(5-dimethylaminomethyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin-Hydrochlorid

Die Mischung aus 1,3 g 1-Benzyl-3-(2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin, 0,38 g 40%iger wäßriger Dimethylaminlösung, 0,19 g Paraformaldehyd und 15 ml Eisessig wurde 30 Minuten auf 85°C erhitzt. Die klare Mischung wurde im Vakuum eingeengt, der ölige Rückstand in 20 ml Wasser aufgenommen und die Lösung durch Zugabe von Natriumbicarbonat neutralisiert. Mit tert-Butylmethylether wurde zweimal ausgeschüttelt. Das nach Trocknen und Einengen der vereinigten organischen Phasen erhaltene rohe Öl wurde aus Essigester als farbloses Hydrochlorid gefällt, das abgesaugt und getrocknet wurde. Ausbeute: 1,1 g.
Schmp.: 203°C

Beispiel 5

1-Benzyl-3-(5-hydroxymethyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin

Zur Suspension von 1,49 g 1 Benzyl-3-(5-formyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin in 70 ml Ethanol wurden 0,34 g Natriumborhydrid gegeben und das Gemisch wurde ohne Kühlung gerührt. Die Innentemperatur stieg auf 35°C und es trat eine leichte Gasentwicklung auf. Die flüchtigen Anteile wurden nach 30 Minuten im Vakuum abgezogen und der verbleibende Rückstand mit Wasser verrührt, wobei sich ein farbloser Niederschlag bildete, der abgesaugt und getrocknet wurde. Ausbeute: 1,36 g.
Schmp.: 171°C

Beispiel 6

rac-1-Benzyl-3-(5-(1-hydroxypropyl)-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin

Die Lösung von 1,42 g 1-Benzyl-3-(5-formyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin in 25 ml absolutem Tetrahydrofuran (THF) wurde auf 0°C abgekühlt. Dazu tropfte man die Lösung von 0,72 g Ethylmagnesiumbromid in 5 ml THF. Nach erfolgter Zugabe wurde ohne Kühlung 30 Minuten weitergerührt, mit Wasser versetzt und im Vakuum eingeengt. Der verbliebene Rückstand wurde in Wasser aufgenommen und daraus das Produkt durch Extraktion mit Essigester abgetrennt. Nach dem Trocknen über Natriumsulfat wurde der Essigester abgedampft und der Rückstand aus n-Heptan unter Zusatz von 10% Dipropylether umkristallisiert. Ausbeute: 1,1 g.

Schmp.: 97°C

Beispiel 7

1-Benzyl-3-(2-furyl)-4,6-dihydroxy-pyrnzolo[3,4-b]-pyridin

Die Mischung aus 12 g 5-Amino-1-benzyl-3-(2-furyl)-pyrazol, 9,6 g Malonsäurediethylester und 70 ml Diphenylether wurde 3 Stunden auf 200°C erhitzt. Nach dem Abkühlen auf Raumtemperatur wurde mit 20 ml tert-Butylmethylether verdünnt und der Feststoff abgesaugt. Er wurde mit 100 ml Methanol verrührt und erneut abgesaugt. Ausbeute: 12,3 g.
Schmp.: 313°C

Beispiel 8

5-Amino-1-tert-butyl-3-(2-furyl)-pyrazol

Die Herstellung der Verbindung erfolgte analog dem Beispiel 1.
Schmp.: 77°C

Beispiel 9

1-tert-Butyl-3-(2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin

Die Herstellung erfolgte ausgehend von 5-Amino-1-tert-butyl-3-(2-furyl)-pyrazol analog dem Beispiel 2.
Schmp.: Öl

Beispiel 10

1-tert-Butyl-3-(5-formyl-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin

Die Herstellung erfolgte ausgehend von 1-tert-Butyl-3-(2-furyl)-4,6-dimethylpyrazolo[3,4-bipyridin analog dem Beispiel 3.
Schmp.: 103°C

Beispiel 11

1-Benzyl-3-(2-furyl)-pyrazolo[3,4-b]-pyridin

Zur Lösung aus 2,4 g 5-Amino-1-benzyl-3-(2-furyl)-pyrazol in 20 ml Ethanol und 7 ml 2 N Salzsäure wurden 1,5 g Zinkchlorid und 1,8 g 1,1,3,3-Tetramethoxypropan gegeben und die Mischung 3 Stunden bei 80°C gerührt. Danach wurden die flüchtigen Anteile im Vakuum abgedampft, der Rückstand in Wasser aufgenommen und mit Essigester extrahiert. Das nach dem Trocknen über Natriumsulfat und Einengen verbliebene Öl wurde säulenchromatographisch gereinigt (Kieselgel, Laufmittel: Methylenchlorid:Methanol 99 : 1). Ausbeute: 0,47 g.
Schmp.: Öl

Beispiel 12

rac-1-tert-Butyl-3-(5-(1-hydroxypropyl)-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]-pyridin

Die Verbindung wurde ausgehend von 1-tert-Butyl-3-(5-formyl-2-furyl)-4,6-dimethylpyrazolo[3,4-b]-pyridin analog dem Beispiel 6 hergestellt und säulenchromatographisch gereinigt (Kieselgel, Laufmittel: Methylenchlorid: Methanol 98 : 2).
Schmp.: Öl

Beispiel 13

1-Benzyl-3-(2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 3-Methyl-2,4-pentandion analog dem Beispiel 2.
Schmp.: 126°C

Beispiel 14

1-Benzyl-3-(2-furyl)-4-methyl-6-hydroxy-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von Acetessigsäureethylester analog dem Beispiel 2.
Schmp.: 248°C

Beispiel 15

1-Benzyl-3-(5-formyl-2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 1-Benzyl-3-(2-furyl)-4,5,6-trimethylpyrazolo[3,4-b]pyridin analog dem Beispiel 3.
Schmp.: 127°C

Beispiel 16

1-Benzyl-3-(5-hydroxymethyl-2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 1-Benzyl-3-(5-formyl-2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin analog dem Beispiel 5.
Schmp.: 190°C

Beispiel 17

rac-1-Benzyl-3-(5-(1-hydroxy-1-phenyl-methyl)-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von Phenylmagnesiumbromid analog dem Beispiel 6.
Schmp.: 153°C

Beispiel 18

rac-1-Benzyl-3-(5-(1-hydroxypropyl)-2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 1-Benzyl-3-(5-formyl-2-furyl)-4,5,6-trimethyl-pyrazolo[3,4-b]pyridin analog dem Beispiel 6.
Schmp.: 137°C

Beispiel 19

rac-1-Benzyl-3-(5-(1-hydroxypentyl)-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von Butylmagnesiumchlorid analog dem Beispiel 6.
Schmp.: 97°C

Beispiel 20

rac-1-Benzyl-3-(5-(1-hydroxy-2-methyl-propyl)-2-furyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von Isopropylmagnesiumchlorid analog dem Beispiel 6.
Schmp.: 123°C

Beispiel 21

rac-1-Benzyl-3-(5-(1-hydroxy-4-dimethylamino-but-2-in-1-yl)-2-furyl)-4,5,6-trimethylpyrazolo[3,4-b]pyridin

Die Herstellung erfolgte analog dem Beispiel 6 ausgehend von 1-Benzyl-3-(5-formyl-2-furyl)4,5,6-trimethyl-pyrazolo[3,4-b]pyridin und dem Lithiumsalz des N,N-Dimethylpropargylamins, das aus N, N-Dimethylpropargylamin und n-Butyllithium in Tetrahydrofuran bei –65°C hergestellt wurde.
Schmp.: Öl

Beispiel 22

5-Amino-1-benzyl-3-(2-thienyl)-pyrazol

Die Herstellung erfolgte ausgehend von (2-Thenoyl)-acetonitril analog dem Beispiel 1.
Schmp.: 128°C

Beispiel 23

1-Benzyl-3-(2-thienyl)-4-methyl6-hydroxy-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 5-Amino-1-benzyl-3-(2-thienyl)-pyrazol und Acetessigsäureethylester analog dem Beispiel 2.
Schmp.: 230°C

Beispiel 24

1-Benzyl-3-(2-thienyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 5-Amino-1-benzyl-3-(2-thienyl)-pyrazol analog dem Beispiel 2.
Schmp.: 122°C

Beispiel 25

1-Benzyl-3-(2-thienyl)-4-phenyl-6-methyl-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 5-Amino-1-benzyl-3-(2-thienyl)-pyrazol und Benzoylaceton analog dem Beispiel 2.
Schmp.: 139°C

Beispiel 26

1-Benzyl-3-(2-thienyl)-4,6-dihydroxy-pyrazolo[3,4-b]pyridin

Die Herstellung erfolgte ausgehend von 5-Amino-1-benzyl-3-(2-thienyl)-pyrazol analog dem Beispiel 7.
Schmp.: 290°C (Zers.)

Beispiel 27

1-Benzyl-3-(5-brom-2-thienyl)-4,6-dimethyl-pyrnzolo[3,4b]pyridin

Zur Mischung aus 1,6 g 1-Benzyl-3-(2-thienyl)-4,6-dimethyl-pyrazolo[3,4-b]pyridin und 20 ml Tetrachlorkohlenstoff wurden bei 0°C 0,94 g N-Bromsuccinimid und 1 ml Eisessig gegeben. Dann wurde 4 Stunden am Rückfluß erhitzt und die flüchtigen Anteile wurden im Vakuum abgezogen. Der Rückstand wurde mit Wasser digeriert und nach Filtration aus Isopropanol umkristallisiert. Ausbeute: 1,6 g.
Schmp.: 92°C.

Pharmakologische Untersuchung

Aktivierung der löslichen Guanylatcyclase

Die Aktivierung der löslichen Guanylatcyclase (sGC), die die Umwandlung von Guanosintriphosphat (GTP) in cyclisches Guanosinmonophosphat (cGMP) und Pyrophosphat katalysiert, durch die erfindungsgemäßen Verbindungen wurde mit Hilfe eines Enyzm-Immuno-Assays (EIA) der Firma Amersham quantifiziert. Dazu wurden die Prüfsubstanzen zunächst mit sGC in Mikrotiterplatten inkubiert und dann die Menge des entstandenen cGMP bestimmt.

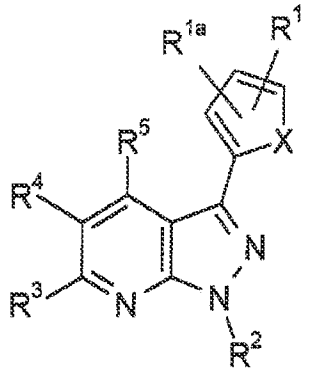
Die eingesetzte sGC war aus Rinderlunge isoliert worden (siehe Methods in Enzymology, Band 195, S. 377). Die Testlösungen (100 µl pro well) enthielten 50 mM Triethanolamin(TEA)-Puffer (pH 7,5), 3 mM $MgCl_2$, 3 mM reduziertes Glutathion (GSH), 0,1 mM GTP, 1 mM 3-Isobutyl-1-methylxanthin (IBMX), geeignet verdünnte Enzymlösung sowie die Prüfsubstanz bzw. bei den Kontrollversuchen Lösungsmittel. Die Prüfsubstanzen wurden in Dimethylsulfoxid (DMSO) gelöst und die Lösung mit DMSO/Wasser verdünnt, so daß die Endkonzentration an Prüfsubstanz in der Testlösung 50 µM betrug. Die DMSO-Konzentration in der Testlösung betrug 5% (v/v). Die Reaktion wurde durch Zugabe der sGC gestartet. Der Reaktionsmix wurde für 15 bis 20 Minuten bei 37°C inkubiert und dann durch Eiskühlung und Zugabe des Stop-Reagenz (50 mM EDTA, pH 8,0) gestoppt. Ein Aliquot von 50 µl wurde entnommen und zur Bestimmung des cGMP-Gehaltes mit dem Acetylierungs-Protokoll des Amersham-cGMP-EIA-Kits eingesetzt. Die Absorption der Proben wurde bei 450 nm (Referenz Wellenlänge 620 nm) in einem Mikrotiterplatten-Lesegerät gemessen. Die cGMP-Konzentration wurde über eine Eichkurve ermittelt, die unter denselben Versuchsbedingungen erhalten wurde. Die Aktivierung der sGC durch eine Prüfsubstanz wird angegeben als n-fache Stimulation der basalen Enzymaktivität, die bei den Kontrollversuchen (mit Lösungsmittel statt Prüfsubstanz) gefunden wurde (berechnet nach der Formel n-fache Stimulierung = $[cGMP]_{Prüfsubstanz}/[cGMP]_{Kontrolle}$).

Es wurden folgende Werte ermittelt:

Verbindung	Konzentration	n-fache Stimulierung
Beispiel 5	50 µM	3-fach
Beispiel 6	50 µM	3-fach
Beispiel 10	50 µM	3-fach
Beispiel 23	50 µM	3-fach

Patentansprüche

1. Verbindungen der Formel I,



I

in der X für O, S, NH oder $N(CH_3)$ steht;
R^1 und R^{1a} unabhängig voneinander für Wasserstoff, Halogen, CO-R^{10} oder (C_1-C_8)-Alkyl stehen, wobei Alkylgruppen gesättigt oder ungesättigt sein können und unsubstituiert oder durch einen oder mehrere gleiche oder verschiedene Reste R^{11} substituiert sein können;
R^2 für (C_1-C_6)-Alkyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl steht;
R^3 und R^5 unabhängig voneinander für Wasserstoff, (C_1-C_6)-Alkyl, unsubstituiertes oder substituiertes Phenyl, in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl, Hydroxy, (C_1-C_6)-Alkoxy oder in der Phenylgruppe unsubstituiertes oder substituiertes Benzyloxy stehen;
R^4 für Wasserstoff, (C_1-C_6)-Alkyl, unsubstituiertes oder substituiertes Phenyl oder in der Phenylgruppe unsubstituiertes oder substituiertes Phenyl-(C_1-C_4)-alkyl steht;
R^{10} für Wasserstoff, (C_1-C_6)-Alkyl oder unsubstituiertes oder substituiertes Phenyl steht;
R^{11} für $R^{12}O$, $R^{12}R^{13}N$, Halogen oder unsubstituiertes oder substituiertes Phenyl steht;
R^{12} für Wasserstoff, (C_1-C_6)-Alkyl, in der Phenylgruppe unsubstituiertes oder substituiertes Benzyl, H-CO oder (C_1-C_6)-Alkyl-CO steht;
R^{13} für Wasserstoff oder (C_1-C_6)-Alkyl steht;
oder R^{12} und R^{13} zusammen mit dem Stickstoffatom, an das sie gebunden sind, einen gesättigten 5-Ring- oder 6-Ring-Heterocyclus bilden, der als zusätzliches Ring-Heteroatom noch ein Sauerstoffatom, Schwefelatom oder ein durch eine Methylgruppe substituiertes Stickstoffatom enthalten kann;
in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

2. Verbindungen der Formel I gemäß Anspruch 1, in der X für O oder S steht, in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

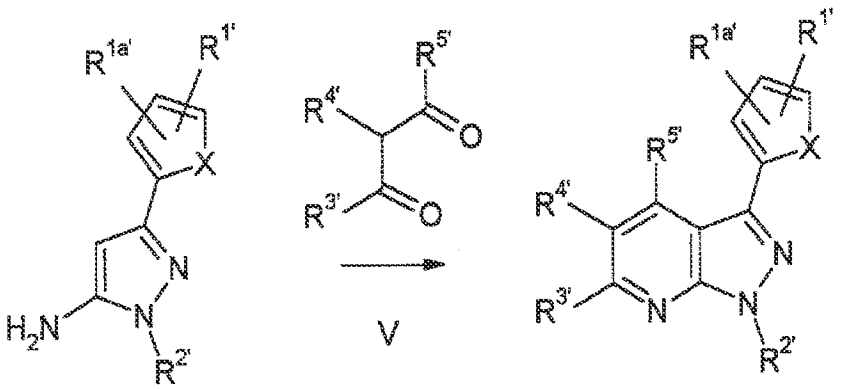
3. Verbindungen der Formel I gemäß Anspruch 1 und/oder 2, in der R^{1a} für Wasserstoff steht und R^1 sich in der 5-Position befindet, in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

4. Verbindungen der Formel gemäß einem oder mehreren der Ansprüche 1 bis 3, in der R^1 für Wasserstoff, Formyl, Halogen, die Gruppe $R^{20}CH(OH)$ oder die Gruppe $((C_1$-$C_6)$-Alkyl$)_2N$-CH_2 steht, wobei R^{20} für Wasserstoff, (C_1-C_6)-Alkyl, das gesättigt ist oder eine Dreifachbindung enthält und das unsubstituiert ist oder durch eine Gruppe $((C_1$-$C_6)$-Alkyl$)_2N$ substituiert ist, oder für unsubstituiertes oder substituiertes Phenyl steht, in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

5. Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 4, in der R^2 für (C_1-C_6)-Alkyl oder im Phenylrest unsubstituiertes oder substituiertes Benzyl steht, in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

6. Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 5, in der R^3 und R^5 unabhängig voneinander für (C_1-C_4)-Alkyl oder Hydroxy stehen, in allen ihren stereoisomeren Formen und Mischungen davon in allen Verhältnissen, sowie ihre physiologisch verträglichen Salze.

7. Verfahren zur Herstellung von Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6, dadurch gekennzeichnet, daß man Verbindungen der Formel IV mit Verbindungen der Formel V zu Verbindungen der Formel I' umsetzt,



IV V I'

wobei in den Formeln IV, V und I' die Reste X, $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$, und $R^{5'}$ die in den Ansprüchen 1 bis 6 für die Reste X, R^1, R^{1a}, R^2, R^3, R^4 und R^5 angegebenen Bedeutungen haben und zusätzlich in den Resten $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$ und $R^{5'}$ funktionelle Gruppen in geschützter Form oder in Form von Vorstufen vorliegen können, und anschließend gegebenenfalls in einem oder mehreren anschließenden Reaktionsschritten durch Umwandlungen der Reste $R^{1'}$, $R^{1a'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$ und $R^{5'}$ in die Reste R^1, R^{1a}, R^2, R^3, R^4 und R^5 aus den Verbindungen der Formel I' die Verbindungen der Formel I herstellt.

8. Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihre physiologisch verträglichen Salze zur Anwendung als Arzneimittel.

9. Pharmazeutisches Präparat, dadurch gekennzeichnet, daß es eine oder mehrere Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihre physiologisch verträglichen Salze neben pharmazeutisch einwandfreien Trägerstoffen und/oder Zusatzstoffen enthält.

10. Verwendung von Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihren physiologisch verträglichen Salzen zur Herstellung eines Medikaments zur Aktivierung der löslichen Guanylatcyclase.

11. Verwendung von Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihren physiologisch verträglichen Salzen zur Herstellung eines Medikaments zur Therapie oder Prophylaxe von Herz-Kreislauf-Erkrankungen, endothelialer Dysfunktion, diastolischer Dysfunktion, Atherosklerose, Bluthochdruck, Angina pectoris, Thrombosen, Restenosen, Myocardinfarkt, Schlaganfällen, Herzinsuffizienz, Pulmonalhypertonie, erektiler Dysfunktion, Asthma bronchiale, chronischer Niereninsuffizienz, Diabetes oder Leberzirrhose oder zur Verbesserung einer eingeschränkten Lernfähigkeit oder Gedächtnisleistung.

12. Verwendung von Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihren physiologisch verträglichen Salzen zur Aktivierung der löslichen Guanylatcyclase.

13. Verwendung von Verbindungen der Formel I gemäß einem oder mehreren der Ansprüche 1 bis 6 und/oder ihren physiologisch verträglichen Salzen zur Therapie oder Prophylaxe von Herz-Kreislauf-Erkrankungen, endothelialer Dysfunktion, diastolischer Dysfunktion, Atherosklerose, Bluthochdruck, Angina pectoris, Thrombosen, Restenosen, Myocardinfarkt, Schlaganfällen, Herzinsuffizienz Pulmonalhypertonie, erektiler Dysfunktion, Asthma bronchiale, chronischer Niereninsuffizienz, Diabetes oder Leberzirrhose oder zur Verbesserung einer eingeschränkten Lernfähigkeit oder Gedächtnisleistung.

- Leerseite -

DESCRIPTION DE19744027

The present invention relates to substituted pyrazolo [3,4-b] pyridine of the formula I,

EMI1.1

in which X, R <1>, R <1a>, R <2>, R <3>, R <4> and R <5> have the meanings indicated below, which are valuable pharmaceutical active compounds for the therapy and prophylaxis of diseases for example, cardiovascular diseases such as hypertension, angina pectoris, heart failure, thrombosis or atherosclerosis.

The compounds of formula I have the ability to modulate the endogenous production of cyclic guanosine monophosphate (cGMP) and are generally suitable for the therapy and prophylaxis of pathological conditions associated with a disturbed cGMP balance.

The invention further relates to processes for preparing compounds of formula I include their use for the therapy and prophylaxis of the designated disease states and for the production of pharmaceuticals therefor, and pharmaceutical preparations which contain compounds of formula I.

cGMP is an important intracellular messenger that triggers a variety of effects on the modulation of cGMP-dependent protein kinases, phosphodiesterases and ion channels.

Examples are smooth muscle relaxation, the inhibition of thrombocyte activation and the inhibition of smooth muscle cell proliferation and leukocyte adhesion. cGMP is produced by particulate and soluble guanylate cyclases (GC) in response to a series of extra-and intracellular stimuli.

In the case of particulate guanylate cyclases stimulation essentially by peptidic signaling molecules, such as the atrial natriuretic peptide or the cerebral natriuretic peptide.

The soluble guanylate cyclases (sGC), where it is cytosolic, heterodimeric heme proteins, in contrast, are essentially regulated by a family of low molecular weight, enzymatically formed factors.

Important stimulant is nitric oxide (NO) or a closely related species.

The importance of other factors such as carbon monoxide or the hydroxyl radical is still largely unknown.

As the activation mechanism of activation by the binding of NO NO to the heme to form a penta-coordinate heme-nitrosyl complex is discussed.

The associated release of bound iron in the basal state histidine converts the enzyme into the activated conformation.

Active soluble guanylate cyclases consist of one each alpha - subunit together - and a beta.

A plurality of subtypes of the subunits are described which differ from one another with respect to sequence, tissue-specific distribution and expression in various stages of development.

The subtypes of alpha 1 and beta 1 is mainly expressed in brain and lung, whereas beta 2 is found mainly in liver and kidney.

In human fetal brain of the alpha subtype 2 could be detected.

The subunits designated as alpha 3 and beta 3 were isolated from human brain and are homologous to alpha 1 and beta 1

More recent works indicate an alpha-2i subunit which contains an insert in the catalytic domain.

All subunits show great homology in the catalytic domain.

The enzymes probably contain one heme per heterodimer, which is about 1 beta-Cys-78 and / or beta 1-105-His bound and is part of the regulatory site.

Under pathological conditions, the formation of guanylate cyclase-activating factors can be reduced or it may be the same by the increased occurrence of free radicals an increased degradation.

The resulting reduced activation of the sGC leads via the attenuation of the respective cGMP-mediated cell response, for example to the increase of the blood pressure, to platelet activation or to increased cell proliferation and cell adhesion.

As a result in the formation of endothelial dysfunction, atherosclerosis, hypertension, stable and unstable angina pectoris, thrombosis, myocardial infarction, stroke, or erectile dysfunction.

The pharmacological stimulation of sGC provides a way for the normalization of cGMP production and thus allows the treatment or

Prevention of the diseases.

Compounds have been used almost exclusively to pharmacological stimulation of sGC far, their effect on an intermediate NO release is based, for example, organic nitrates.

The disadvantage of this treatment is the development of tolerance and effect of attenuation and therefore become necessary higher dosage.

Different does not have a release of NO acting sGC stimulators were described by Vesely in a large number of works.

The compounds, which are mostly involving hormones, plant hormones, vitamins or natural substances such as poisons lizards, however, consistently show only weak effects on cGMP formation in cell lysates (DL Vesely, Eur

J. Clin.

Invest.

15 (1985) 258, DL Vesely, Biochem.

Biophys.

Res

Comm.

88 (1979) 1244).

Stimulation of guanylate cydase by heme-free protoporphyrin IX was by Ignarro et al.

(Adv.

Pharmacol. 26 (1994) 35) detected.

Pettibone et al.

(Eur.

J. Pharmacol. 116 (1985) 307) described for Diphenyliodoniumhexafluorophoshat a hypotensive effect and attributed this to a stimulation of sGC.

Isoliquiritigenin showing a relaxant effect on isolated rat aorta, activated according to Yu et al.

(Brit.

J. Pharmacol. 114 (1995) 1587) also sGC. Ko et 21

(Blood 84 (1994) 4226), Yu et al.

(Biochem.

J. 306 (1995) 787) and Teng et al.

(Brit.

J. Pharmacol. 116 (1995) 1973) showed a sGC-stimulating activity of 1-benzyl-3-(5-hydroxymethyl-2-furyl) indazole and demonstrated an antiproliferative and thrombocyte-inhibiting action.

Various indazoles is described in EP-A-667 345 as inhibitors of platelet aggregation.

In pyridine unsubstituted pyrazolo [3,4-b] pyridine, which at the 1-position of an aryl or heteroaryl group and bear an aryl or heteroaryl at the 3-position are known as antitumor agents and immunostimulants in the Japanese patent application 01 - 190681 described.

Surprisingly, it has now been found that the inventive contrast pyrazolo [3,4-b] pyridine derivatives of the formula I have a very different effect and effect guanylate cyclase activation, because of which they are suitable for the therapy and prophylaxis of diseases associated with are connected to a low level of cGMP.

The present invention thus relates to compounds of formula I

EMI4.1

in which X stands for O, S, NH or N (CH3);

R <1> and R <1a> are each independently hydrogen, halogen, CO-R <10>, or (C1-C8)-alkyl, wherein alkyl groups may be saturated or unsaturated and unsubstituted or substituted by one or more identical or different radicals R <11> may be substituted;

R <2> is (C1-C6) alkyl or phenyl unsubstituted or substituted in the phenyl (C1-C4) alkyl;

R <3> and R <5> independently represent hydrogen, (C1-C6)-alkyl, unsubstituted or substituted phenyl, in which phenyl is unsubstituted or substituted phenyl-(C1-C4)-alkyl, hydroxy, (C1-C6) alkoxy or are in the phenyl unsubstituted or substituted benzyloxy;

R <4> is hydrogen, (C1-C6)-alkyl, unsubstituted or substituted phenyl or in the phenyl group is unsubstituted or substituted phenyl -(C1-C4)-alkyl;

R <10> is hydrogen, (C1-C6)-alkyl or unsubstituted or substituted phenyl;

R <11> R <12> O, R <12> R <13> N, halogen, or unsubstituted or substituted phenyl;

R <12> is hydrogen, (C1-C6)-alkyl, unsubstituted or substituted in the phenyl group, benzyl, H, or-CO (C1-C6)-alkyl-CO;

R <13> is hydrogen or (C1-C6) alkyl;

or R <12> and R <13> together with the nitrogen atom to which they are attached form a saturated 5-membered ring or 6-ring heterocycle as an additional ring hetero atom, an oxygen atom, sulfur atom or by a may include methyl group substituted nitrogen atom;

in all its stereoisomeric forms and mixtures thereof in all ratios, and their physiologically tolerable salts.

Alkyl radicals may be straight or branched.

This also applies when they are substituted, for example by a phenyl or substituted by hydroxyl, or if they are included in other groups, for example in alkoxy groups.

Examples of alkyl groups are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3,3-dimethylbutyl, n-heptyl, n-octyl.

The term alkyl and unsaturated alkyl groups are here to be understood, in particular alkyl radicals or one or two double bonds, contain one or two triple bonds or one double bond and one triple bond.

Examples of such radicals are the vinyl radical, the 2-propenyl radical (allyl radical), the 2-butenyl radical, 3-methyl-2-butenyl, ethynyl of the 2 - propynyl radical (propargyl radical) or the 3-Butinylrest.

Phenyl radicals may be unsubstituted or, for example, be singly or multiply twice or trisubstituted by identical or different substituents, where the substituents can be situated in any desired positions.

Preferred substituted phenyl groups are mono-or di-substituted.

Monosubstituted phenyl radicals can be in the 2-position, the 3-position or the 4 - position to be substituted, phenyl disubstituted in the 2,3-position, 2,4 - position, 2,5-position, the 2,6 - position, 3,4-position or 3,5-position.

In tri-substituted phenyl, the substituents can for example 2,3,4 - position, 2,3,5-position, 2,3,6-position, 2,4,5-position, 2,4,6-position or 3 , 4,5 - position located.

Substituents on substituted phenyl rings are in particular (C1-C4)-alkyl, halogen, (C1-C4)-alkoxy, benzyloxy, amino, ((C1-C4)-alkyl) carbonylamino, mono-and di-((C1-C4)-alkyl)-amino, nitro, cyano, trifluoromethyl, hydroxycarbonyl, aminocarbonyl (= carbamoyl), and ((C1-C4) - alkoxy)-carbonyl.

The explanations also apply to such phenyl phenyl, phenylalkyl contained in or benzyl.

Preferred phenylethyl radicals are phenylalkyl and in particular the benzyl radical.

Examples of heterocycles which R <12> and R may <13> together with the nitrogen atom carrying them, are pyrrolidine, piperidine, piperazine, morpholine and thiomorpholine.

These heterocycles may also be, for example, by one or more, for example one, two, three or four alkyl groups, especially methyl groups, and substituted in case of Thiomorpholins be oxidized to the sulfoxide or sulfone on the sulfur.

Halogen is fluorine, chlorine, bromine or iodine, preferably fluorine or chlorine.

Are alkyl radicals R <11> substituted by radicals, it can be R <11> substituted, for example by one, two, three, four or more identical or different radicals.

Preferably such substituted groups contain one, two or three, particularly preferably one or two identical or different radicals R <11>.

In the substituted alkyl, the substituents can be located in any positions.

The two substituents R <1> and R <1a> can be in any position of the heterocycle, ie in positions 3, 4 and 5 of the furan ring, thiophene or pyrrole ring.

The compounds of formula I may, when appropriately substituted exist in stereoisomeric forms.

If the compounds of formula I, these can independently have one or more asymmetric centers in the S configuration or the R configuration.

The invention includes all possible stereoisomers, for example enantiomers and diastereomers, and mixtures of two or more stereoisomeric forms, for example mixtures of enantiomers and / or diastereomers, in all ratios.

Enantiomers are therefore in enantiomerically pure form, both as a left and as a right-handed antipodes, in the form of racemates, and as mixtures of the two enantiomers in all ratios of the invention.

In the presence of cis / trans isomerism both the cis form and the trans form and mixtures of these forms are the subject of the invention.

The preparation of individual stereoisomers can be carried out, if desired, by separation of a mixture according to customary methods, for example by chromatography or crystallization, by use of stereochemically uniform starting substances in the synthesis or by stereoselective synthesis.

If necessary, can be done before separation of stereoisomers derivatization.

For example, a compound of formula I, which includes, for example in the group R <1> is a chiral carbon atom and a hydroxy group, are first converted to the hydroxyl group in the ester of an optically active acid, and the resulting diastereomeric esters may then by customary methods, are separated, for example by crystallization or chromatography, and the esters are then saponified again.

The separation of a stereoisomer mixture can take place at the stage of the compounds of the formula I or at the stage of an intermediate in the course of the synthesis.

If mobile hydrogen atoms, the present invention also includes all tautomeric forms of the compounds of the formula I, for example lactam / lactim tautomers.

Examples of such tautomers forms 1a and 1b may be mentioned of compounds of formula I, <3> in which R is hydroxy.

Corresponding tautomeric forms are possible, for example when R <5> in formula I is hydroxy.

EMI8.1

If the compounds of formula I contain one or more acidic or basic groups, also the corresponding physiologically or toxicologically acceptable salts, the invention, in particular the pharmaceutically usable salts thereof.

Thus, the compounds of formula I which contain one or more acidic groups, for example hydroxyl groups in the pyridine ring or COOH substituent in phenyl rings are present in these groups, for example as alkali metal salts, alkaline earth metal salts or as ammonium salts and can be used according to the invention.

Examples of such salts are sodium salts, potassium salts, calcium salts, magnesium salts or salts with ammonia or organic amines such as ethylamine, ethanolamine, triethanolamine or amino acids.

Compounds of formula I which contain one or more basic, that is protonatable, groups can be present in the form of their acid addition salts with inorganic or organic acids and are used according to the invention, for example as salts with hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid , p-toluenesulfonic acid, naphthalenedisulfonic acids, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, malic acid, sulfamic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, etc.

If the compounds of formula I both acidic and basic groups in the molecule, so in addition to the salt forms include internal salts or betaines to the invention.

Salts can be obtained from the compounds of the formula I by customary processes known to the expert, for example by combination with an organic or inorganic acid or base in a solvent or dispersant, or by anion exchange or cation exchange from other salts.

The present invention also includes all salts of the compounds of formula I, because of low physiological Verträglichkeit not directly suitable for use in pharmaceuticals but are suitable, for example, as intermediates for chemical reactions or for the preparation of physiologically tolerable salts.

The present invention furthermore includes all solvates of compounds of formula I, for example hydrates or adducts with alcohols, and also derivatives of the compounds of the formula I, for example esters, prodrugs and metabolites which act like the compounds of formula I.

In formula I, X preferably represents O or S, more preferably O.

R <1> is preferably hydrogen, formyl, halogen, the group R <20> CH (OH) or a group ((C1-C6) alkyl) 2N-CH2, wherein R <20> is hydrogen, (C1- C6) alkyl, which is saturated or containing one triple bond, and which is unsubstituted or is substituted by a group ((C1-C6) alkyl) 2N, or represents unsubstituted or substituted phenyl, and wherein in a group ((C1- C6) alkyl) 2N, the alkyl radicals may be the same or different.

More preferably R <1> is hydrogen, formyl or the group R <21> CH (OH), wherein R is <21> is hydrogen, (C1-C4) alkyl or unsubstituted phenyl.

Preferably, the radical R <1> is at the 5-position of the heterocycle, that the furan ring, thiophene ring or pyrrole ring.

R <1a> is preferably hydrogen or (C1-C4)-alkyl, more preferably hydrogen.

R <2> is preferably (C1-C6) alkyl or phenyl unsubstituted or substituted in the phenyl (C1-C2) alkyl, particularly preferably (C1-C6) alkyl or unsubstituted or substituted in the phenyl benzyl.

Most preferably, R <2> is unsubstituted benzyl or tert-butyl.

R <3> and R <5> are preferably independently of one another represent hydrogen, (C1-C6) - alkyl, unsubstituted or substituted phenyl, or hydroxy, particularly preferably hydrogen, (C1-C4)-alkyl or hydroxy, in particular hydrogen, methyl or hydroxy, and most preferably (C1-C4)-Akyl hydroxy or, especially, methyl or hydroxy.

R <4> is preferably hydrogen or (C1-C6) alkyl, more preferably hydrogen or (C1-C4)-alkyl, very particularly preferably hydrogen.

It is also preferred if one or more of R <3>, R <4> and R <5> is other than hydrogen.

Preferred compounds of formula I are those in which have one or more of the groups contained therein preferred meanings, all combinations are covered by preferred substituent.

Also all preferred compounds of the formula I the present invention comprises all their stereoisomeric forms and mixtures thereof in all ratios, and their physiologically tolerable salts.

A group of preferred compounds of formula I formed by those compounds in which in the formula I

X is O or S;

R <1> is hydrogen, formyl, halogen, the group R <20> CH (OH) or a group ((C1-C6) alkyl) 2N-CH2, where R <20> is hydrogen, (C1-C6) alkyl, which is saturated or contains a triple bond, and which is unsubstituted or substituted by a group ((C1-C6) alkyl) 2 N groups, or is unsubstituted or substituted phenyl, and R <1> is in the 5 - position;

R <1a> is hydrogen;

R <2> is (C1-C6) alkyl or phenyl unsubstituted or substituted in the phenyl (C1-C2) alkyl;

R <3> and R <5> independently represent hydrogen, (C1-C6)-alkyl, unsubstituted or substituted phenyl or hydroxy;

R <4> is hydrogen or (C1-C6) alkyl;

in all its stereoisomeric forms and mixtures thereof in all ratios, and their physiologically tolerable salts.

The novel compounds of formula I can, for example, analogously to in J. Het.

Chem 12 (1975) 1303 and J. Het.

Chem 15 (1978) 319 method described are manufactured, which is described below and is also a subject of the present invention.

Starting materials for the first step of preparing the beta-ketonitriles of formula II which can be reacted with the hydrazines of the formula III or their salts of the aminopyrazoles of formula IV.

EMI11.1

In the formulas II, III and IV, X, R <1>, R <1a '>, and R <2'>, as defined above for X, R <1>, R <1a> and R <2> specified meanings and additionally, <2 '> functional groups are present in protected form or in the form of precursors in the radicals R <1'>, R <1a '>, and R.

Suitable protecting groups or favorable precursors for functional groups in these radicals are known in the art.

For example, a carbonyl group in these radicals initially present in protected form, for example in the form of an acetal or ketal, or an amino group can be present in acylated form, or it may be a hydrogen atom as a precursor for a group is received that in a subsequently performed electrophilic substiiution reaction is introduced, or modifications can be carried out by other functional groups in accordance with the invention according to the invention groups.

Suitable compounds of the formulas II and III for the preparation of the novel compounds of formula I are commercially available or can be obtained with methods according or analogous to those described in the literature, for example in the standard works, Houben-Weyl, Methoden der Organischen Chemie, Thieme-Verlag, Stuttgart, or Organic Reactions, John Wiley & Sons, New York.

The heterocyclic Acylacetonitrile of formula II may be obtained, for example, by acylation of acetonitrile or ethyl cyanoacetate with heterocyclic esters or acyl chlorides in the presence of a base such as lithium diisopropylamide, and, in the case of the cyanoacetic ester, subsequent removal of the ester group.

The hydrazines of formula III may be obtained for example by alkylation of appropriate hydrazine or by reduction of hydrazones.

The reactions of beta-ketonitriles of formula II with hydrazines of the formula III or salts thereof are preferably carried out in a solvent or dispersant.

Suitable solvents are, for example, water, alcohols such as methanol, ethanol, n-propanol, isopropanol, or butanols, ethers such as diethyl ether, dipropyl ether, dibutyl ether, tert-butyl methyl ether, tetrahydrofuran or dioxane, monoethers and diethers of ethylene glycol and of di-and triethylene glycol such as ethylene glycol monomethyl ether , ethylene glycol monoethyl ether, ethylene glycol dimethyl ether, ethylene glycol monobutyl ether, diethylene glycol monomethyl ether or diethylene glycol dimethyl ether, esters such as ethyl acetate or butyl acetate, amides such as dimethylformamide, dimethylacetamide, N-methylpyrrolidone or hexamethylphosphoramide nitriles such as acetonitrile, acids such as acetic acid, sulfoxides and sulfones such as dimethyl sulfoxide or sulfolane, hydrocarbons and chlorinated hydrocarbons such as petroleum fractions , benzene, toluene, xylene, chlorobenzene, dichlorobenzene, methylene chloride or chloroform.

Also mixtures of two or more solvents are used, for example mixtures of water and alcohols or mixtures of water and acids.

Preferred solvents are alcohols such as methanol and ethanol.

The reaction of compounds of formulas II and III can be carried out in a wide temperature range.

In general, it is carried out at temperatures from 0 ° C to 150 ° C., preferably at temperatures of 20 ° C to 120 ° C.

It is particularly preferred to carry it out under reflux at the boiling point of the solvent or solvent mixture used, for example at the boiling point of methanol or ethanol is.

The reaction time depends on the individual case and depends for example on the reactivity of the reactants and the reaction conditions.

In general, when the reaction is carried out in methanol or ethanol at boiling temperature, the reaction after 1 to 10 hours.

The workup of the reaction mixture can be effected according to standard procedures and the product purified, if desired, by conventional purification methods, for example by recrystallization, distillation, sublimation, or chromatography.

As a reaction of a carbonyl compound with an amino compound of the reaction of the compounds of the formulas II and III can be accelerated by the addition of both base and the addition of acids.

Suitable acids are, for example, organic carboxylic acids and sulfonic acids such as acetic acid, trifluoroacetic acid, methanesulfonic acid or p-toluenesulfonic acid, inorganic acids such as hydrochloric, sulfuric or phosphoric acid, acidic salts such as ammonium salts or hydrogen phosphates, or acidic ion exchangers.

Suitable bases are for example, hydroxides, carbonates, hydrogen carbonates, acetates or alcoholates of alkali metals and alkaline earth metals, for example sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium acetate, sodium methylate, sodium carbonate, potassium carbonate, potassium tert-butoxide, basic ion exchangers, or amines such as triethylamine or pyridine.

Free hydrazines of the formula III are used, it is in many cases particularly advantageous to carry out the reaction with the compounds of formula II under acidic catalysis.

The catalysts exemplified acids come into consideration.

It may also be advantageous to set a certain pH value, or to operate in the presence of a buffer system.

It is preferred to carry out the reaction of compounds of formula II with free hydrazines of the formula III in the presence of acetic acid.

The type and amount of added acid catalyst depend on the individual case and depends for example on the reactivity of the reactants, the solvent or the intended temperature.

For example, when an acid such as acetic acid is used, it may act both as a solvent and as a catalyst depending on the amount used.

If instead of a free hydrazine, an acid addition salt of hydrazine used as a R <2 '>-substituted hydrazinium or hydrazinium, so that an acidic compound is already incorporated in the reaction mixture, which can act catalytically.

When using a Hydraziniumsalzes it is often particularly advantageous to trap a part of the inserted through this acid by addition of a certain amount of a base, for example by addition of sodium acetate or other deadening material to the reaction mixture.

In individual cases, rather than react with the carbonyl group in the compound of formula II with the nitrile group, depending on the reaction conditions and the reactivity of the reactants, the NH2 group of the hydrazine of formula III.

In this case, the reaction can also lead to the undesired isomers of the aminopyrazole of the formula IV in which the amino group is in the 5-position in the 3-position and the heterocycle.

The reaction progress can be optionally controlled by the choice of reaction conditions.

, The reaction of the compounds of formulas II and III of the isomeric pyrazoles mixtures, these can be separated into the components by customary methods, for example by recrystallization, distillation, sublimation, or chromatography.

In the second step of the preparation of the compounds of formula I then the aminopyrazoles of formula IV with 1,3-dicarbonyl compounds of formula V are converted to the Pyrazolopyridinen of formula I '.

EMI15.1

According to the above explanations of the formulas II, III and IV are also in the formulas V and I 'the radicals X, R <1'>, R <1a>, R <2 '>, R <3'>, R < 4 '> and R <5'> for the above X, R <1>, R <1a>, R <2>, R <3>, R <4> and R <5> the meanings indicated and can in addition to the radicals R <1 '>, R <1a>, R <2'>, R <3 '>, R <4'> and R <5 '> turn functional groups are present in protected form or in the form of precursors.

From the compounds of formula I 'can then optionally compounds of formula I are obtained by reacting, in one or more subsequent reaction steps which are present in protected form or in the form of precursor groups into the desired, in the above definitions of R <1> , R <1a>, R <2>, R <3>, R <4> and R transferred <5> groups, or said functional groups modified according to the invention.

If the compounds of formula I are prepared in which the radicals R <3> and R <5> in the formula I or of R <3 '> and R <5'> in the formula I 'for both radicals from the of hydrogen, alkyl, phenyl and phenylalkyl, it is the compounds of formula V to dialdehydes beta, beta-beta-diketones or ketoaldehydes.

If the compounds are prepared in which one of R <3> and R <5> in the formula I and the radicals R <3 '> and R <5'> in the formula I 'is hydroxy or a subsequent one in step reaction of a hydroxy group is available alkoxy or benzyloxy, it is the compounds of formula V to beta - Aldehydsäurederivate or beta-keto acid derivatives.

As the acid, in particular the ester derivatives are contemplated, for example, (C1-C4)-alkyl esters such as the methyl ester or the ethyl ester.

If the compounds are prepared in which the radicals R <3> and R <5> in the formula I and the radicals R <3 '> and R <5'>, in the formula I 'are both hydroxy or therefrom at a later step are available alkoxy or benzyloxy, it is for the compounds of formula V to malonic acid.

Even as malonic turn in particular, the (C1-C4)-alkyl esters such as methyl acetate or ethyl esters into consideration.

All these compounds of the formula V are commercially available in large quantities or can be obtained by or in analogy to methods that are described in the literature, for example the above-mentioned standard works, in detail.

Aldehyde and ketone V can be present in the compounds of formula in the form of acetals or ketals, for example in the form of geminal dimethoxy compounds, the geminal Diethoxyverbindungen or dioxolanes from which the aldehyde or ketone groups are only released during the reaction, for example by acid.

The reactions of compounds of formula IV with dicarbonyl compounds of formula V are preferably carried out in a solvent or dispersant.

Suitable solvents here are for example water, alcohols, ethers, monoethers and diethers of ethylene glycol and of di-and triethylene glycol, amides, acids, sulfoxides and sulfones, hydrocarbons and chlorinated hydrocarbons.

The above examples of this solvent also apply here.

Depending on the individual case, it may here also be applied at higher temperatures than in the first step to work with, so come addition to the solvents exemplified above also appropriate higher-boiling solvents are, for boiling alcohols such as decanol or higher boiling ethers such as diphenyl ether.

It can also be used in turn mixtures of two or more solvents.

A preferred solvent is acetic acid (Essigäure).

The reaction of the compounds of formulas IV and V is generally carried out at temperatures from 0 ° C to 200 ° C., preferably at temperatures from 50 ° C to 200 ° C.

It is particularly preferred to carry it out under reflux at the boiling point of the solvent used, but may for example also be carried out under pressure in an autoclave.

The reaction time depends again on the individual case and depends for example on the reactivity of the reactants and the reaction conditions.

Generally, the reaction of 1 to 12 hours is completed.

The workup of the reaction mixture can be carried out according to standard procedures and the product purified, if desired, by conventional cleaning methods.

The reaction of the compounds of formulas IV and V can, both the addition of the addition of bases or acids can be accelerated.

In many cases, particularly advantageous and therefore preferred is the use of acid catalysts.

The above explanations for catalysis and the aforementioned examples of acids and bases apply here.

A particularly preferred catalyst for the reaction of compounds of formulas IV and V is acetic acid, which in turn can be used as a particularly favorable solvent and as a catalyst.

Can also in the reactions of the compounds of formulas IV and V in an individual case, depending on the reaction conditions and the reactivity of the reactants occur isomer: is not symmetric, the compound of formula V can in addition to the compound of formula I ', the corresponding pyrazolopyridine arise in the radicals R <3 '> and R <5'> are interchanged.

, The reaction of the compounds of the formulas IV and V is a mixture of the two isomeric compounds, these can be separated into the components by customary methods, for example by recrystallization, distillation, sublimation, or chromatography.

In the compounds of formula I 'can, as mentioned above, the radicals R <1'>, R <1a '>, R <2'>, R <3 '>, R <4'> and R <5 ' > in the definition of R <1>, R <1a>, R <2>, R <3>, R <4> and R are <5>, is reacted so that the reaction products obtained by the described methods of synthesis of the formula I 'compounds of this invention of formula I present.

But it may be made in the synthesis obtained by the described process compounds of the formula I 'in a variety of ways also structural variations.

As I said, it may involve the release of functional groups that were present during the synthesis in a protected form.

It is also possible in accordance with the invention compounds of formula I 'according to conventional chemical methods, additional functional groups can be introduced or existing functional groups or structural elements can be modified according to conventional methods into other structural elements or functional groups in compounds of the invention.

These methods are techniques well known and described in detail for example in the standard works already mentioned, so for example, in Houben-Weyl, Methods of Organic Chemistry, Thieme-Verlag, Stuttgart, or Organic Reactions, John Wiley & Sons, New York, or in Jerry March, Advanced Organic Chemistry, 4th

Edition, John Wiley & Sons, New York.

If necessary adjustment of the reaction conditions on the reactivity of the compounds of formula I 'to those skilled in preparing any problems.

The following are some coming into consideration types of reactions are exemplified.

A particularly valuable type of reaction for the introduction of radicals R <1> or R <1a> in the preparation of compounds of formula I is an electrophilic aromatic substitution on the furan ring, thiophene ring or pyrrole ring is bonded at the 3-position of the pyrazolopyridine, further in a reaction step after the reaction of the compounds of formulas IV and V is performed.

This can be introduced into the molecule functional group that can be subsequently modified varied.

A particularly convenient and preferred way for making a variety of inventive compounds is from compounds of formula II from the one of the radicals R <1 '> and R <1a'> is hydrogen or alkyl and the other is hydrogen, and in particular both R <1 '> and R <1a'> are hydrogen.

This path leads to the first method described above to give compounds of formula I 'in which one of the radicals R <1'> and R <1a '> is hydrogen or alkyl and the other is hydrogen, and in particular both R < 1 '> and R <1a'> are hydrogen.

In these compounds of formula I ', in other words only after the structure of the pyrazolopyridine system are in this preferred way then by electrophilic aromatic substitution in the furan ring, thiophene ring or pyrrole ring to R <1> and / or R <1a>-standing functional groups inserted, can be modified even subsequently.

Thus, in the formulas II, IV and I 'standing for hydrogen first R <1'> and / or R <1a '> may than the above-mentioned precursors of the subsequently introduced R <1> and / or R <1a be> viewed.

This path leads to a group of compounds according to the invention in which at least one of R <1> and R <1a> is other than hydrogen.

As electrophilic aromatic substitutions are, for example such as the Vilsmeier formylation formylation, acylation, halogenation, for example with N-halosuccinimides, such as the chlorine methylation or Mannich aminoalkylation reaction, for example with formaldehyde and a secondary amine, into consideration.

Such reactions are carried out, the skilled person is well known, is described in detail in the standard works, and it is clear from the examples.

Carbonyl groups in acyl groups or, in particular in aldehyde groups can be introduced for example by electrophilic aromatic substitution in the molecule can be reduced to alcohol groups by standard procedures, for example by reduction with complex hydrides such as sodium borohydride or lithium borohydride in alcohols or ethers.

Furthermore, organometallic compounds, for example Grignard reagents or organolithium compounds to carbonyl groups are added.

For example, as by reduction or by reaction with organometallic compounds, an aldehyde group CHO into the group R <20> CH (OH) are converted.

Reductions and reactions with organometallic compounds can also be stereoselective.

Or hydroxy, or carbonyl compounds, for example, sulfonic acid esters such as mesylates or tosylates by hydroxy can also be reduced to the level of the hydrocarbon.

An alcohol group CH (OH) can also in turn be oxidized to a carbonyl group CO, as R <10> CO is present, for example, in a standing for R <1> group, for example with the aid of dimethyl sulfoxide and an activating agent according to standard methods or by Oppenauer -oxidation.

Hydroxyalkyl compounds can be converted, for example, with inorganic acid halides such as thionyl chloride or thionyl bromide in the halogen compounds, in which in turn exchange reactions can be carried out with alcohols to form ethers, with halides of halogen exchange, or with amines.

To carry out such exchange reactions hydroxyalkyl compounds can be, for example, activated initially by reaction with sulfonyl chlorides such as tosyl chloride or mesyl chloride.

Amino compounds can also be obtained directly from carbonyl compounds.

Hydroxyalkyl compounds can be converted to carboxylic acids in the presence of an acid activating agent or with reactive carboxylic acid derivatives such as acid anhydrides, mixed acid anhydrides or acid chlorides in the esters.

Accordingly aminoalkyl can be acylated to amides in this way.

Hydroxyl groups can still be etherified with alkyl halides and benzyl halides, for example, or the sulfonic esters of alkyl alcohols and benzyl alcohols.

This also applies to hydroxyl groups, R <3> and / or R <5> are for residues in the pyridine ring.

Furthermore, various modifications can be carried out by substituents on phenyl rings.

In all of these reactions are standard reactions that are familiar to the skilled worker and where more details can be found for example in the standard works.

The novel compounds of the formula I effect on the activation of soluble guanylate cyclase (sGC), an increase in the cGMP concentration and are therefore valuable agents for the therapy and prophylaxis of diseases which are associated with a low or decreased cGMP level or by a such cause or to their treatment or prevention of an increase in the existing cGMP level is desired.

The activation of sGC by the compounds of formula I can be investigated, for example, in the activity assay described below.

Diseases and pathological conditions which are associated with a low cGMP level or in which an increase in the cGMP level is desired and can be used for whose therapy and prophylaxis compounds of the formula I are, for example cardiovascular disorders such as endothelial dysfunction , diastolic dysfunction, atherosclerosis, hypertension, stable and unstable angina pectoris, thrombosis, restenosis, myocardial infarction, stroke, congestive heart failure or pulmonary hypertension, or erectile dysfunction, for example, bronchial asthma, chronic renal failure and diabetes.

Compounds of formula I may be also used in the treatment of cirrhosis of the liver and in part due to their synergistic effect with the retrograde messenger substance NO for improving restricted learning ability or memory power.

The compounds of formula I and their physiologically tolerable salts can thus be used as pharmaceuticals on their own, in mixtures with one another or in the form of pharmaceutical preparations in animals, preferably in mammals, and in particular in humans.

The present invention therefore also provides the compounds of formula I and their physiologically tolerable salts for use as pharmaceuticals, their use for the normalization of a disturbed cGMP balance and in particular their use in the therapy and prophylaxis of the abovementioned diseases and to their use for the preparation of of medication for it.

Furthermore, the present invention provides pharmaceutical preparations which contain as active ingredient an effective dose of at least one compound of the formula I and / or a physiologically tolerable salt thereof in addition to customary pharmaceutically innocuous excipients and additives.

The drug can be administered orally, for example in the form of pills, tablets, coated tablets, dragees, granules, hard and soft gelatin capsules, aqueous, are for example in the form of suppositories, alcoholic or oily solutions, syrups, emulsions or suspensions, or rectally .

However, administration can also take place parenterally, for example subcutaneously, intramuscularly or intravenously in the form of injection solutions or infusion solutions.

Further suitable administration forms are, for example, percutaneous or topical administration, for example in the form of ointments, tinctures, sprays or transdermal therapeutic systems, or the inhalative administration in the form of nasal sprays or aerosol mixtures, or, for example, microcapsules, implants or rods.

The preferred form of application depends for example on the disease being treated and its severity.

The pharmaceutical preparations normally contain 0.5 to 90 percent by weight of the compounds of formula I and / or their physiologically tolerable salts.

The pharmaceutical preparations can be effected in known manner.

Given one or more compounds of the formula I and / or their physiologically tolerable salts, together with one or more solid or liquid pharmaceutical excipients and / or auxiliaries and, if desired, or in combination with other active pharmaceutical ingredients with therapeutic or prophylactic action into a suitable administration form .

Brought dosage form which can then be used as pharmaceuticals in human medicine or veterinary medicine.

For example, the production of pills, tablets, coated tablets and hard gelatine capsules can be lactose, starch, for example maize starch, or starch derivatives, talc, stearic acid or its salts, etc..

Excipients for soft gelatin capsules and suppositories are, for example, fats, waxes, semi-solid and liquid polyols, natural or hardened oils, etc.

Suitable excipients for the production of solutions, for example injection solutions, or of emulsions or syrups are, for example, water, physiological saline, alcohols such as ethanol, glycerol, polyols, sucrose, invert sugar, glucose, mannitol, vegetable oils, etc. are

The compounds of formula I and their physiologically tolerable salts can also be lyophilized and the lyophilizates obtained used, for example for the production of injection or infusion preparations.

Suitable excipients for microcapsules, implants or rods are, for example, copolymers of glycolic acid and lactic acid.

The pharmaceutical preparations, in addition to the active ingredients and excipients comprise customary additives, for example fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, dispersants, preservatives, sweeteners, coloring, flavoring or flavoring agents, thickeners, diluents, buffers, and also solvents or solubilizers or agents for achieving a depot effect, salts for varying the osmotic pressure, coating agents or antioxidants.

The dosage of the administered compound of formula I and / or a physiologically acceptable salts thereof depends on the individual case and, as usual, to the individual requirements for optimum effect.

So it depends on the nature and severity of the disease being treated and on the sex, age, weight and individual responsiveness of the treated human or animal, of the potency and duration of action of the compounds used, whether the therapy is acute or chronic or operated prophylaxis is, or whether in addition to compounds of formula I other active compounds are administered.

In general, a daily dose of about 0.01 to 100 mg / kg, preferably 0.1 to 10 mg / kg, in particular 0.3 to 5 mg / kg (in each case mg per kg body weight) when administered to an approximately 75 kg adult weighing appropriate to achieve effective results.

The daily dose can be administered in a single dose or, in particular when larger amounts are administered, into several, for example two, three or four individual doses are split.

In some cases it may be necessary depending on individual behavior to deviate from the stated daily dose up or down.

The pharmaceutical preparations normally contain from 0.2 to 500 mg, preferably 1 to 200 mg of active ingredient of formula I and / or its physiologically tolerable salts per dose.

The compounds of the formula I activate the soluble guanylate cyclase.

Due to this property they can be apart used as pharmaceutically active compounds in human and veterinary medicine as a scientific tool or as a tool for biochemical investigations in which such influence of guanylate cyclase is intended, as well as for diagnostic purposes, for example in the in vitro diagnosis of cell or tissue samples.

Further, the compounds of formula I and their salts, as already mentioned above, serve as intermediates for preparing further pharmaceutical active compounds.

The following examples illustrate the invention without limiting it.

Example 1

5-Amino-1-benzyl-3-(2-furyl)-pyrazole

The mixture of 2.7 g (2-Furoyl) acetonitrile, 3.9 g benzylhydrazine dihydrochloride, 3.3 g of sodium acetate and 50 ml of ethanol was stirred and heated at reflux for 6 hours.

The reaction mixture was cooled and suction filtered, the insoluble fraction.

The filtrate was concentrated in vacuo to give a solid residue remained which was recrystallized from isopropanol and gave a colorless product.

Yield: 2.4 g

Mp: 148 ° C.

Example 2

1-Benzyl-3-(2-furyl) -4,6-dimethyl-pyrnzolo [3,4-b] pyridine

7.2 g of 5-amino-1-benzyl-3-(2-furyl)-pyrazole and 3.4 ml acetylacetone was dissolved in 30 ml glacial acetic acid and this mixture was heated to boiling for 6 hours.

The volatiles were distilled off in vacuo, and this process is repeated after the addition of 30 ml of toluene.

The remaining residue was recrystallized from isopropanol and delivered a finely crystalline, colorless product.

Yield: 6.3 g

Mp: 83 ° C

Example 3

1-Benzyl-3-(5-formyl-2-furyl) -4,6-dimethyl-pyrazolo [3,4-b] pyridine

2.5 g of phosphorus oxychloride was dissolved in 25 ml of anhydrous dimethylformamide (DMF) under ice-cooling for 45 minutes at room temperature, allowed to stand.

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(54) Title: ATRIOPEPTINS, GUANYLATE CYCLASE ACTIVATORS, AND PHOSPHODIESTERASE INHIBITORS AS TREATMENT FOR GLAUCOMA, HYDROCEPHALUS AND CEREBRAL EDEMA (CRANIAL FLUID VOLUME DYSFUNCTION)

(57) Abstract

Method of treating cranial fluid volume dysfunctions such as edema, hydrocephalus, or glaucoma in an individual, comprising administering compounds which effect an increase in cyclic GMP at the site of the dysfunction or at the site of synthesis or removal of the accumulating fluid.

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TITLE: ATRIOPEPTINS, GUANYLATE CYCLASE ACTIVATORS, AND PHOSPHODIESTERASE INHIBITORS AS TREATMENT FOR GLAUCOMA, HYDROCEPHALUS AND CEREBRAL EDEMA (CRANIAL FLUID VOLUME DYSFUNCTION)

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Application Serial No. 006,405, filed January 23, 1987 which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method of treating cranial fluid volume dysfunctions including edema, hydrocephalus and glaucoma.

Description of the Background Art

Because the brain is encased within a rigid skull and lacks a true lymphatic drainage system, it is critically vulnerable to damage from edema. However, compared with peripheral tissues, relatively little is known about intracranial regulation of water and electrolytes. Extracellular fluid movement into and out of the brain occurs primarily at the level of the blood-brain barrier (capillary endothelium), blood-cerebrospinal fluid (CSF) barrier (choroid plexus epithelium), and CSF outflow system (dural sinus/arachnoid villae). Smith et. al., J. Neurochem., 37:117 (1981); Johanson, Encycl. Neurosci., (G. Adelman, Ed.) Birkhauser Boston, in press.

Pathological conditions associated with fluid accumulation in the cranium include cerebral (brain) edema and hydrocephalus, among others. Cerebral edema is a distinct and separate pathological entity from peripheral edema, and may result from a variety of causes such as stroke (including hemorrhage), anoxia, trauma, tumor, or infection. In some cases (e.g., pseudotumor cerebri or Reye's syndrome) the cause is as yet unknown.

The components of the intracranial compartment are brain, cerebrospinal fluid, and blood. Because the skull limits the total intracranial contents, the volume of the compartment is compromised with expanding lesions within the cranial cavity. The brain is virtually incompressible, so the CSF and blood serve as the main buffers of changing intracranial volume. Increases in intracranial pressure may be caused by such diverse pathologic processes as head trauma, cerebral hemorrhage, encephalitis, and brain edema. Increased intracranial pressure may not be harmful in itself, but secondary damage results either as a consequence of precipitiously decreased global cerebral perfusion or herniation of brain tissue. Approximately 50% of patients who die as a result of closed head injury do so because of uncontrolled elevations in intracranial pressure.

The ability of cerebral vasculature to dilate or constrict in response to decreased or increased perfusion pressure, respectively, may also be impaired by head trauma. Occassionally, large-vessel vasospasm may also occur after acute head injury.

Intracranial tumors often cause edema of the surrounding brain tissue. Circulatory slowing and altered permeability of vessels lead to local vasogenic edema of

the brain. The increased brain volume occasioned by the tumor and surrounding edema may raise the pressure in one of the cranial compartments to the point that the brain tissue is displaced into an adjacent compartment. In this way brain herniations occur. Regional edema causes a rapidly evolving impairment of the function of the part of the brain involved.

Pathologic intracranial infections may also lead to intracranial edema. The early reaction to bacterial invasion of the brain includes localized inflammatory necrosis and edema. Persistence or progression of high intracranial pressure may cause deepening coma and threat of herniation.

Hydrocephalus is a condition of increased intracranial pressure caused by obstruction to the movement or impairment of the reabsorption of cerebrospinal fluid (CSF). This can result from congenital defects, infections, cerebral hemorrhage, inflammatory conditions, and other conditions. Cerebrospinal fluid is normally secreted by the choroid plexus, a tissue located in the cerebral ventricles.

Glaucoma is a condition of the eye associated with high pressure due to an impediment in the outflow of the aqueous fluid (aqueous humor), which is normally secreted by the ciliary process (a tissue similar in

function to the choroid plexus). In 90% of cases the cause is unknown, while in 5%, the condition is secondary to some disease process that blocks the outflow channels. Glaucoma occurs in 2% of all patients over 40; it may be asymptomatic and unrecognized before it progresses to rapid vision loss. The normal pressure is about 15 mmHg. Pressures of 20-30 mmHg may damage the optic nerve and lead to blindness.

Prior to the present invention, methods of treating cerebral edema have included administration of urea, mannitol, or cortisone derivatives. These treatments are often inadequate and patients can go on to develop severe neurological sequelae or even death. Even surgical fluid drainage (ventriculostomy) often can not prevent these sequelae. Pharmacological treatment of hydrocephalus has also been quite disappointing. Patients often require a permanent surgical shunt, a procedure which has serious side effects, including infection and subdural hematoma. In the area of glaucoma, some advances have been made with the introduction of beta-adrenergic blockers. However, these agents can have serious pulmonary and cardiovascular side effects, including asthma and congestive heart failure.

Recently, it has been suggested that a group of peptides, released from atrial cardiac myocytes, are key hormones for regulating fluid volume in the periphery. Cantin and Genest, Endocrine Rev., 6:107 (1985); deBold, Science,230: 767 (1985). These peptides are known as atrial natriuretic peptides, atrial natriuretic factors (ANF), or atriopeptins (ANP), and have been isolated from a variety of species, including man. In response to fluid overload, atriopeptins are released into the circulation and cause rapid diuresis and natriuresis through both direct and indirect effects on the kidney. Atriopeptins are also known to induce systemic vasodilation through an endothelial-independent mechanism. Windquist, Life Sci.,37:1081 (1985). Reports of studies in peripheral tissue suggest that the atriopeptin receptor occupancy is associated with the intracellular production of guanosine 3',5'- monophosphate (cyclic GMP). Waldman et. al., J. Biol. Chem., 259:14332 (1984); Winquist et. al., Proc. Natl. Acad. Sci. USA, 81: 7661 (1984). Atriopeptin receptors have been identified by autoradiographic studies in kidney and (peripheral) vasculature as well as in other tissues including brain (hypothalamus and circumventricular organs), pituitary, intestine, adrenal, and ciliary body. Napier et al., Proc. Natl. Acad. Sci.(USA),

81:5946 (1984); Gibson et al., J. Neurosci., 6:2004 (1986).

Many hormones act through "second messengers" such as cyclic AMP (cAMP) or cyclic GMP (cGMP). An accepted model of hormone action involves the binding of a hormone to a hormone-specific cell membrane-bound receptor which activates a hormone-sensitive adenylate (for cAMP) or guanylate (for cGMP) cyclase to a form capable of converting ATP (or GTP) in the cytoplasm of the cell into cAMP (or cGMP). The cAMP (or cGMP) then relays the signal brought by the hormone from the membrane to the interior of the cell. Agonists of the hormone are, by definition, capable of eliciting the same response (see, for example, Nathanson and Greengard, Scientific American, 237:108-119 (1977) for a discussion involving cyclic nucleotides).

Once formed inside cells, cAMP and cGMP are broken down by a group of enzymes called cyclic nucleotide phosphodiesterases (hereafter called phosphodiesterase). Pharmacological inhibition of phosphodiesterase results in prolonged and augmented levels of cAMP and cGMP within cells, and can cause physiological responses similar to those of the original hormone.

SUMMARY OF THE INVENTION

The present invention arose out of the observations by the inventor that blood-brain barrier tissues are end organs for atriopeptins and that atriopeptin receptors in the secretory epithelial cells of the choroid plexus of the brain and the ciliary process of the eye are coupled to the activation of guanylate cyclase activity. Based upon these observations, the inventor hypothesized that compounds which are interactive with the atriopeptin receptors, or other nitrogen-containing guanylate cyclase activators, or compounds which are phosphodiesterase inhibitors, might be useful in the treatment of glaucoma and various conditions associated with pathological intracranial fluid accumulation. Investigation of this hypothesis has led to the present invention which relates to the treatment of fluid volume dysfunctions in the brain and eye (cranial fluid volume dysfunction) with at least one compound selected from atriopeptins, their analogues and agonists, other nitrogen-containing guanylate cyclase activators, and those agents which are inhibitors of phosphodiesterase enzymes (those enzymes which are capable of hydrolyzing cGMP).

Thus, in one embodiment, the present invention provides a method of treating fluid volume dysfunction of the cranium in an individual in need of such treatment which comprises administering a fluid volume decreasing amount of an atriopeptin, an analogue or agonist thereof, other nitrogen-containing guanylate cyclase activators, or an agent capable of inhibiting a phosphodiesterase enzyme which degrades cGMP to said individual.

In addition to drugs which act directly on atriopeptin receptors, the inventor has developed additional pharmacological strategies which involve manipulation of the intracellular second messenger system (cyclic GMP) involved in atriopeptin action. Drugs acting on cyclic GMP (cGMP) have physiological effects similar to those of the atriopeptins. In addition, such drugs can be designed to be well absorbed through the oral or topical routes of administration. This considerably broadens the scope of the approach to treating cranial volume dysfunctions which involve a number of different types of chemical compounds, including for example, nitroglycerine, sodium nitrate, hydralazine, and minoxidil. This group of nitrogen-containing guanylate cyclase activating agents includes the nitro compounds, which stimulate cyclic GMP formation in brain and eye secre-

tory tissues. When administered topically, i.e., as eye drops, at very low concentrations, these guanylate cyclase activators, nitroglycerine, for example, cause a substantial decrease in intraocular pressure without observable side effects on the eye. In addition, the topical administration decreases IOP without decreasing systolic, diastolic, or mean systemic blood pressure and without affecting cardiac pulse rate.

These agents may be synergistic, i.e., the administration in combination of an atriopeptin or atriopeptin agonist and a phosphodiesterase inhibitor or another nitrogen-containing guanylate cyclase activator and atriopeptin, for example, may result in the correlated action of both compounds which, together, have greater total effect that the sum of their individual effects.

Description of the Drawings

Figure 1 demonstrates the effect of rat atrial natriuretic peptide 1-28 (rANP) on membrane bound guanylate cyclase (Figure 1A and Figure 1B) and adenylate cyclase (Figure 1C) activity in various brain barrier tissues and tissue fractions from rabbit compared with activity in cerebrum and cerebellum.

Figure 2 shows the appearance and hormone responsiveness of intact isolated and purified choroid epithelial cells.

Figure 3 shows the inhibitory effect of intraventricular administration of rANP on CSF production rate in a rabbit with chronically implanted lateral ventricular and cisternal catheters. The increase in optical density shown indicates a decrease in the rate of fluid production.

Figure 4 demonstrates atriopeptin-activated guanylate cyclase activity in membrane preparations from rabbit kidney. Values shown are the means ± range for duplicate guanylate cyclase determinations, each assayed by radioimmunoassay (RIA) in triplicate. Control activity was 7.1 pmol/mg protein/min.

Figure 5 demonstrates atriopeptin-activated guanylate cyclase activity in membrane preparations from isolated rabbit ciliary processes. Values shown are the means ± range for duplicate guanylate cyclase determinations, each assayed by radioimmunoassay (RIA) in triplicate. Control activity was 75 ± 10 pmol/mg protein/min.

Figure 6 demonstrates the effect of unilateral intravitreal injection of 0.3 nmoles of rANP 1-28 on intraocular pressure in a group of eight albino rabbits.

The contralateral eye received intravitreal injection of vehicle alone. Filled circles represent the IOP in ipsilateral eye; open circles represents the IOP in contralateral eye.

Figure 7 demonstrates the effect of unilateral intravitreal injection of vehicle (artificial aqueous humor) on IOP in a group of five albino rabbits. Contralateral eyes received nothing. There were no statistically significant differences between ipsilateral and contralateral IOP and no significant changes in IOP following injection.

Figure 8 demonstrates that topical application of inhibitors of PDE cause a decrease in intraocular pressure in the rabbit eye.

A potent PDE inhibitor of the arylxanthine class, 1,3-dibutly-xanthine, was applied unilaterally and topically to rabbit eyes in a 0.5% solution. There was a small decrease in the IOP in the contralateral eye, possibly due to systemic absorption. The IOP was decreased 4 mm (Hg) in the ipsilateral eye.

Figure 9 demonstrates the effect of 1-methyl-3-isobutyl-xanthine on IOP and PDE inhibition in ciliary process.

Figure 10 demonstrates the effect of 1,2,3-propanetriol trinitrate (nitroglycerine) on the activation of guanylate cyclase in the ciliary process of the rabbit.

Figure 11 demonstrates the effect of the administration of nitroglycerine as an eye drop preparation on the IOP in rabbits. The decrease in IOP is not accompanied by a concomitant decrease in systemic blood pressure.

Figure 12 additionally shows the absence of effect on pulse rate or systemic blood pressure from the administration of an eye drop preparation of nitroglycerine in rabbits.

Figure 13 illustrates that doses of nitroglycerine eye drops (50μl of 0.1% aqueous solution) caused no additional decrease in IOP and that lower doses were less effective.

Figure 14 illustrates the effect of the administration of a nitroglycerine eye drop preparation (50μl of 0.1% aqueous solution in one eye) in a volunteer human. Importantly, although the nitroglycerine eye drops decreased IOP, they did not affect systemic blood pressure.

Figure 15 demonstrates that a 50μl 0.1% aqueous eye drop solution of minoxidil, a nitrogen-containing

guanylate cyclase activator, decreases the IOP in rabbits.

Figure 16 illustrates that a 50µl 0.1% aqueous eye drop solution of sodium nitrate, another nitrogen-containing guanylate cyclase activator, lowers the IOP in rabbits.

Figure 17 demonstrates that a 0.1% eye drop preparation of hydralazine decreases the IOP in rabbits.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The method of the present invention is useful for treatment of cranial fluid volume dysfunction of any origin in an individual. By the term "cranial fluid volume dysfunction" is intended those pathological conditions associated with an overproduction or decreased rate of removal of fluid from the cranium including the eye.

Typical cranial fluid volume dysfunctions include, but are not limited to, brain edema, hydrocephalus, and glaucoma.

The term "individual" is intended to include any animal, preferably a mammal, and most preferably, a human.

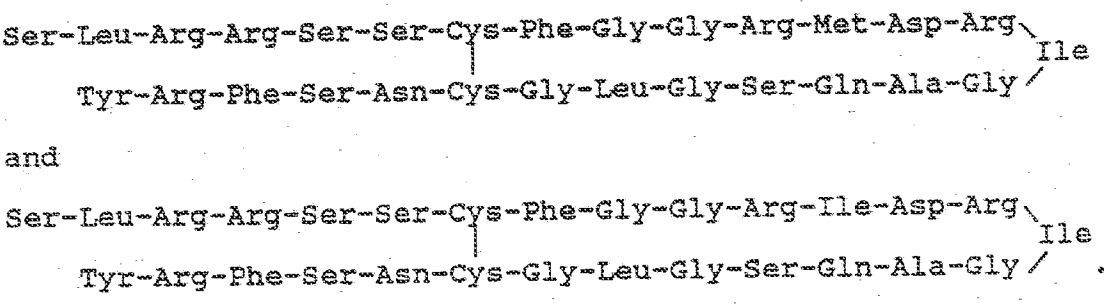
Compounds useful in the practice of the present invention include those compounds which act directly on

the atriopeptin receptors of the brain (including membranes, blood vessels, choroid plexus and CSF reabsorptive areas), spinal cord, and ciliary process and trabecular meshwork of the eye to activate guanylate cyclase (hereinafter atriopeptin-sensitive guanylate cyclase activators) and further include those compounds which act through the mechanism of inhibition of phosphodiesterase activity relating to degradation of cGMP.

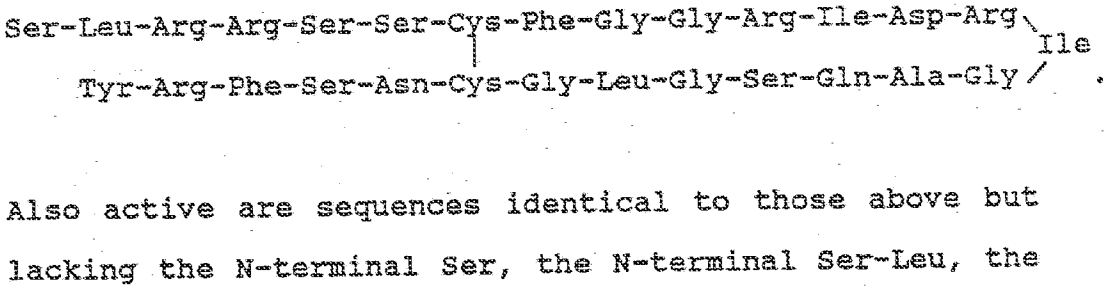
The compounds useful in the present invention which act directly upon the atriopeptin receptor sites to activate guanylate cyclase include, but are not limited to, atriopeptins, atriopeptin analogues, and atriopeptin agonists. In addition, nitrogen-containing guanylate cyclase activators have been discovered to be useful in the present invention.

The term atriopeptin is meant to include atrial natriuretic factors and their precursor polypeptides, as well as peptides having atriopeptin activity, regardless of the source. Atriopeptin precursors are polypeptides which include an atriopeptin amino acid sequence within a longer sequence of amino acids which precursors may or may not exhibit atriopeptin activity in vitro. Typical atriopeptins include naturally occurring as well as synthetic atriopeptins and active fragments thereof.

Among the atrial natriuretic factors which may be used in the present invention are peptides or their precursors, with amino acid sequences such as the following naturally occurring sequences isolated from the human and rat, respectively:



and



Also active are sequences identical to those above but lacking the N-terminal Ser, the N-terminal Ser-Leu, the N-terminal Ser-Leu-Arg, the N-terminal Ser-Leu-Arg-Arg, or the C-term Tyr. Also active are longer sequences such as those above additionally consisting of Arg-Pro-Gly-Ala at the N-terminal end.

Typical analogues of atriopeptin useful in the present invention include, but are not limited to peptides which may mimic the naturally occuring atriopeptins or vary by one or more amino acids and which demonstrate biological activity substantially similar to that of atriopeptins, such as

Tyr-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-Asp-Arg
                    | \Ile
Arg-Phe-Ser-Asn-Cys-Gly-Leu-Gly-Ser-Gln-Ala-Gly/

Typical atriopeptin agonists are compounds which demonstrate biological activity substantially similar to that of atriopeptins. Such atriopeptin agonists include compounds which bind to the atriopeptin receptor as well as compounds which activate the atriopeptin-sensitive guanylate cyclase. Typical atriopeptin agonists would include, but are not limited to, compounds such as nonpeptide analogues interacting with the receptor, and nitro compounds such as sodium azide, sodium nitrite, or nitroglycerine, which directly interact with guanylate cyclase.

In addition, nitrogen-containing guanylate cyclase activators, including inorganic nitrates like sodium nitrate, organic nitrates such as nitroglycerine and pentaerythritol tetranitrate, and non-nitrate nitrogen-containing compounds such as hydralazine and minoxidil are also useful in the present invention.

A suitable screening method for determining whether a given compound is an atriopeptin agonist or analogue comprises measuring guanylate cyclase activity of the atriopeptin-sensitive guanylate cyclase in broken cell preparations of choroid plexus (or epithelial cells) by a modification of the technique of Waldman et. al..

Said modified technique is described in detail in Example 1.

Generally, the broken cell preparations are prepared according to the method described in a paper by Nathanson et al., (Molecular Pharmacology 20:68 (1981), which is herein incorporated by reference. Choroid epithelial cells are prepared as described in Example 1. To measure activity of atriopeptins, analogues, or agonists thereof, washed particulate cell preparations are prepared by using the pellet obtained following high speed centrifugation. To measure direct activators of guanylate cyclase, the crude broken-cell preparation without centrifugation is used. Guanylate cyclase activity is measured in appropriate buffer-containing GTP, cofactors, tissue fraction, and the compound to be tested. If necessary, the compounds to be tested are initially solubilized and appropriate solvent controls are run in parallel. The enzyme reaction is initiated by addition of GTP, stopped by heating, and centrifuged. Cyclic GMP can be measured by any test which indicates the presence thereof, typically by the radioimmunoassay as described in Example 6. Normally, the solution mixture contains a phosphodiesterase inhibitor such as theophylline, so as to provide linear measurements with respect to time and enzyme concentration. The deter-

mination of the constant K_a, which is the concentration of agonist necessary for half-maximal activation of cyclase activity, is carried out by measuring cyclase activity in the preparation, and plotting the activity (above control activity) versus the semilogarithm of the particular agonist concentration. This is done for a series of increasing concentrations until maximal activity (V_{max}) is reached. K_a^B, where B is the test compound, is compared with the constant (K_a^{ANF}) determined in an analogous way using rat atrial natriuretic factor (rANF) as a standard. The ratio K_a^{ANF}/K_a^B is then an indication of whether the compound (B) is better (ratio greater than 1) or worse (ratio smaller than 1) than rANF. Maximal activation of enzyme activity as a percentage of maximal activation seen in the presence of rANF can be denoted as % V_{max}.

Typical compounds capable of inhibiting a phosphodiesterase enzyme useful in the method of the present invention include those compounds which prevent or greatly decrease the hydrolysis of endogenous cGMP produced by activation of guanylate cyclase. The inhibition of cGMP phosphodiesterase may be either through a competitive or non-competitive mode. Further, the cGMP phosphodiesterase inhibited should be that found in the particular cranial tissue of the species

being treated. Thus, the testing of any particular PDE inhibitor can be carried out on PDE isolated from or found in choroid plexus (or epithelial cells) or ciliary process.

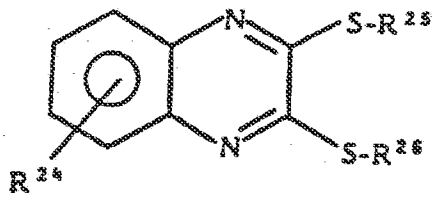
The ability of any compound to inhibit cGMP phosphodiesterase (PDE) activity in broken-cell preparations of choroid epithelial cells or ciliary process can be determined either 1) by measuring the decrease in rate of hydrolysis of an added amount of cyclic GMP by PDE (see, Methods Section of Nathanson et al., Mol. Pharmacol. 12:390-398 (1975)), or 2) by measuring the rate of accumulation of one of the breakdown products of cyclic GMP, such as 5'-GMP or guanosine (see method of Filburn et al., Anal. Biochem. 52:505-516 (1973)). Both of these are herein incorporated by reference.

Generally, any compound capable of maximally inhibiting PDE activity by at least 50% (V_{max}-inhibition) and preferably by at least 80% is preferred. Also, in terms of the concentration of the compound required for such inhibition, this can be quantitated by determining the IC_{50}-inhibition, i.e., the concentration of the compound required to cause 50% of the maximal inhibition caused by the compound at any concentration. Generally, any compound with an IC_{50}-inhibition for PDE

of less than 10mM and preferably less than 2.5mM is preferred.

Of particular interest are purine derivatives, such as theophylline, xanthine, methylxanthine, isobutylmethylxanthine (IBMX), and lower alkyl or substitution homologues or analogues thereof. See, e.g. Kramer, et al., Biochem., 16:3316 (1977); Garst et al., J. Med. Chem., 19:499 (1976); Amer et al., J. Pharm. Sci., 64:1 (1975); or Beavo et al., Mol. Pharm., 6:597 (1970). For the purposes of this invention, halide, hydroxy, keto, lower alkoxy, lower straight alkyl, lower branched alkyl, amino, lower alkylamino, lower halo alkyl, fluorine, chlorine, bromine, iodo, azido, nitro, mercapto, alkene-oxy, cyano, alkyl-cyano, phenyl, benzyl, substituted benzyl, or the like substituents on any of the aforementioned compounds are equivalent if they do not substantially block the agonistic activity of the atriopeptinergic agonist.

Of interest are also the phosphodiesterase inhibitors described by Rojakovick et al., (Pesticide Biochemistry and Physiology, 610-19 (1976)) which belong to the family of quinoxaline dithiols. These compounds, denoted as oxythioquinox, SAS 2185, 1948, 2501, 2061, 2551 or 2079, are those of the formula:



where R^{24} can be hydrogen, lower alkyl, lower alkoxy or trifluoromethyl, R^{25} and R^{26} can be the same or different and selected from the group of H, $COOR^{27}$, where R^{27} is lower alkyl; or both R^{25} and R^{26} taken together may form a group of the formula -CO-, bridging both S atoms.

Another family of PDE inhibitors are the benzylisoquinoline derivatives, such as papaverine (See, for example, U.S. Patent 3,978,213 to Lapinet et al., which relates to the cosmetic use of mixtures of cyclic AMP and phosphodiesterase inhibitors; or Amer et al., supra).

Another family of PDE inhibitors are the substituted pyrrolidones, such as 4-(3-cyclopentyloxy-4-methylphenyl)-2-pyrrolidine (ZK62711). See Schwabe et al., Mol. Pharmacol. 12:900-910 (1976).

Another family of PDE inhibitors are the 4-(3,4-dialkoxybenzyl)-2-imidazolidinones, such as (4-(3-

butoxy-4-methoxbenzyl)-2-imidazolidinone (Ro 20-1724). See Sheppard et al., Biochem. J. 120:20P (1970).

Another family of PDE inhibitors are the benzodiazepine derivatives, such as diazepam. See Dalton et al., Proc. Soc. Exp. Bio. Med. 145:407-10 (1974).

Another family of PDE inhibitors are the tricyclic agents, such as the phenothiazines. See Honda et al., Biochem. Biophys. Acta 161:267 (1968).

Another family are various purine-ribose derivatives, including puromycin and derivatives of cyclic nuclectides (other than cyclic AMP or active cyclic AMP analogues). See Amer et al., J. Pharm. Sci. 64:1-37 (1975) Table VI.

Another PDE inhibitor is SQ20009: (1-ethyl-4-isopropylidene-hydrazino-14-pyrazolo(3,4)pyridine-5-carboxylate ethyl ester. See Beer et al., Science 176:428 (1972).

Other PDE inhibitors include M&B22948, dilazep, MY5445 and OPC3689. See, Hidaka et al., TIPS, pp237-239 (June 1984) and Bergstrand et al., Molec. Pharmacol., 13:38-43 (1977).

In general, any compound which inhibits PDE as described above and which, at the same concentration, does not substantially block the activity of the guanylate cyclase activator or atriopeptin agonist in

stimulating guanylate cyclase or atriopeptin-sensitive guanylate cyclase (as measured above) and is non-toxic to the individual to be treated, can be used.

The PDE inhibitor may be present alone or in combination with other active or non-active compounds.

The molecular inhibition of PDE in vitro by a PDE inhibitor correlates with the molecular inhibition of the enzyme in vivo. However, it may be that a compound which is an excellent PDE inhibitor in vitro does not show good in vivo activity. Other factors, such as possible metabolism, transport or absorption of the compound may influence its overall effectiveness. One of skill in the art, however, can by a simple preliminary trial measuring intracranial pressure or intraocular pressure, ascertain quite quickly and routinely whether a chosen agent is useful in vivo.

Administration of the compounds useful in the method of the present invention may be by topical, parenteral, oral, intranasal, eye drop, intravenous, intramuscular, subcutaneous, or any other suitable means. The dosage administered may be dependent upon the age, weight, kind of concurrent treatment, if any, and nature of the cranial fluid dysfunction. The compounds useful in the method of the present invention may be employed in such forms as eye drops for topical

administration, capsules, liquid solutions, suspensions, or elixirs, for oral administration, or sterile liquid forms such as solutions or suspensions for parenteral administration. Any inert carrier is preferably used, such as saline, or phosphate-buffered saline, or any such carrier in which the compounds used in the method of the present invention have suitable solubility properties for use in the method of the present invention.

Typical dosages will vary with the potency of the drugs for activating guanylate cyclase or inhibiting PDE. For eye drop preparations of guanylate cyclase activators or PDE inhibitors, 0.01%-2.0% (gm/100 ml) is typical. For oral organic nitrate guanylate cyclase activators, doses are typically 0.1-30 mg. For oral PDE inhibitors, doses are typically 0.1-300 mg. For i.v. organic nitrate guanylate cyclase activators, doses are typically 0.1-50 μg/min. For atriopeptin analogues, doses vary from 0.1 μg-1.0 mg.

Having now generally described the invention, the same may be further understood by reference to the following examples, which are not intended to be limiting unless so expressly stated.

EXAMPLE 1

The effect of rANP on membrane-bound guanylate cyclase and adenylate cyclase activity in various brain-barrier tissues and tissue fractions from rabbit was compared with its activity in cerebrum and cerebellum. Guanylate cyclase was measured as the rate of cyclic GMP formation by modifications of the technique of Waldman et al., supra, incorporated herein by reference. Briefly, tissues were homogenized (10 mg wet weight per milliliter) in 50 mM tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, and 250 mM sucrose and centrifuged at 100,000g to obtain a P1 pellet. Reaction tubes contained (in 0.3 ml), 50 mM tris, pH 7.6, 6 mM $MnCl_2$, 0.5 mM 3-isobutyl, 1-methylxanthine (IBMX), 10 mM theophylline, 3 mM GTP, hormone (in 0.03 ml containing 2.5 mM ascorbic acid and 0.1% bovine serum albumin), and 0.06 ml of P1 fraction (40 to 60 ug of protein). The reaction (4 minutes at 30°C) was started by addition of GTP and terminated by addition of 0.3 ml of 150 mM sodium acetate (pH 4.0) and boiling for 3 minutes. Cyclic GMP formed was subsequently measured by radioimmunoassay as described in example 6, infra. Under these conditions, guanylate cyclase activity was linear with time and tissue concentration. Adenylate cyclase activity in P1 fractions was measured according to the

method described by Nathanson, et al., Science; 204:843 (1979); Mol. Pharmacol. 18:199 (1980), see Figure 1C . Figure 1B demonstrates choroid epithelial cells isolated according to the method described by Gabuzola, et al., Soc. Neurosci. Abstr., 10, 899 (1984); Nathanson, et al., Soc. Neurosci. Abstr., 12, 1257 (1986), incorporated herein by reference, but with a lower concentration (0.025%) of trypsin and a rotating tissue tumbler which allowed cleaner fractionation of epithelial cells from erythrocytes and vascular components. In Figure 1A large stimulations were observed in fractions highly enriched in intraparenchymal cerebral microvessels compared with little or no stimulation in ventral portion of dural sinus (containing arachnoid villi), in pia arachnoid membrane, and in cerebrum. Membrane fractions from cerebellum showed a moderate amount of stimulation. The presence of atriopeptin-activated guanylate cyclase in cerebral microvessels suggests a role for atriopeptins in regulating cerebral water since these microvessels are the site of the blood-brain barrier.

Figure 1B shows that rat ANP also activated guanylate cyclase in membrane fractions from whole choroid plexus (middle curve). Fractionation of choroid indicated that purified choroid epithelium (site of the

blood-CSF barrier) contained most of the rANP receptors, with few present in choroid stoma which contains vascular elements. Figure 1C demonstrates that the rat ANP stimulation in whole choroid was selective for guanylate cyclase and not adenylate cyclase, causing a small inhibition of the latter enzyme. Adenylate cyclase activity could be stimulated by isoproterenol through β-adrenergic receptors known to be present in this tissue. Values shown are the mean $\pm$ range for duplicate enzyme determinations, each assayed for cyclic AMP or cyclic GMP in triplicate. For the results shown in Fig. 1, highly enriched suspensions of choroid epithelial cells from intact choroid were prepared and the identity of the cells was confirmed through immunohistochemical studies showing the presence (>95%) of morphologically typical epithelial cells strongly stained with a polyclonal antibody to the peripheral form of Na- and K-activated adenosine triphosphatase (Na, K-ATPase) Sweadner, et al., J. Biol. Chem., 260:9016 (1985), which only faintly labels endothelial and stromal cells, and with antibody to the protein, DARPP-32, which solely labels epithelial cells (Fig. 2A-2C). Nestler et al., Science, 225:1357 (1984). When isolated as described hereinabove, the choroid epithelium is a nearly homogenous population of cells that can

be identified by cell markers and sustained in short-term culture. Stimulation by rANP was selective for guanylate cyclase and did not activate adenylate cyclase in the same brain-barrier preparations, although adenylate cyclase was stimulated by isoproterenol (Fig. 1C). As shown in Fig. 1C, high concentrations of rANP caused a small inhibition of adenylate cyclase. Stimulation of guanylate cyclase in barrier tissues was also selective for the intact 28-amino acid peptide. Thus, in choroid plexus, rANP fragment 1-11 was about 10% to 15% as active as the intact peptide and rANP fragment 13-28 caused almost no stimulation of guanylate cyclase, a pattern of activity nearly identical to that found in rabbit kidney. For rANP 1-28, the range of K_a values (0.5 to 10 nM) that was observed for guanylate cyclase activation in cerebral microvessels and choroid plexus was similar to the range of binding affinities (0.1 To 2 nM) reported in other tissues for radiolabeled atriopeptins. Figure 1a shows that rat atrial natriuretic peptide (rANP) 1-28 [Ser-Leu-Arg-Arg-atriopeptin III (APIII)] was a potent activator of guanylate cyclase activity in purified rabbit cerebral microvessels [maximum velocity of enzyme activity (V_{max}), 215% of control; activation constant (K_a), 0.5 nM]. From other studies, it is known that such microvessels consist of a

high percentage of endothelial cell-containing cerebral capillaries. A similar degree of activation of guanylate cyclase by rANP was observed in microvessels prepared from pig brain.

Figure 1B (middle curve) shows that rANP also stimulated enzyme activity in membrane fractions prepared from whole rabbit choroid plexus obtained from lateral, third, and fourth ventricles. In five separate experiments, the K_a for activation of the enzyme in the choroid (9.8 ± 5.1 nM; SEM) was similar to that (K_a = 5 ± 2 nM; SEM, N = 4) observed in rabbit kidney, a tissue known to be rich in atriopeptin receptors, and somewhat greater than that observed for atriopeptin stimulation in the rabbit cerebellum (Fig. 1A), a tissue known to be enriched (relative to other brain areas) in cyclic GMP. The V_{max} for stimulation of basal activity in the choroid plexus averaged 58% ± 16% (SEM, N = 5).

However, no stimulation of guanylate cyclase activity in membrane fractions prepared from rabbit or pig cerebral cortex occurred (Fig. 1A). In membranes prepared from the ventral portion of pig or rabbit superior sagittal dural sinus, which contains arachnoid villi, rANP caused a small stimulation of guanylate cyclase but only at high concentrations (K_a > 1000 nM). There was also a very small (15%) degree of enzyme

stimulation in the pia-arachnoid, a preparation consisting of both pia-arachnoid membrane and small extraparenchymal cerebral arterioles and veins.

EXAMPLE 2

Purified secretory epithelial cells were isolated and maintained in tissue culture medium in the absence of any hormones. After 3 hours, rANP was added either alone or in the presence of phosphodiesterase (PDE) inhibitors to a suspension of choroid epitheliel cells incubated at 37°C in artificial CSF. Figure 2A demonstrates the appearance by phase contrast microscopy of a small group of epithelial cells after isolation and purification. Figure 2B shows the same cells as in figure 2A which have been stained with a rabbit polyclonal antibody (1:150 dilution) to the alpha form of Na,K-ATPase, Sweadner et al., J. Biol.Chem., 260:9016 (1985) followed by second antibody (1:100 dilution). Plasma membrane fluorescence (see also figure 2E) was characteristic of choroid epithelium as demonstrated with rhodamine optics. Figure 2C shows epithelial cells which were also immunstained with mouse monoclonal antibody to DARPP-32, Nestler et al., Science, 225:1357 (1984), followed by second antibody (1:100 dilution). More diffuse staining was found only in epithelium and

not in vascular or stromal components of choroid epithelial cells as determined by fluorescein optics. Figure 2D shows a suspension of choroid epithelial cells incubated at 37°C in artificial CSF. After exposure for 5 minutes to 1 uM rANP, the cells showed a marked increase in intracellular cyclic GMP content, an effect that was potentiated by the phosphodiesterase inhibitors theophylline (10 mM) and IBMX (0.5 mM). The synergistic effect of phosphodiesterase inhibitors and rANF is demonstrated in Fig. 2D where the cGMP accumulation after treatment with the combination is greater than would be expected from the summation of the effects of phosphodiesterase inhibitors and rANF added individually. For one experiment, the mean ± range is shown for duplicate determinations, each assayed in triplicate for cyclic GMP content. In four separate experiments, the degree of stimulation by rANP alone varied from 290% to 1460%. Figure 2E demonstrates another group of epithelial cells showing bright plasma membrane immunostaining of Na,K-ATPase. Figure 2F shows the same cells immunostained with antibody to DARPP-32. Fewer than 5% of cells were DARPP-negative.

After exposure for 5 minutes to 1 uM rANP the cells showed a marked increase in intracellular cyclic GMP content, an effect that was potentiated by the phos-

phodiesterase inhibitors theophylline (10 mM) and IBMX (0.5 mM). For one experiment, the mean ± range is shown for duplicate determinations, each assayed in triplicate for cyclic GMP content. In four separate experiments, the degree of stimulation by rANP alone varied from 290% to 1460%. After the cells were incubated for 5 minutes, they were killed and their cyclic GMP content was determined. The isolated epithelial cells showed more than an eightfold increase in cyclic GMP content when incubated with rANP alone (Fig. 2D). Basal cyclic GMP content was increased by the PDE inhibitors, and the combination of rANP and PDE inhibitors caused more than 30-fold increase in cyclic GMP content. (Similar but somewhat larger increases were seen after 15 minutes of incubation.) These marked increases in cyclic GMP provide further evidence that the choroid epithelium is an atriopeptin end organ.

EXAMPLE 3

The effects of rANP on CSF production measured by ventricular-cisternal perfusion in living rabbits demonstrated that atriopeptins affect the secretory function of these cells, which produce CSF as shown on Fig. 3. Drug was given intraventricularly, either by bolus injection or by continuous addition (during a 10-minute

period) to the CSF perfusion system. Ventricular-cisternal perfusion of Blue dextran was carried out by a closed system with active pumping both into and out of the catheters at a rate of 30 l/min. Output dye concentration was monitored continuously with an in-line photocell; and a separate, contralateral venticular catheter independently measured intracranial pressure. Positive responses were observed in 13 of 14 experiments, in 3 of which rANP (30 pmol) was given by bolus intraventricular injection and in 10 of which rANP was given by continuous intraventricular infusion at 3 to 30 pmol/min for 10 minutes. Drug was administered in an artificial CSF containing 0.01% rabbit serum albumin (RSA) and 0.25 mM ascorbate. In addition, all plastic surfaces were pretreated with 0.1% RSA to reduce peptide loss by adsorption.Out of 14 rabbits in which CSF production could be adequately assessed throughout the experiment, 13 showed a decrease in the rate of CSF production. In one animal, there was no change in CSF secretion rate, and in no case was there an increase in CSF production. Figure 3 shows a positive response to a bolus injection of 30 pmol of rANP into the lateral ventricle. During a period of 100 minutes, secretion rate dropped by 70%. In the 13 positive responses, the mean decrease observed was 35.3% $\pm$ 6.9% (SEM) from a

starting basal CSF secretion of 8.3 ± 0.8 l/min. In other experiments, intravenous injection of similar doses of rANP caused no change in peripheral blood pressure, suggesting that the decreased CSF production rate observed was not due to a systemic effect on vascular perfusion pressure.

EXAMPLE 4

Ciliary process tips were obtained from male, 3-4 kg, New Zealand white rabbits, immediately following sacrifice with an overdose of ether, as described in Nathanson, Invest. Ophthal. Vis. Sci., 21:7981 (1982). The tissue was minced and homogenized (10 mg/ml) by hand in a glass-glass homogenizer in a buffer consisting of 50 mM Tris HCl, pH 8.0; 1mM EDTA, and 250mM sucrose. The homogenate was diluted 30-fold with buffer and centrifuged at 100,000 x g, to obtain a P1 fraction. The pellet was rehomogenized in the original volume and left on ice until use. P1 fractions were also prepared from wedges of rabbit kidney taken from the same animals. In some experiments, P1 fractions were prepared from iris and from ciliary body, as described in Nathanson, Proc. Natl. Acad. Sci. USA, 77:7420 (1980). Briefly, iris was removed from its attachment to the ciliary body, cleaned of adhering ciliary process

tissue, washed, and homogenized as above. The ciliary muscle was cleaned of most remaining ciliary process tissue, freed from the underlying sclera by blunt and sharp dissection, and then washed and homogenized.

EXAMPLE 5

Atriopeptin-activated guanylate cyclase activity was measured as the rate of conversion of GTP to cyclic GMP. For this assay, reaction tubes were prepared to contain (in 0.3 ml final volume): 50mM Tris HCl, pH 7.6; 6mM $MnCl_2$; 1mM 3-isobutyl-1-methylxanthine (IBMX); 10mM theophylline; 3mM GTP; hormone (in 0.03ml containing 2.5mM ascorbic acid and 0.1% rabbit serum albumin); and tissue P1 fraction (0.06ml). The reaction was started by the addition of GTP. Tubes were incubated for 4 min at 30°C, and the reaction stopped by addition of 0.3ml of 150mM sodium acetate (pH 4.0), followed by boiling for 3 min. Under these conditions, guanylate cyclase activity was linear with respect to time and tissue concentration.

EXAMPLE 6

The cyclic GMP was typically measured by radioimmunoassay (RIA) using a polyclonal antibody (NEX137, New England Nuclear Corp.). This polyclonal antibody has

both a great deal of sensitivity (ED_{50} of 25 fmoles at final dilution) as well as selectivity (less than 0.03% crossover with cyclic AMP). This degree of selectivity for cyclic GMP, coupled with the finding that the specific activity of ciliary process guanylate cyclase is quite high (Table 1), indicated that there was no significant cross-contamination of guanylate cyclase activity with adenylate cyclase activity.

For RIA, each tube contained (in 0.5ml), 50mM sodium acetate buffer (pH 6.2), 2mM EDTA, 1mg/ml bovine serum albumin, 0.1 ml of sample (or diluted sample), 8,000-15,000 cpm (about 5 fmole) of ^{125}I succinyl cyclic GMP tyrosine methyl ester, and antibody to bind 25-35% of the label. Prior to the addition of label and antibody, sample tubes underwent an acetylation reaction with acetic anhydride and triethylamine, according to the method of Harper and Brooker, J. Cyclic Nucl. Res., 1:207 (1975) in order to increase the sensitivity of the assay. (Acetyl-cyclic GMP has a greater affinity for the antibody than cyclic GMP). After addition of label and antibody, the mixture was incubated at 4°C for 24-48 hours, then the binding reaction terminated by addition of 0.1ml of a mixture of 1gm/10ml of Norit XG charcoal in 50 mM sodium acetate buffer (pH 6.2) containing 0.2g/10ml of bovine serum albumin. (The charcoal-BSA-

buffer were pre-mixed for 1 hr prior to addition). The mixture was vortexed, allowed to stand for 10 min at 4°C, then centrifuged at 2000 x g for 30 minutes and an aliquot of the supernatant taken for measurement of bound cyclic GMP. Using these conditions, the assay was sensitive over the range of 2.5 to 500 femtomoles. Activation constants (K_a) were determined from dose-response curves utilizing 12-14 data points (6-7 concentrations of hormone, in duplicate), each point representing the mean of triplicate RIA determinations.

EXAMPLE 7

The measurement of intraocular pressure (IOP), was performed using procedures similar to those described in Nathanson, Brit. J. Pharmacol., 73:97 (1981) and Nathanson, Current Eye Res., 4:191 (1985). Briefly, male (3-5 kg), New Zealand white rabbits were housed under standard conditions and exposed to a 12 hr light-dark cycle. IOP was measured with a Perkins applanation tonometer after topical anesthesia with 0.4% benoxinate (and 0.25% sodium fluorescein). The tonometer had been calibrated previously by connecting the anterior chamber of enucleated rabbit eyes to a manometer (and reservoir) and taking tonometer readings at different pressures. A number of preliminary IOP readings were made in order to

accommodate the animals to the measurement procedure. Readings were then taken just prior to drug or vehicle injection and at 3, 9, 20, 27, 44, 51, 72, and 144 hours post-injection.

Following topical anesthesia with 0.4% benoxinate, rabbits received rat atrial natriuretic peptide 1-28 (rANP) via a 10 microliter, intravitreal injection, through a 30g 1/2" needle, the tip of which was positioned at the approximate center of the eye. Drug was dissolved in a solution of artificial aqueous humor containing (in mmol/liter): NaCl 130, KCl 2.7, NaH_2CO_3 18.3, $MgCl_2$ 1.33, $CaCl_2$ 1.5, Glucose 10, HEPES 20, and L-ascorbic acid, 2.5. The contralateral eye received a 10 microliter injection of vehicle alone. In order to evaluate the possible effects of intravitreal injection, per se, on IOP, additional rabbits received a 10 microliter intravitreal injection of vehicle in one eye only.

EXAMPLE 8

Atriopeptin-activated guanylate cyclase activity was measured, as described in Example 5, in membranes from rabbit kidney and ciliary process prepared as in Example 4.

In particulate fractions of rabbit kidney, guanylate cyclase activity was present at high specific activity and could be activated by rANP at low concentrations. In four separate experiments, the K_a for hormone activation ranged from 1 to 9 x 10^{-9}M, with a V_{max} which varied from 63 to 151% stimulation of basal activity. Figure 4 shows a typical response. Values shown in figure 4 are the means ± range for duplicate guanylate cyclase determinations, each assayed by radioimmunoassay (RIA) in triplicate. Control activity was 7.1 pmol/mg protein/min.

In the rabbit ciliary process, guanylate cyclase in P1 fractions was extremely active, with basal levels (49.4 ± 12.7 pmol/mg protein/min; ±SEM, N=4 separate experiments) considerably exceeding that found in kidney (10.3 ± 2.5 pmol/mg protein/min; N=4). This high concentration of guanylate cyclase activity also exceeded that (20.4 ± 4.3; N=5) which previously reported for choroid plexus and for other atriopeptin receptor tissues.

In addition, at very low concentrations, rANP caused significant stimulation of basal guanylate cyclase activity in ciliary process (Figure 5). In four separate experiments, the K_a for hormone stimulation of the enzyme ranged from 0.4 to 4 x 10^{-9}M, with a V_{max}

which varied from 24 to 337% stimulation of basal activity. This range of activation is similar, also, to the values obtained from studies of the vasorelaxant properties of atriopeptins in rabbit aorta, where the EC_{50} for Arg-Arg-APIII has been reported to range from 1 x 10^{-10} to 1 x 10^{-9} M.

Table 1 shows that activation of ciliary process guanylate cyclase by rANP was selective for the complete peptide; rANP fragment 13-28 showed partial activity and rANP fragment 1-11 was almost devoid of activity.

Table 1: Stimulation of Guanylate Cyclase Activity By Intact rANP 1-28 and By Atriopeptin Fragmants 1-11 and 13-28

	Vmax Stimulation (%) of Guanylate Cyclase	
Peptide	Ciliary Process	Kidney
rANP 1-11	4 ± 6	25 ± 6
rANP 13-28	51 ± 5	54 ± 4
rANP 1-28	124 ± 5	129 ± 25

A complete dose response curve was run for each peptide. The V_{max} values shown are the mean ± range of duplicate determinations, each assayed by RIA in triplicate .

In tissue distribution studies, the greatest hormone-stimulated activity was in the isolated ciliary

processes. Ciliary body and iris showed significantly less rANP-stimulated activity.

This distribution of activity is similar to that reported for beta$_2$-adrenergic receptors associated with the activation of adenylate cyclase.

EXAMPLE 9

This example demonstrates the effects of exogenous rANP on intraocular pressure. Figure 6 shows the effects of unilateral intravitreal injection of 0.3 nmoles of rANP (1-28) on intraocular pressure in a group of eight albino rabbits. The contralateral eye received intravitreal injection of vehicle alone. Over a period of 9 hours, the ipsilateral eyes underwent a marked decline in pressure of about 5mm (Hg). This degree of hypotension persisted until about 48 hours post-injection and then underwent a slow recovery over the following four days. The decrease in pressure, compared with pre-injection IOP, was statistically significant at all time points from 3 to 72 hours post-injection. Values shown are mean ± SEM. Filled circles represent the IOP in the ipsilateral eye; open circles represent the IOP in the contralateral eye. *($p < .05$); **($p < .01$), for paired Students T-test relative to preinjection pressure (time 0). The IOP at 3, 9, 20, 27, 44, and

51 hours was also significantly decreased relative to the pressure present at the same time in the contralateral, vehicle-injected eyes. During the entire period, no affect on pupillary diameter of either the ipsilateral or contralateral eye was noted.

Following injection of rANP in the ipsilateral eye, IOP in the contralateral, vehicle-injected eye also showed a decrease, although it was smaller, of somewhat slower onset, and recovered by 72 hours. This contralateral decrease was statistically significant ($p < .05$ vs. pre-injection IOP) at 20 and 44 hours following injection. The dose of rANP which caused a marked and prolonged decrease in ipsilateral IOP, if distributed uniformly within a sphere the size of a rabbit eye, would yield a concentration ($2-3 \times 10^{-7}$M) similar to that which caused maximal stimulation of ciliary process guanylate cyclase activity (Fig.5).

EXAMPLE 10

A separate group of 5 rabbits received a unilateral intravitreal injection of vehicle alone in order to evaluate the possible effects of intravitreal injection, per se, on IOP. Figure 7 shows that there was no significant decrease in IOP, relative to starting IOP, at any of the time points observed, in either the

ipsilateral (injected) or contralateral (non-injected) eye; nor, at a given time point, was there any significant difference between ipsilateral and contralateral eyes. At 9 hours (the only measurement made during the normal dark cycle of the rabbits) there was a small, non-significant increase in IOP. Also, on a given day, afternoon measurements of IOP tended to be somewhat higher than morning measurements. These small daily changes may have been due to a circadian effect.

EXAMPLE 11

Inhibitors of PDE also cause a decrease in intraoccular pressure in rabbit eye.

A potent PDE inhibitor of the arylxanthine class, 1,3-dibutylxanthine, was applied unilaterally and topically to rabbit eyes in a 0.5% solution. There was a small decrease in the IOP in the contralateral eye, possibly due to systemic leakage. The IOP was decreased 4 mm (Hg) in the ipsilateral eye (Figure 8). This example demonstrates that inhibition of the enzyme which hydrolyzes cGMP mimics the reduction in IOP caused by atriopeptins.

EXAMPLE 12

Figure 9A shows the effect of topical administration of another PDE inhibitor, 1-methyl-3-isobutyl-xanthine on IOP. Again there was about a 4 mm (Hg) decrease in intraocular pressure. Figure 9B shows the effect of 1-methyl-3-isobutyl-xanthine on inhibiting cGMP phosphodiesterase in ciliary process. This xanthine inhibited the enzyme with an IC_{50} (concentration necessary for 50% enzyme inhibition) of 25 uM.

EXAMPLE 13

Figure 10 shows that 1,2,3-propanetriol trinitrate (1,2,3-PTT), i.e., nitroglycerine, activates guanylate cyclase in the ciliary process of the rabbit. Experimental procedures set forth in Example 1 were repeated, substituting nitroglycerine for rANP and using a crude broken-cell preparation as opposed to a washed-membrane preparation. The results demonstrate that nitroglycerine caused a significant stimulation of basal guanylate cyclase activity in the ciliary process at low concentrations.

EXAMPLE 14

The effect of nitroglycerine eye drops on rabbit intraocular pressure was also measured. The IOP of 8 conscious, male albino rabbits (3-4 kg) was measured by applanation pnumatonometry after topical anesthesia with 0.4% benoxinate. Nitroglycerine in phosphate buffered saline, pH 7.4, was given unilaterally, in two 25µl drops spaced five minutes apart. Blood pressure and pulse rate were measured by a photoelectric detector using an automated external tail cuff. Figure 11 shows the results of the topical administration of a 0.03% (gm/100ml) nitroglycerine solution on IOP. A marked decrease in IOP (5.2mm Hg at maximum), which was greatest at one hour and still significantly reduced at six hours, can be seen. By 24 hours, pressure had returned to normal. Importantly, there was no significant change in pulse rate or in systolic, diastolic or mean blood pressure, as can also be seen in Figure 12.

As Figure 13 illustrates, higher doses of nitroglycerine caused no additional decrease in IOP, and lower doses were less effective. Each curve demonstrates the ± mean of eight rabbits at various times after administration of 50µl eye drops containing nitroglycerine in neutral aqueous solution at the dose shown. At higher doses, contralateral eyes also showed

a decrease in pressure, although significantly less, ranging from 40-75% of that seen ipsilaterally. At no dose did conjunctival inflammation nor any change in pupillary diameter occur. At higher doses (0.1%), there was a slight, transient (five minutes) hyperemia of the conjunctival vessels.

This experiment indicates that direct topical ocular administration of a guanylate cyclase activator as an eye drop can decrease IOP without any significant change in systemic cardiovascular parameters. Thus, administration of nitroglycerine as an eye drop involves a more selective delivery to end organ tissues in the eye.

EXAMPLE 15

The effect of administration of a nitroglycerine eye drop preparation (50µl of 0.1% aqueous solution in one eye) in a volunteer human is illustrated in Figure 14. IOP was measured by applanation pnumatonometry and blood pressure and pulse were measured by arm cuff. Topical administration of nitroglycerine significantly decreased IOP by formula mmHg without change in systemic blood pressure (shown) or pulse (not shown).

EXAMPLE 16

The inventor has found a number of other compounds which also increase guanylate cyclase and decrease IOP when applied as an eye drop.

As Table 2 indicates, minoxidil (Compound "M") also increases guanylate cyclase activity alone, and to an even greater extent when combined with rANP. Figure 15 illustrates the effect of a 50µl of 0.1% aqueous solution of minoxidil on intraocular pressure in 12 rabbits. Minoxidil caused a significant decrease in IOP.

TABLE 2

CILIARY PROCESS
GUANYLATE CYCLASE ACTIVITY

(Percent Increase)

COMPOUND "M" (10^{-4}M)	14 ± 4
rANP 1-28 (10^{-6}M)	60 ± 27
COMPOUND "M" + rANP	210 ± 22

As Table 3 indicates, another compound, sodium nitrate (Compound "N") was found to increase ciliary process guanylate cyclase activity and Figure 16 illustrates the effect of sodium nitrate given as 50µl

in a 0.1% eye drop preparation. Experimental procedures were similar to those described in Example 14. Sodium nitrate lowers IOP in rabbits.

TABLE 3

CILIARY PROCESS
GUANYLATE CYCLASE ACTIVITY

(Percent Increase)

COMPOUND "N"	46
rANP 1-28	74

Finally, hydralazine, a drug thought to work through activation of guanylate cyclase activation, when given to rabbits as a 0.1% eye drop preparation in normal saline, decreases IOP. Experimental procedures used were similar to those described in Example 14. Figure 17 illustrates this effect.

Having now fully described the invention, it may readily be seen by those of skill in the art that the present invention can be performed utilizing equivalent agents without affecting the scope of the invention or any embodiment thereof.

I claim:

1. A method of treating cranial fluid volume dysfunction in an individual, comprising administering a fluid volume decreasing amount of a compound effective to increase the amount of cGMP at the site of the dysfunction.

2. A method of treating cranial fluid volume dysfunction in an individual, comprising administering a fluid volume decreasing amount of a compound selected from the group consisting of (A) an atriopeptin-sensitive guanylate cyclase activator, (B) a phosphodiesterase inhibitor and (C) other nitrogen-containing guanylate cyclase activators to an individual in need of said treatment.

3. The method of claim 2 wherein said atriopeptin-sensitive guanylate cyclase activator is selected from the group consisting of

1) an atriopeptin,

2) an atriopeptin agonist, and

3) an atriopeptin analogue.

4. The method of claim 3 wherein said atriopeptin-sensitive guanylate cyclase activator is atriopeptin.

5. The method of claim 3 wherein said atriopeptin-sensitive guanylate cyclase activator is an atriopeptin agonist.

6. The method of claim 3 wherein said atriopeptin-sensitive guanylate cyclase activator is an atriopeptin analogue.

7. The method of claim 2 wherein said phosphodiesterase inhibitor is an arylxanthine.

8. The method of claim 2 wherein said nitrogen-containing guanylate cyclase activators is selected from the group consisting of:

1) nitroglycerine'

2) sodium nitrate;

3) minoxidil, and

4) hydralazine.

9. A method of decreasing intraocular pressure comprising topically administering an intraocular pressure decreasing amount of a nitrogen-containing guanylate cyclase activator.

10. The method of claim 9 wherein said nitrogen-containing guanylate cyclase activator is selected from the group consisting of:

1) nitroglycerine;

2) minoxidil;

3) sodium nitrate; and

4) hydralazine.

11. The method of any of claims 1, 2, or 9 wherein said individual is an animal.

12. The method of claim 8 wherein said animal is a mammal.

13. The method of claim 9 wherein said mammal is a human.

14. The method of any of claims 1, 2, or 9 wherein said cranial fluid volume dysfunction is selected from the group consisting of glaucoma, hydrocephalus, and brain edema.

15. The method of claim 11 wherein said brain edema results from trauma, hypoxia, stroke, cerebral hemorrhage, infection tumor, pseudotumor cerebri (benign intracranial hypertension) or Reye's syndrome.

16. A method of treating brain edema in an individual, comprising administering a brain edema-decreasing amount of an atriopeptin-sensitive guanylate cyclase activator to an individual in need of said treatment.

17. A method of treating brain edema in an individual, comprising administering a brain edema decreasing amount of an atriopeptin, an agonist or an analogue of said atriopeptin to an individual in need of said treatment.

18. A method of treating brain edema in an individual, comprising administering a brain edema-

decreasing amount of a cGMP phosphodiesterase inhibitor to an individual in need of said treatment.

19. A method of treating hydrocephalus in an individual, comprising administering a hydrocephalus reducing amount of an atriopeptin-sensitive guanylate cyclase activator to an individual in need of said treatment.

20. A method of treating hydrocephalus in an individual, comprising administering a hydrocephalus reducing amount of an atriopeptin, an agonist or an analogue of said atriopeptin to an individual in need of said treatment.

21. A method of treating hydrocephalus in an individual, comprising administering a hydrocephalus reducing amount of a cGMP phosphodiesterase inhibitor to an individual in need of said treatment.

22. A method of treating glaucoma in an individual, comprising administering a glaucoma reducing amount of an atriopeptin-sensitive guanylate cyclase activator to an individual in need of said treatment.

23. A method of treating glaucoma in an individual, comprising administering a glaucoma reducing amount of an atriopeptin, an agonist or an analogue of said atriopeptin to an individual in need of said treatment.

24. A method of treating glaucoma in an individual, comprising administering a glaucoma reducing amount of an of a cGMP phosphodiesterase inhibitor to an individual.

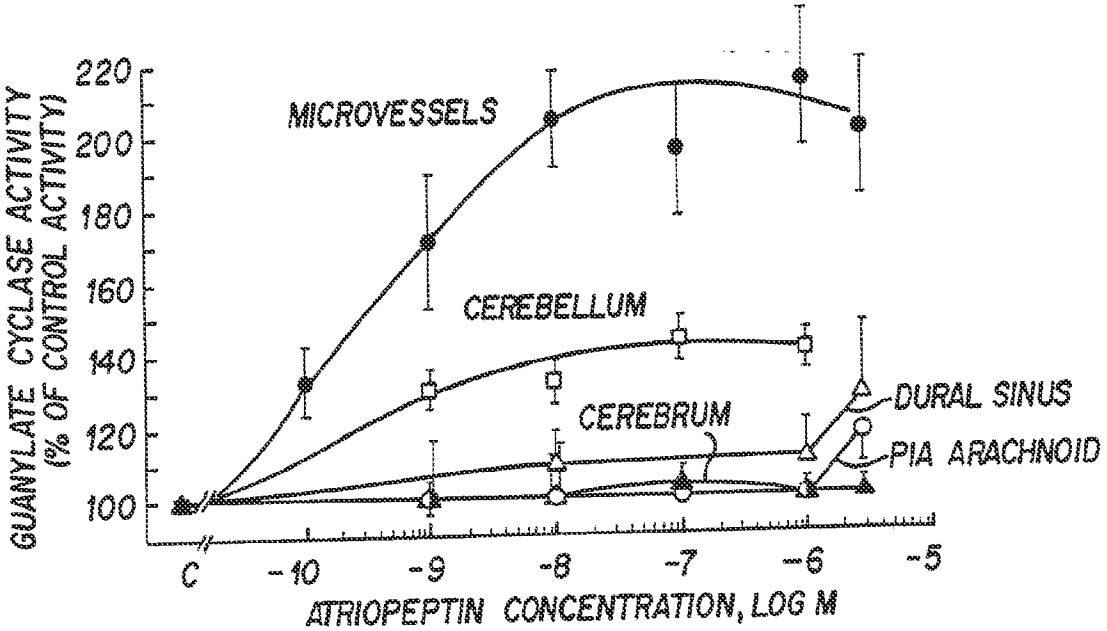

GUANYLATE CYCLASE ACTIVITY
(% OF CONTROL ACTIVITY)
220
200
180
160
140
120
100
MICROVESSELS
CEREBELLUM
CEREBRUM
DURAL SINUS
PIA ARACHNOID
C
-10
-9
-8
-7
-6
-5
ATRIOPEPTIN CONCENTRATION, LOG M

FIG. 1A

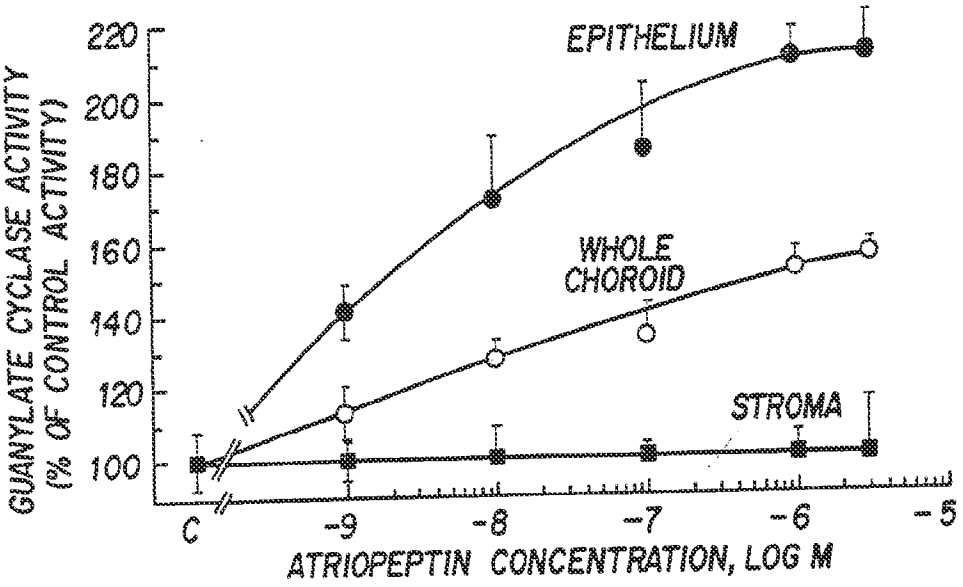

GUANYLATE CYCLASE ACTIVITY
(% OF CONTROL ACTIVITY)
220
200
180
160
140
120
100
EPITHELIUM
WHOLE
CHOROID
STROMA
C
-9
-8
-7
-6
-5
ATRIOPEPTIN CONCENTRATION, LOG M

FIG. 1B

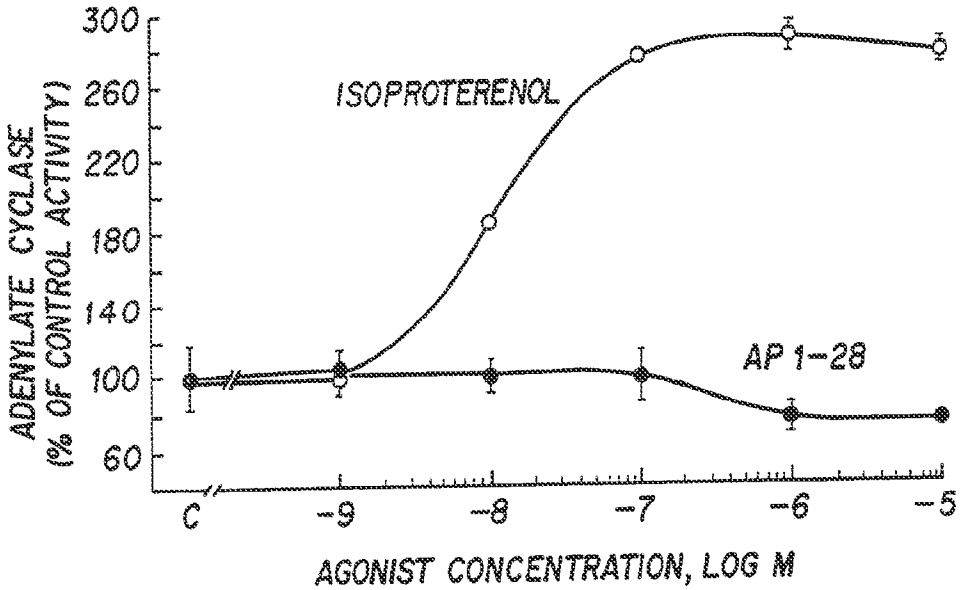

ADENYLATE CYCLASE (% OF CONTROL ACTIVITY)
300
260
220
180
140
100
60
ISOPROTERENOL
AP 1-28
C
-9
-8
-7
-6
-5
AGONIST CONCENTRATION, LOG M

FIG. 1C

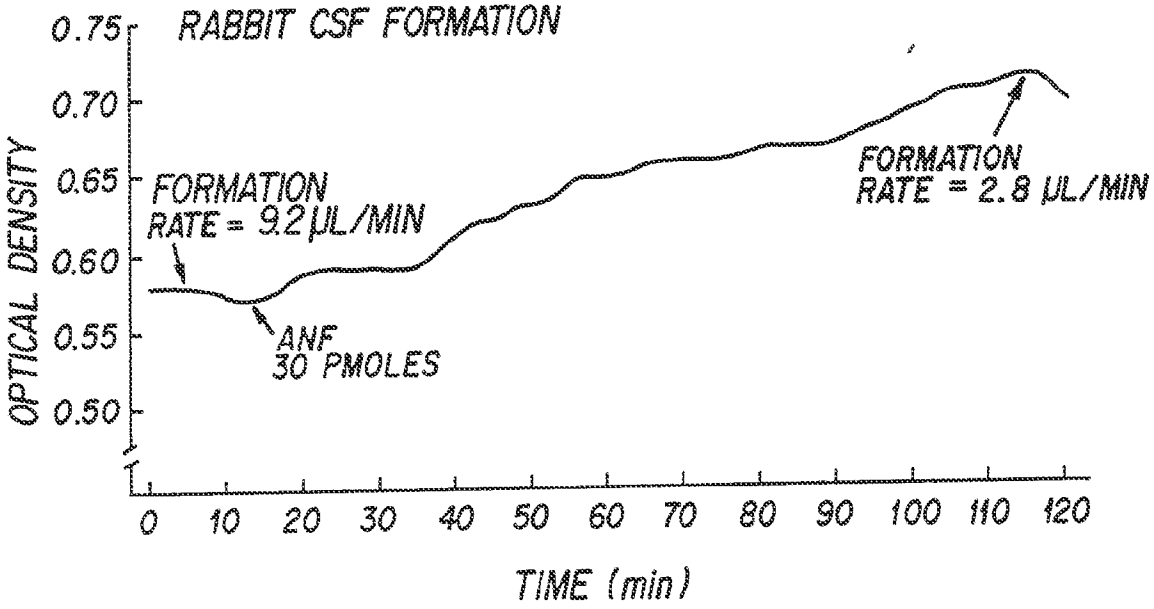

RABBIT CSF FORMATION
OPTICAL DENSITY
0.75
0.70
0.65
0.60
0.55
0.50
FORMATION RATE = 9.2 µL/MIN
ANF 30 PMOLES
FORMATION RATE = 2.8 µL/MIN
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME (min)

FIG. 3

FIG. 2c

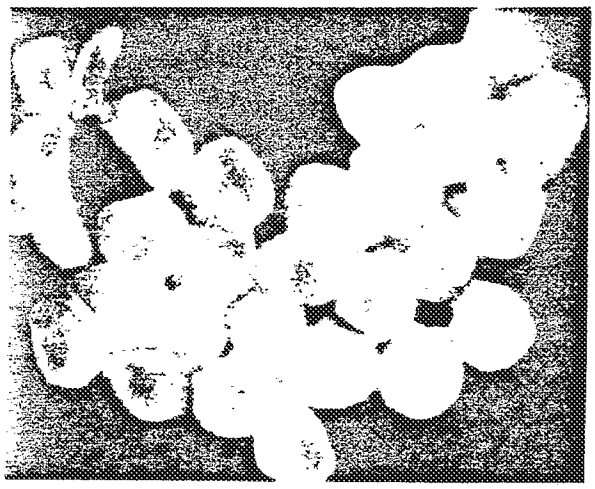


FIG. 2b

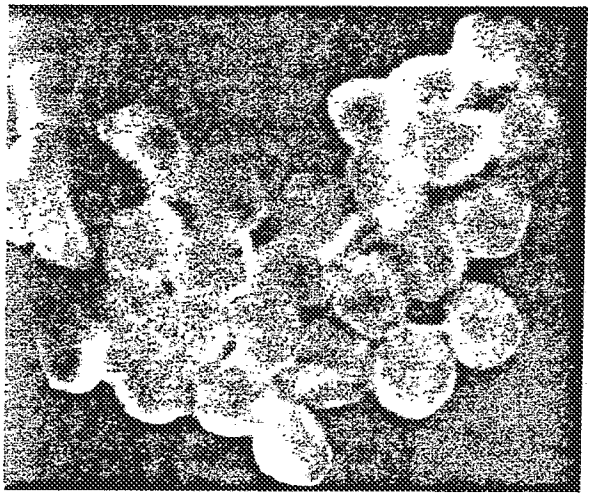


FIG. 2a

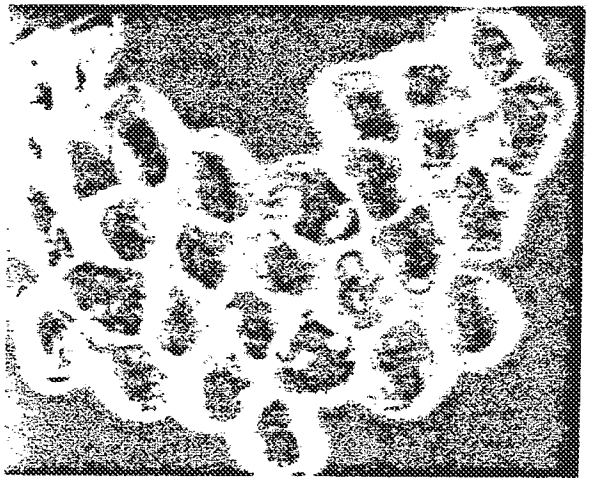


FIG. 2f

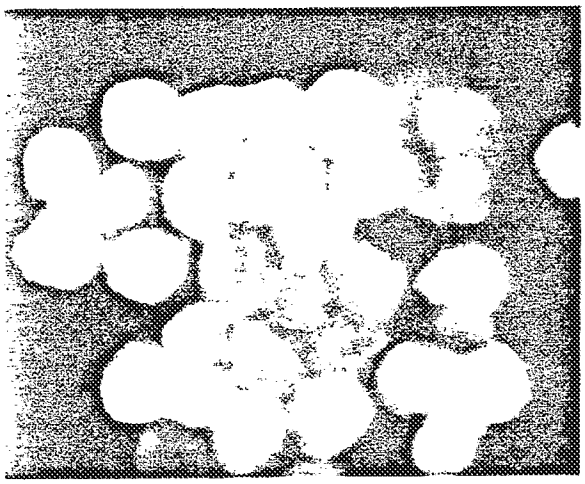


FIG. 2e

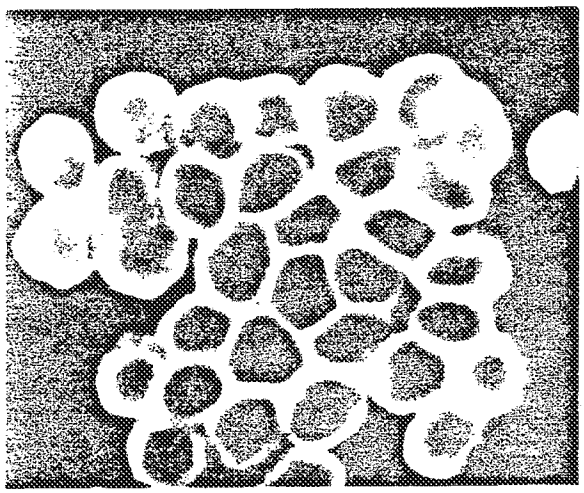
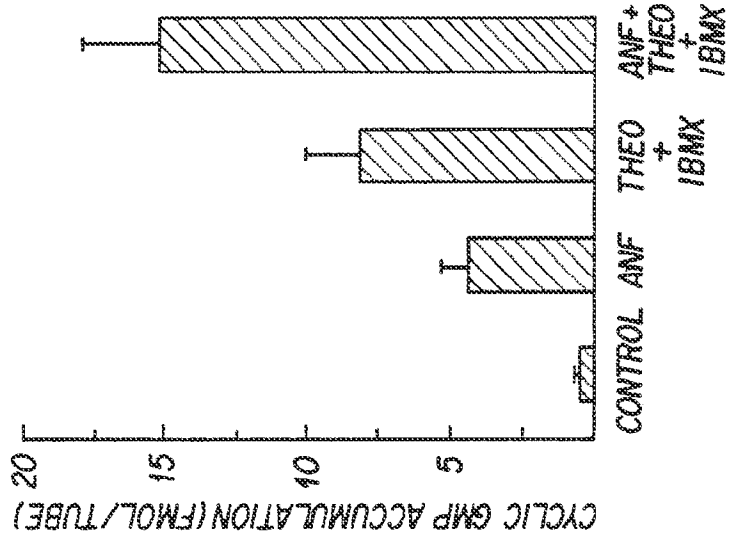


FIG. 2d



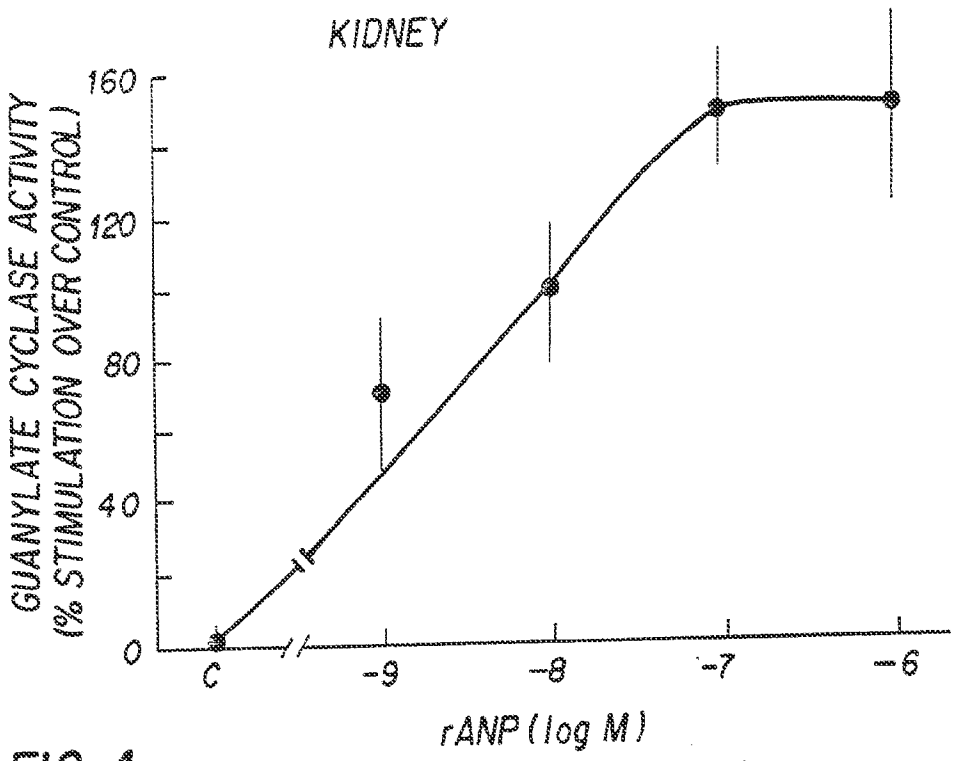

KIDNEY
GUANYLATE CYCLASE ACTIVITY
(% STIMULATION OVER CONTROL)
160
120
80
40
0
C
-9
-8
-7
-6
rANP (log M)

FIG. 4

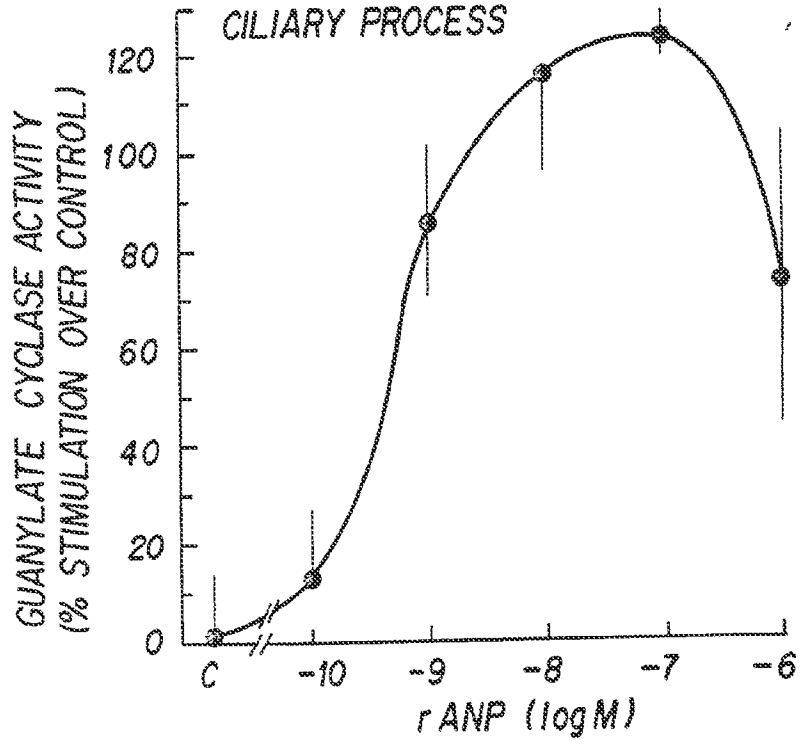

CILIARY PROCESS
GUANYLATE CYCLASE ACTIVITY
(% STIMULATION OVER CONTROL)
120
100
80
60
40
20
0
C
-10
-9
-8
-7
-6
r ANP (log M)

FIG. 5

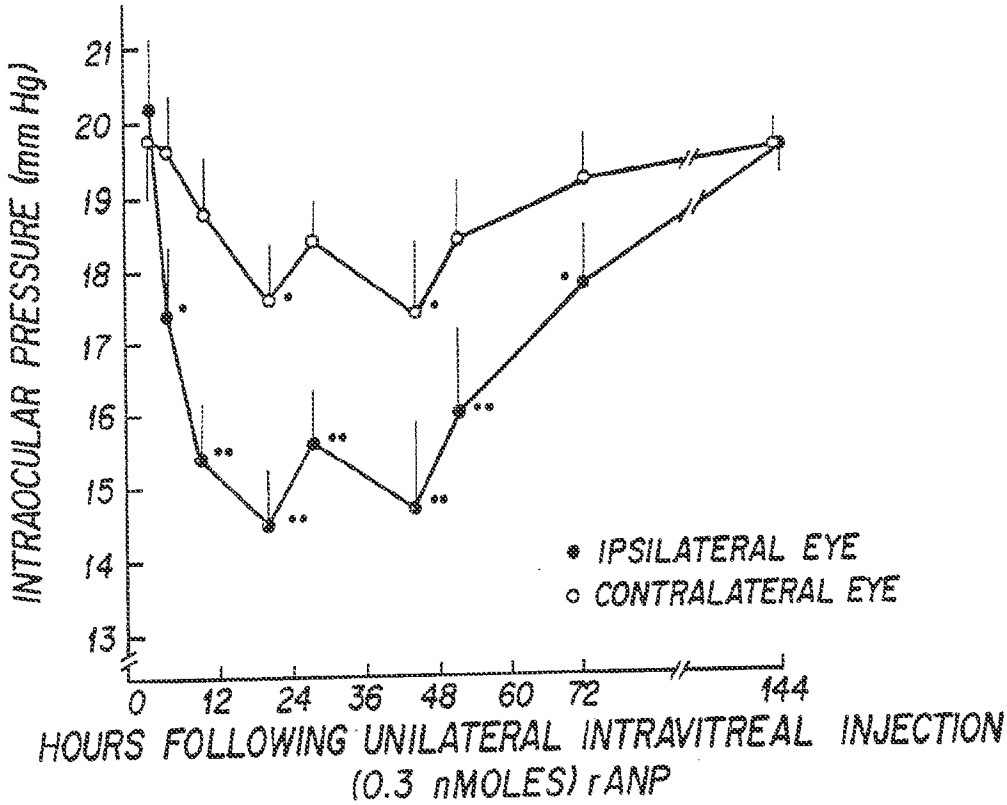

INTRAOCULAR PRESSURE (mm Hg)
21
20
19
18
17
16
15
14
13
0
12
24
36
48
60
72
144
• IPSILATERAL EYE
○ CONTRALATERAL EYE
HOURS FOLLOWING UNILATERAL INTRAVITREAL INJECTION
(0.3 nMOLES) rANP

FIG. 6

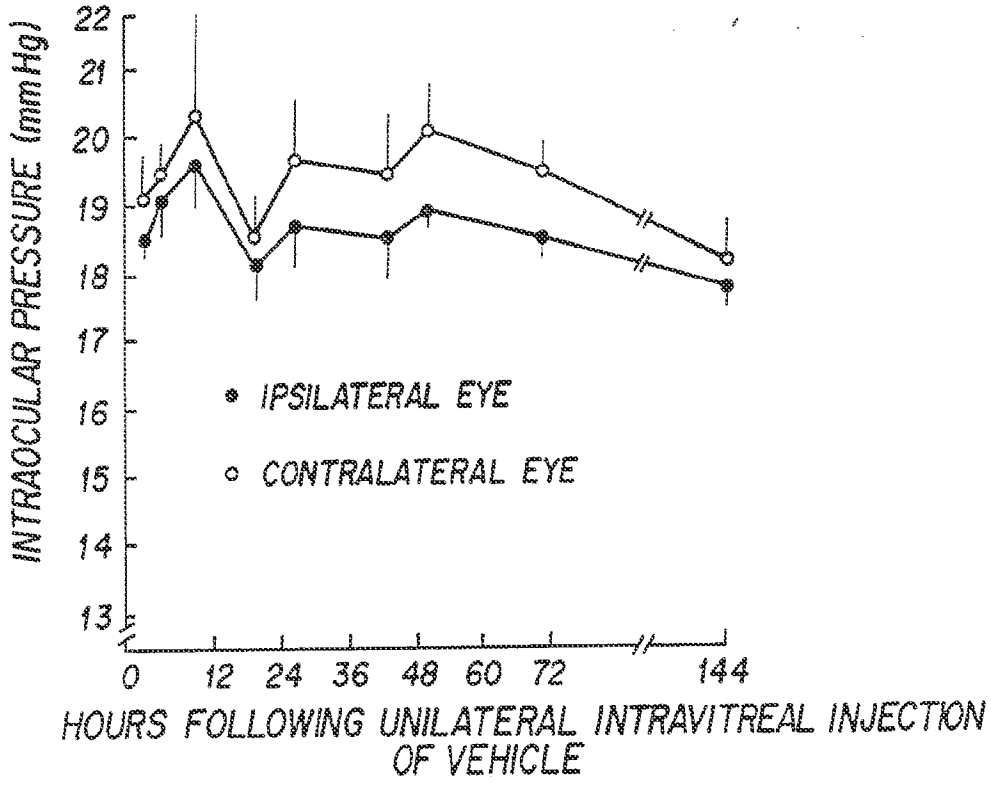

INTRAOCULAR PRESSURE (mm Hg)
22
21
20
19
18
17
16
15
14
13
0
12
24
36
48
60
72
144
• IPSILATERAL EYE
○ CONTRALATERAL EYE
HOURS FOLLOWING UNILATERAL INTRAVITREAL INJECTION
OF VEHICLE

FIG. 7

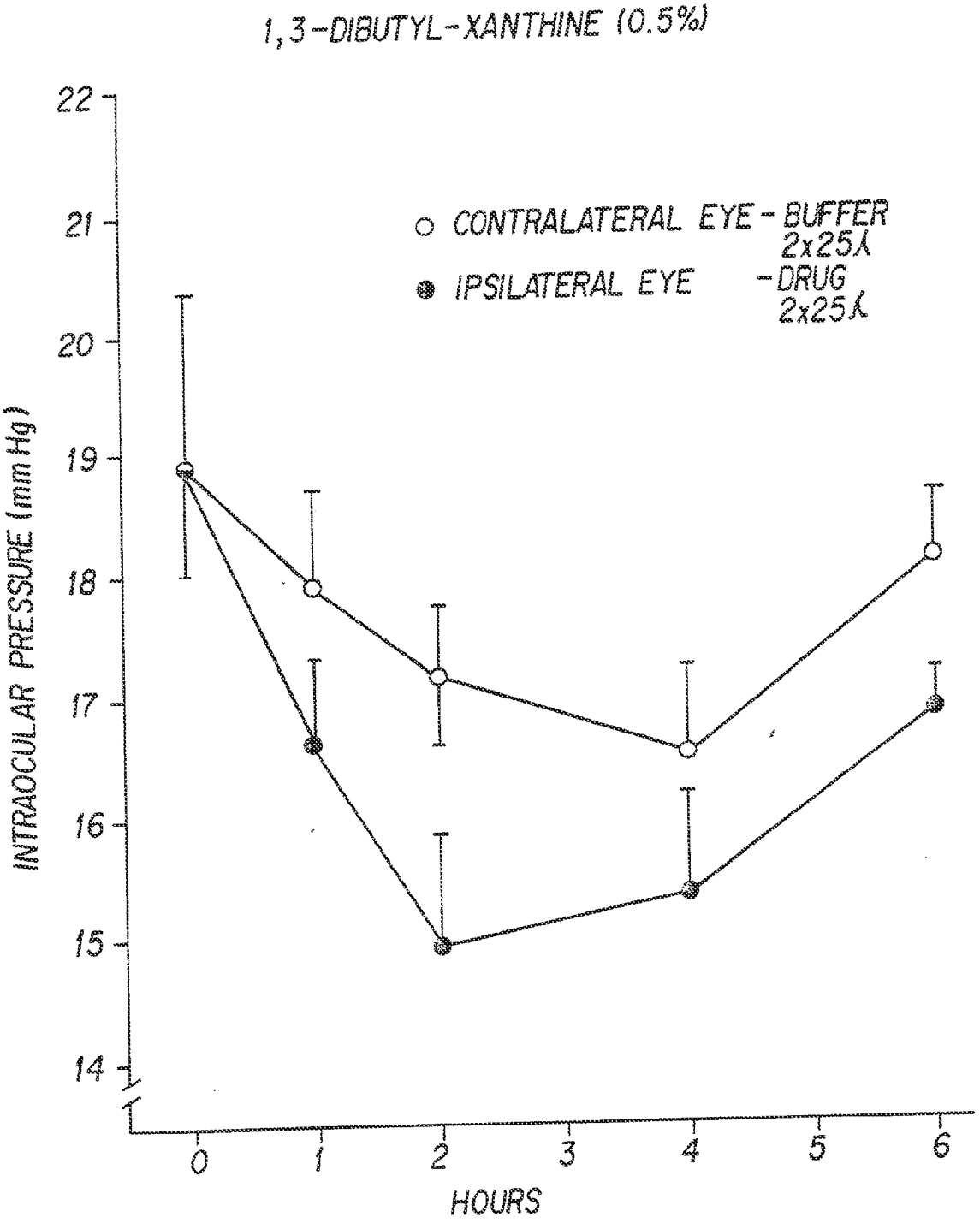

1,3-DIBUTYL-XANTHINE (0.5%)
CONTRALATERAL EYE - BUFFER 2x25λ
IPSILATERAL EYE - DRUG 2x25λ
INTRAOCULAR PRESSURE (mm Hg)
22
21
20
19
18
17
16
15
14
0
1
2
3
4
5
6
HOURS

FIG. 8

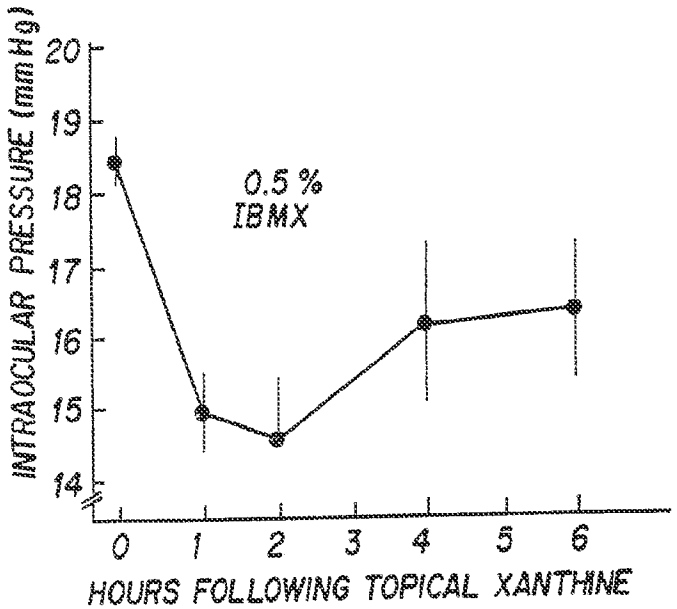

INTRAOCULAR PRESSURE (mm Hg)
20
19
18
17
16
15
14
0.5 %
IBMX
0
1
2
3
4
5
6
HOURS FOLLOWING TOPICAL XANTHINE

FIG. 9A

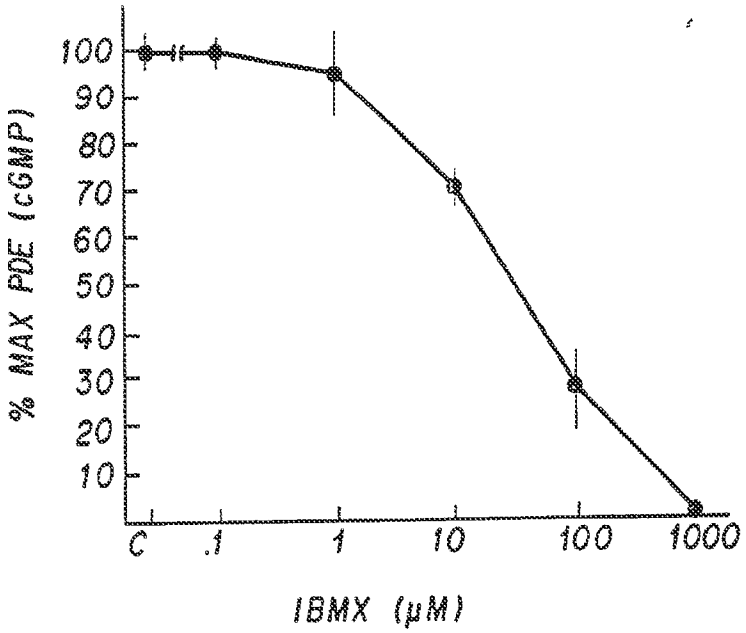

% MAX PDE (cGMP)
100
90
80
70
60
50
40
30
20
10
C
.1
1
10
100
1000
IBMX (μM)

FIG. 9B

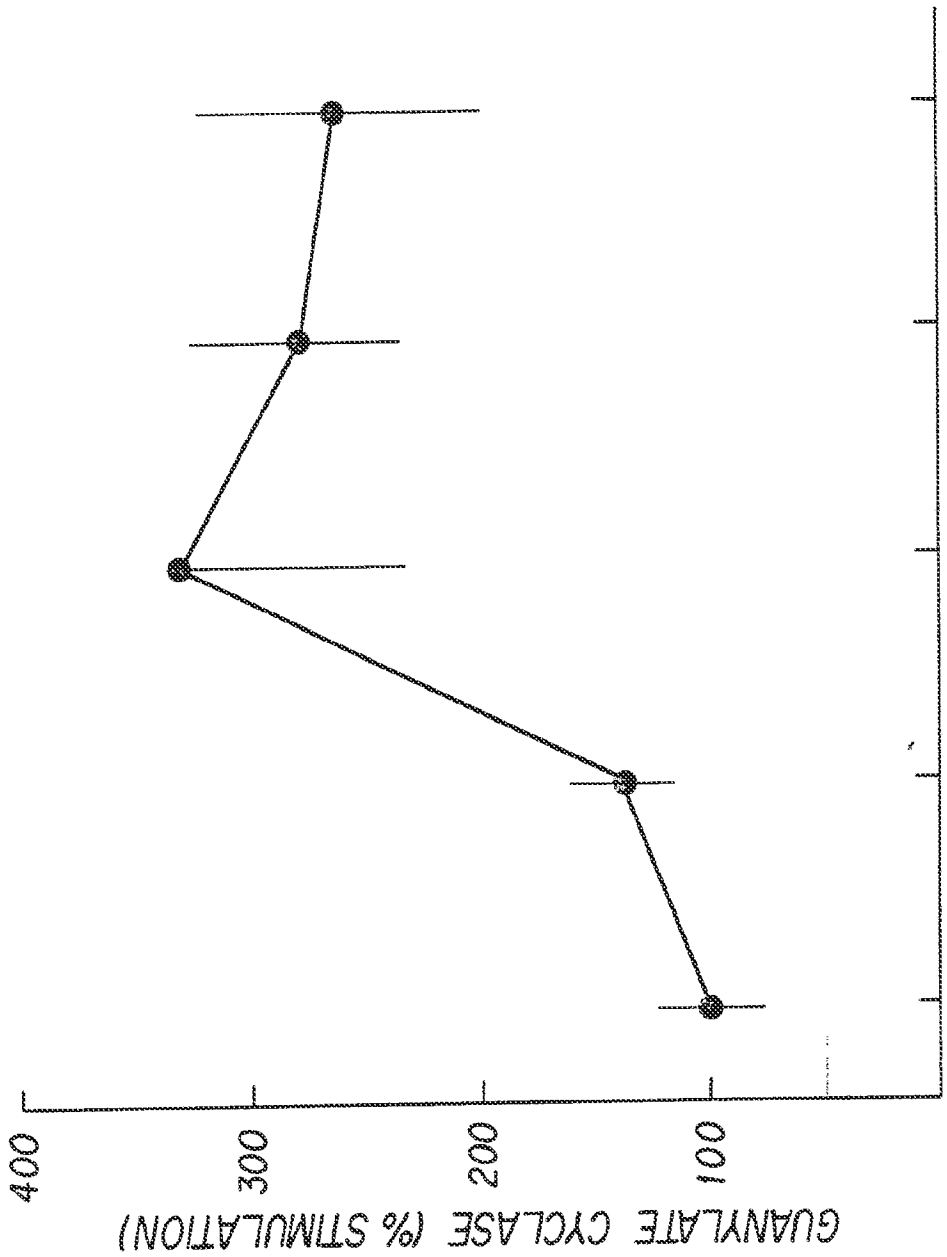

GUANYLATE CYCLASE (% STIMULATION)
400
300
200
100
C
-7
-6
-5
-4
1, 2, 3 – PTT CONCENTRATION (LOG M)

FIG. 10

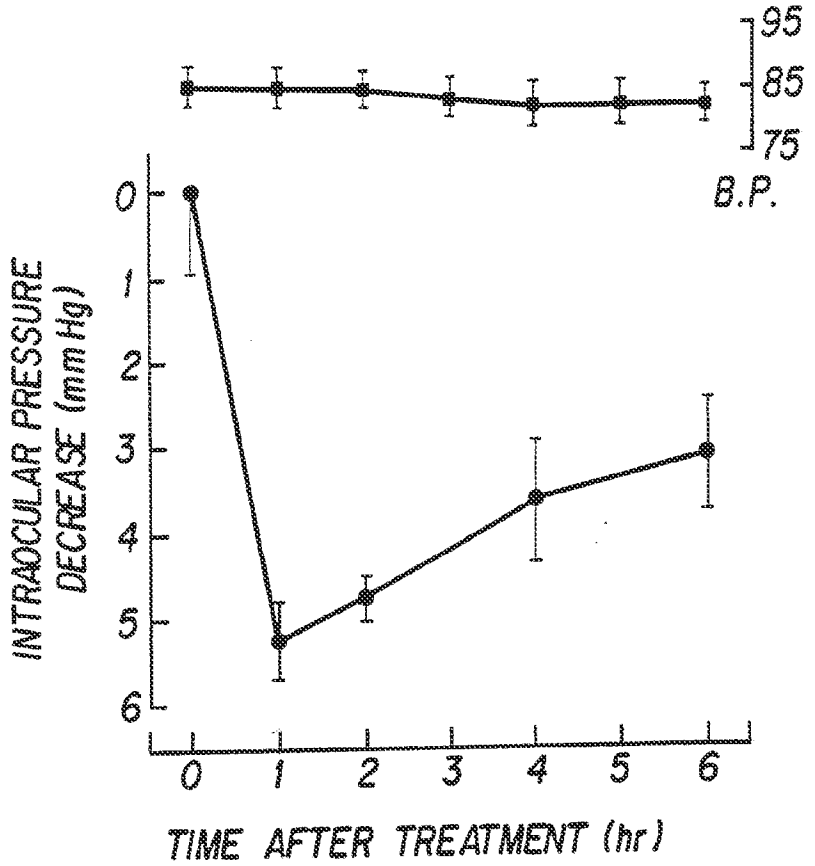

95
85
75
B.P.
INTRAOCULAR PRESSURE DECREASE (mm Hg)
0
1
2
3
4
5
6
0 1 2 3 4 5 6
TIME AFTER TREATMENT (hr)

FIG.11

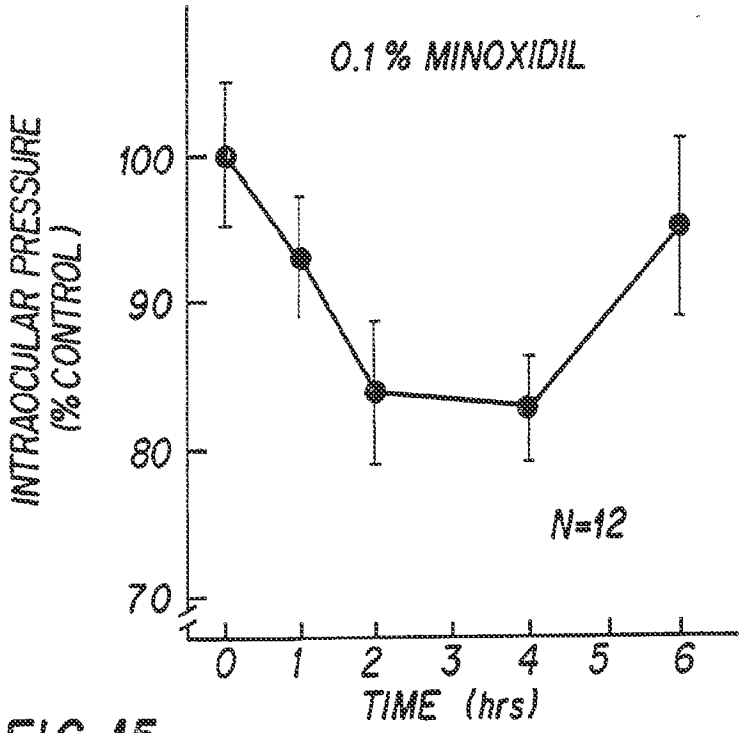
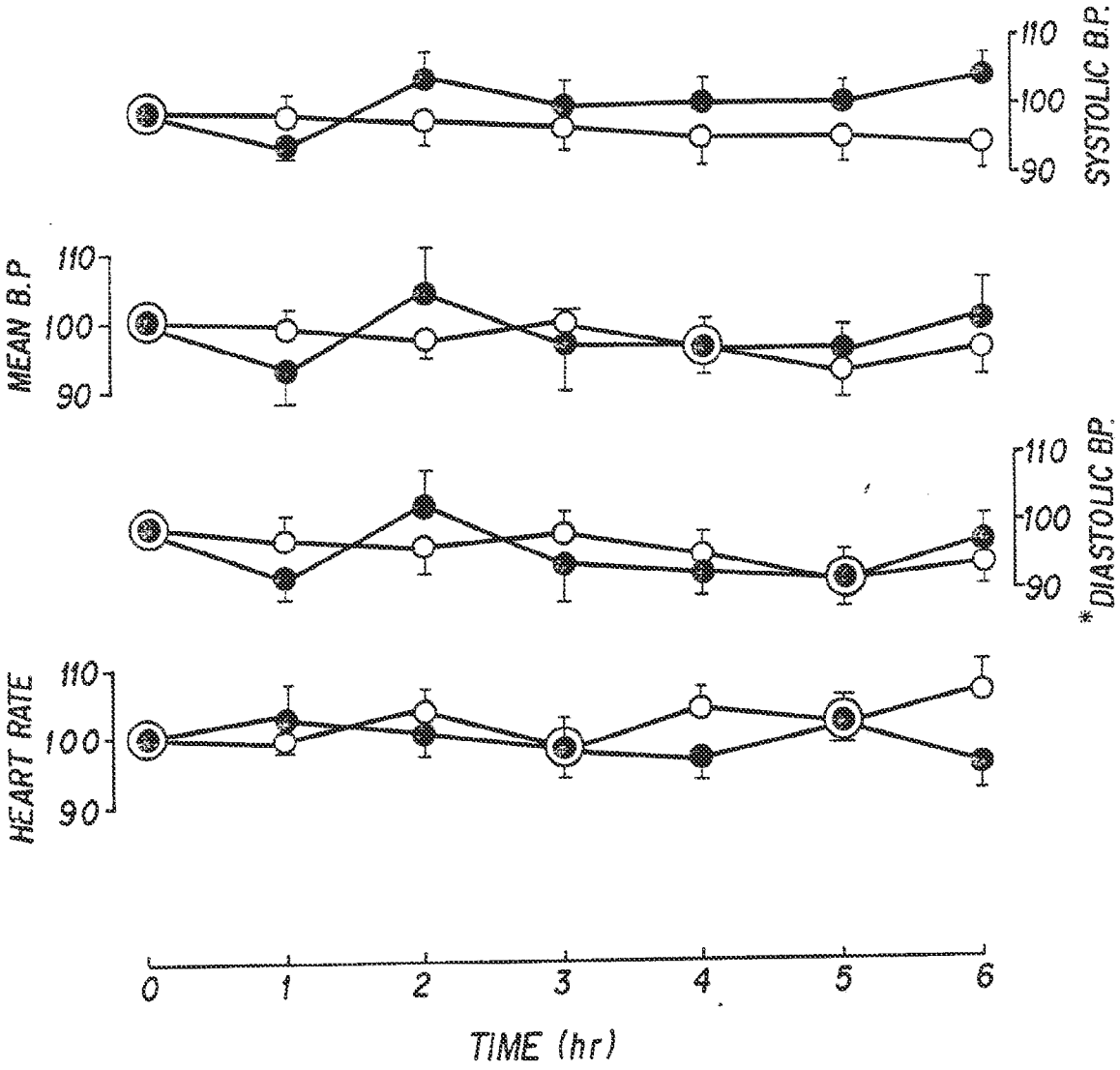

0.1% MINOXIDIL
INTRAOCULAR PRESSURE (% CONTROL)
100
90
80
70
N=12
0 1 2 3 4 5 6
TIME (hrs)

FIG. 15

EFFECT OF TOPICAL NITROGLYCERINE (0.03%) ON HEART RATE AND BLOOD PRESSURE

● CONTROL
○ NITRO

100% SYSTOLIC = 88 ± 2 mmHg
100% MEAN = 64 ± 1 mmHg
100% DIASTOLIC = 52 ± 2 mmHg
100% H R = 212 ± 10 bpm



$$^{*}\text{DIASTOLIC} = \frac{3\,\text{MEAN} - \text{SYSTOLIC}}{2}$$

FIG. 12

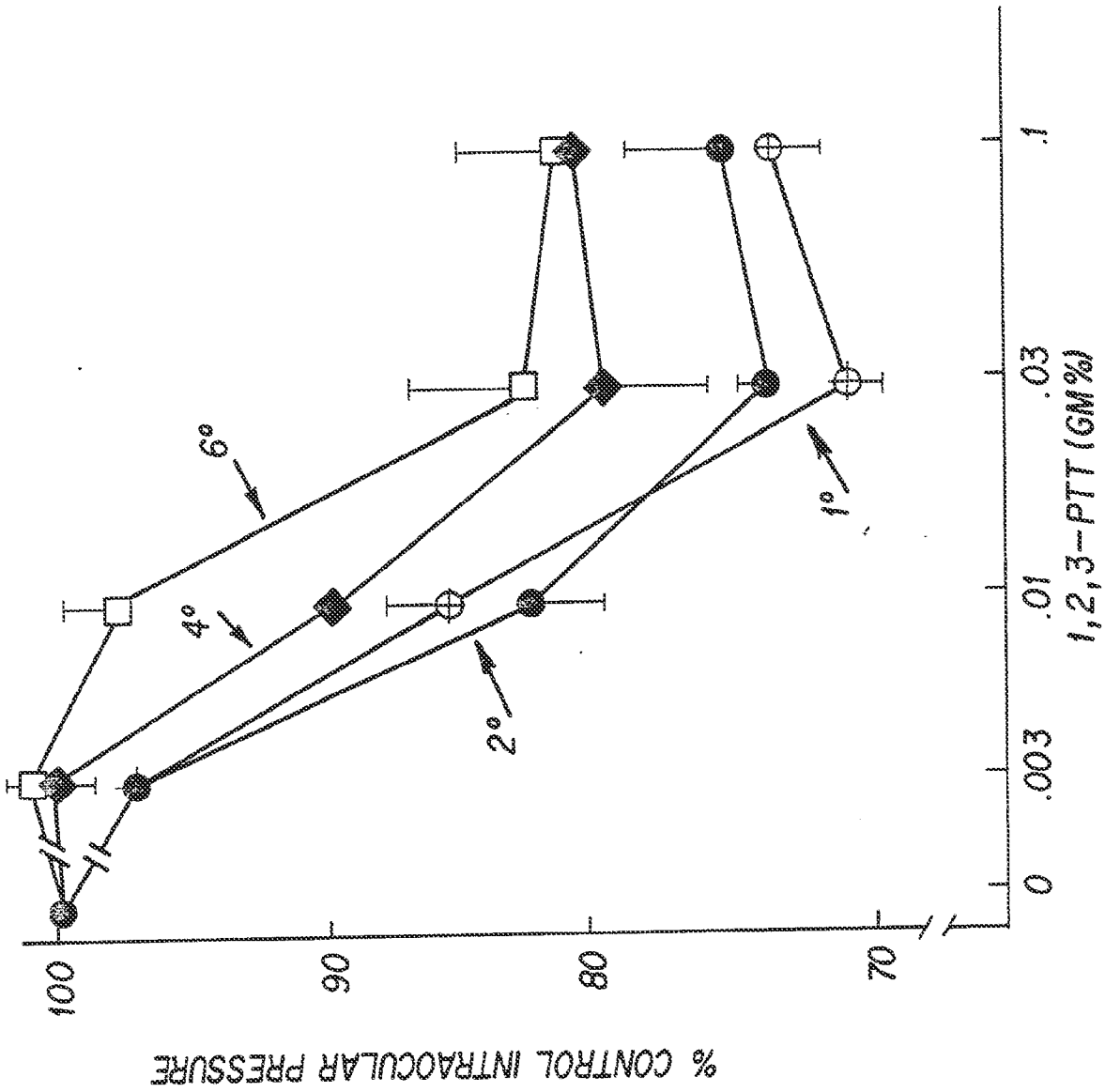

% CONTROL INTRAOCULAR PRESSURE
100
90
80
70
0
.003
.01
.03
.1
1,2,3-PTT (GM%)
6°
4°
2°
1°

FIG.13

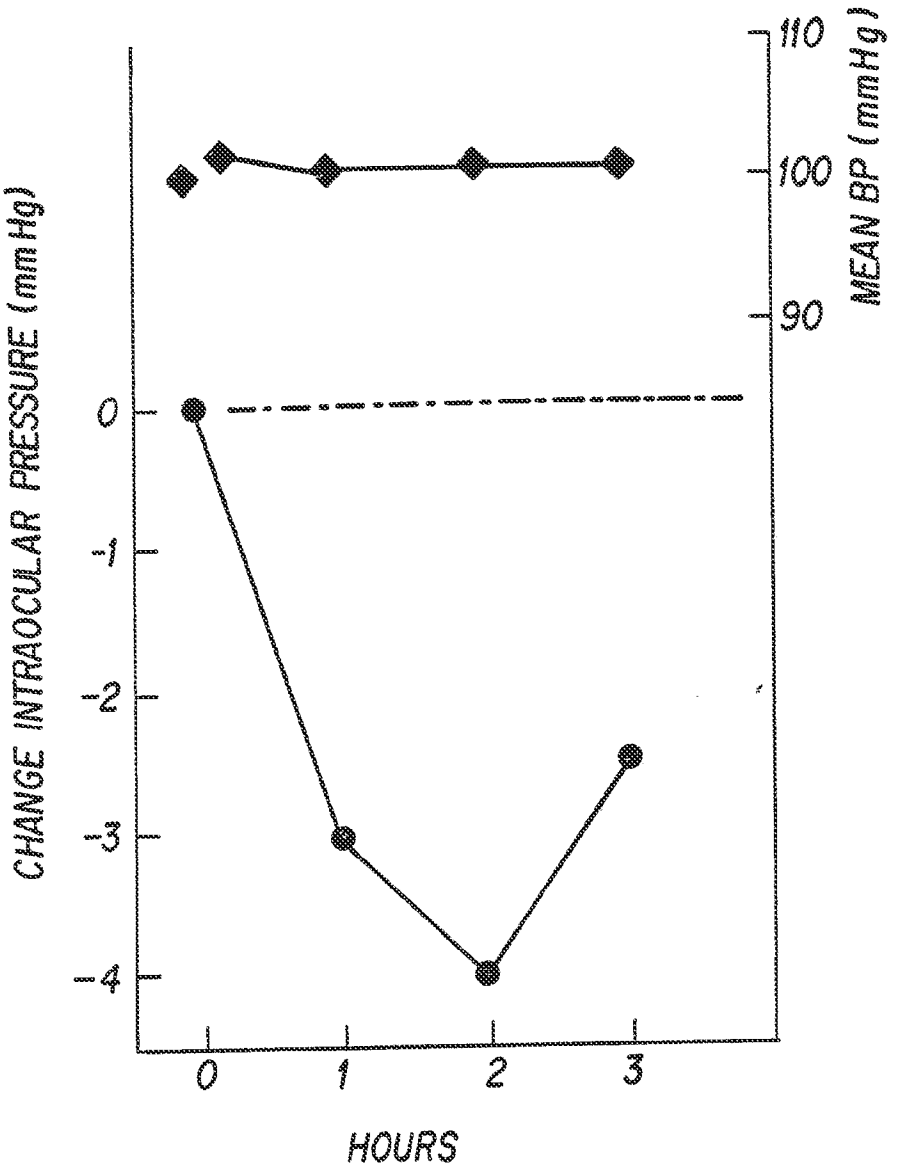

1, 2, 3 - PTT - HUMAN - TOPICAL 0.1 %
CHANGE INTRAOCULAR PRESSURE (mm Hg)
MEAN BP (mmHg)
110
100
90
0
-1
-2
-3
-4
0
1
2
3
HOURS

FIG. 14

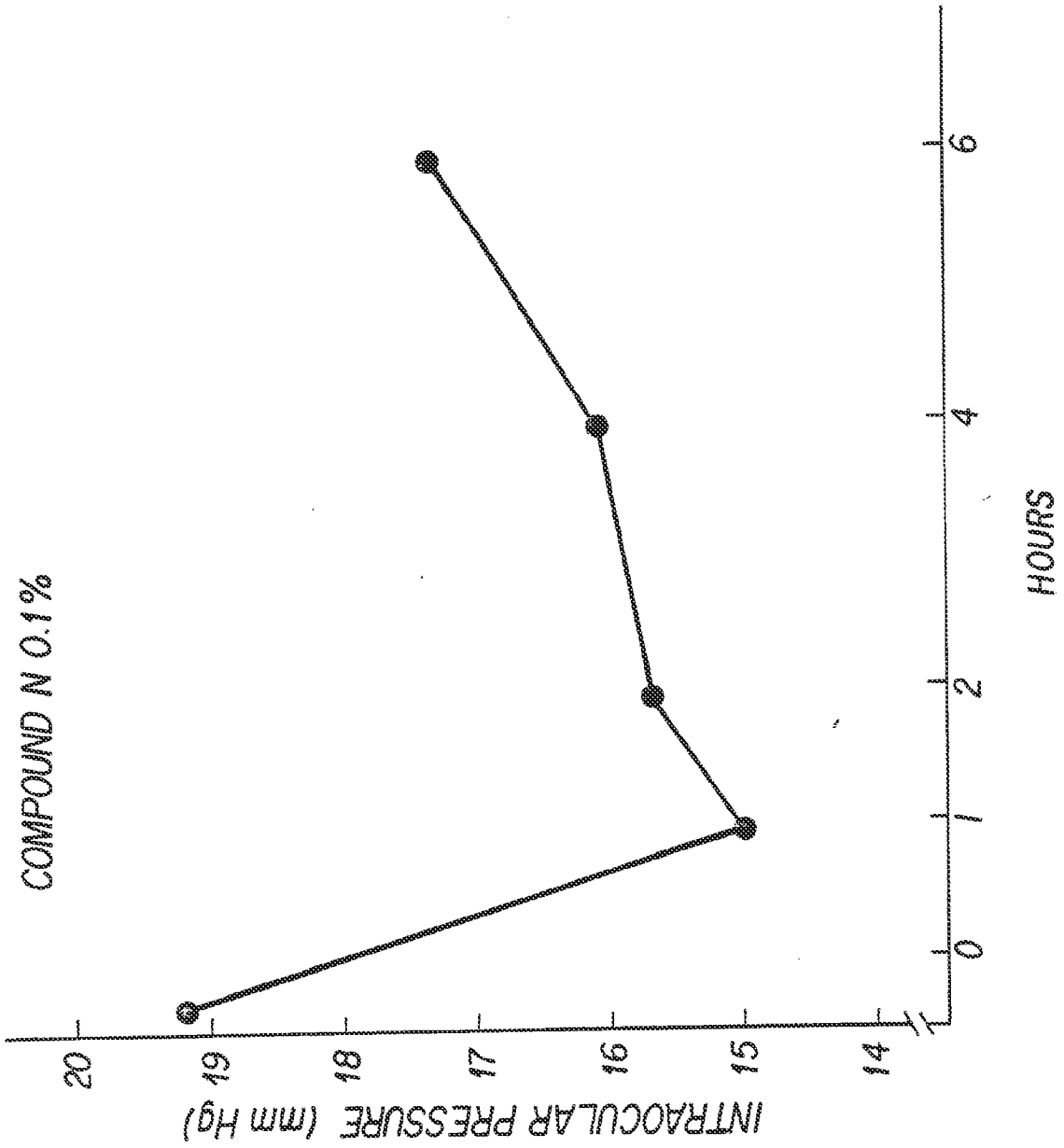

COMPOUND N 0.1%
INTRAOCULAR PRESSURE (mm Hg)
20
19
18
17
16
15
14
0
1
2
4
6
HOURS

FIG.16

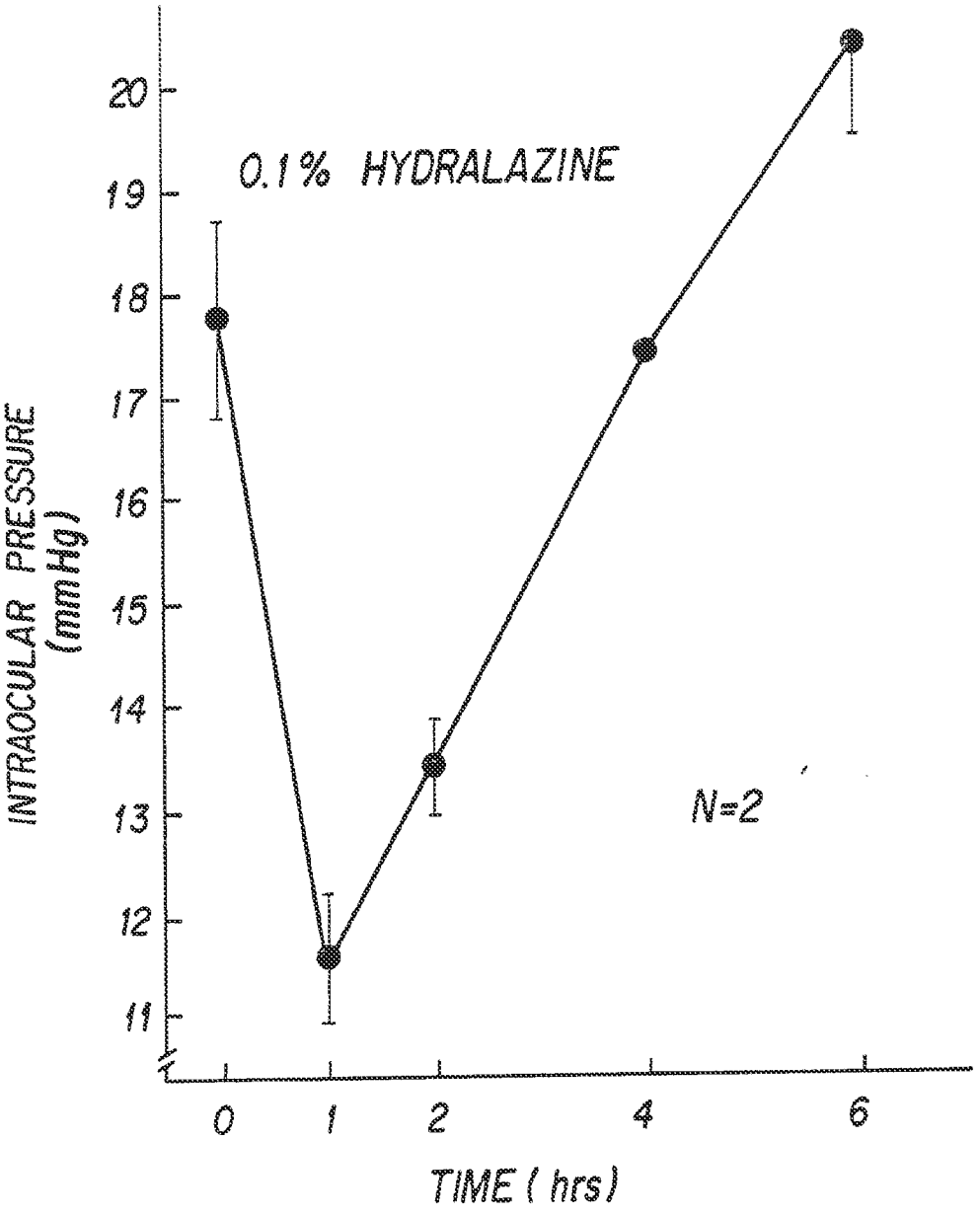

0.1% HYDRALAZINE
N=2
INTRAOCULAR PRESSURE (mmHg)
20
19
18
17
16
15
14
13
12
11
0
1
2
4
6
TIME (hrs)

FIG. 17

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00168

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL4: A61K 37/02, 37/48, 37/64
U.S. CL 514/2, 233, 261

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/324, 325, 325; 514/2, 11, 13, 233, 261, 913

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	PRO. NATIONAL ACADEMY SCIENCE USA, VOLUME 81, PAGES 7661-7664, ISSUED 1984, "ATRIAL NATRIURETIC FACTOR ELICITS AN ENDOTHELIUM-INDEPENDENT RELAXATION AND ACTIVE PARTICULATE GUANYLATE CYCLASE IN VASCULAR SMOOTH MUSCLE" WINQUIST, R. (DEPT. OF CARDIOVASCULAR PHARMACOLOGY MERCK INST. FOR THERAPEUTIC RESEARCH), SEE THE ENTIRE ARTICLE.	1-24
A	LIFE SCIENCES, VOLUME 37, PAGES 1081-1087, NO. 12 ISSUED 1985, "THE RELAXANT EFFECTS OF ATRIAL NATRIURETIC FACTOR ON VASCULAR SMOOTH MUSCLE", WINQUIST, R. (DEPT. OF CARDIOVASCULAR PHAR. MERCK INST. FOR THERAPEUTIC RESEARCH), SEE THE ENTIRE ARTICLE.	1-24
A	ENDOCRINE REVIEWS, VOLUME 6, NO. 2, PAGES 107-127, ISSUED 1985, "THE HEART AND THE ATRIAL FACTOR", CANTIN, M. (CLINICAL RESEARCH INST. OF MONTREAL), SEE THE ENTIRE ARTICLE.	1-24

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 MARCH 1988	13 MAY 1988
International Searching Authority	Signature of Authorized Officer
ISA/US	THERESA D. WESSENDORF

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	THE JOURNAL OF NEUROSCIENCE, VOLUME 6, NO. 7, ISSUED 1986, PAGES 2004-2011, "AUTORADIOGRAPHIC LOCALIZATION AND CHARACTERIZATION OF ATRIAL NATRIURETIC PEPTIDE BINDING SITES IN THE RAT CENTRAL NERVOUS SYSTEM AND ADRENAL GLAND"., GIBSON, T. (DEPT. OF PHAR., SCHOOL OF MED., UNIV. OF PA., PHILADELPHIA, PA), SEE THE ENTIRE ARTICLE	1-24
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(57) Abstract

S-nitrosothiols exert a potent relaxant effect, mediated both by guanylate cyclase, and a cGMP-independent mechanism, upon non-vascular smooth muscle. Such types of smooth muscle include airway, gastrointestinal, bladder, uterine and corpus cavernosal. Thus, S-nitrosothiols may be used for the treatment or prevention of disorders associated with relaxation of smooth muscle, such as airway obstruction, and other respiratory disorders, bladder dysfunction, premature labor and impotence. Additionally, S-nitrosothiols may be used to alleviate smooth muscle contraction and spasm, and thus facilitate procedures involving diagnostic instrumentation, such as endoscopy, bronchoscopy, laparoscopy and cystoscopy. S-nitrosothiols also increase the binding affinity between hemoglobin and oxygen, and therefore, may be used to improve hemoglobin-oxygen binding, and oxygen transport to bodily tissues.

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S-NITROSOTHIOLS AS SMOOTH MUSCLE RELAXANTS AND THERAPEUTIC USES THEREOF

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Application Serial No. 07/804,665, filed December 11, 1991, which is a continuation-in-part of U.S. Application Serial No. 676,691, filed March 29, 1991, abandoned.

Background of the Invention

This invention was made with government support under R01-HL40411, HL43344, and R04870, awarded by The National Institutes of Health. The government has certain rights in the invention.

Field of the Invention

This invention relates to the use of low molecular weight S-nitrosothiols, such as S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-homocysteine, S-nitroso-cysteine, S-nitroso-penicillamine and S-nitroso-captopril, to relax non-vascular smooth muscle. Types of smooth muscle include airway, gastrointestinal, bladder uterine, and corpus cavernosum. The invention also relates to the use of S-nitrosothiols for the treatment or prevention of disorders which involve non-vascular smooth muscle, such as

respiratory disorders, gastrointestinal disorders, urological dysfunction, impotence, uterine dysfunction or premature labor. The invention also relates to the use of S-nitrosothiols to ameliorate smooth muscle contraction or spasm and thus, facilitate diagnostic or therapeutic procedures, such as bronchoscopy, endoscopy, laparoscopy, and cystoscopy. S-nitrosothiols may also be used to increase hemoglobin-oxygen binding, and thus enhance oxygen transport to bodily tissues.

Brief Description of the Background Art

The endothelium secretes a vascular relaxing factor, known as endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO), or a closely related derivative thereof. (Palmer *et al., Nature 327*:524-526 (1987); Ignarro *et al., Proc. Natl. Acad. Sci. USA 84*:9265-9269 (1987)). Under physiologic conditions, however, NO is exceedingly unstable, reacting essentially instantaneously with oxygen, superoxide anion, and redox metals (Lancaster *et al., Proc. Natl. Acad. Sci. USA 87*:1223-1227 (1990); Ignarro *et al., Circ. Res. 65*:1-21 (1989); and Gryglewski *et al., Nature 320*:454-456 (1986)). This fact has lead to the supposition that, in order to exert its effect on vascular smooth muscle, NO must be stabilized *in vivo* in a form that preserves its biological activity.

S-nitrosothiols (RS-NO) are adducts that form readily under physiologic conditions from the reaction of NO with reduced low molecular weight thiols (Oae *et al., Org. Prep. Proc. Int. 15(3)*:165-198 (1983)). These compounds have half-lives that are significantly greater than that of NO and, like EDRF, possess vasorelaxant activity that is mediated through activation of guanylate cyclase (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)).

The relaxant effect of S-nitrosothiols on blood vessels, and the mechanism by which this effect is exerted, is reasonably well understood in

the art. However, the role of NO, or involvement of the guanylate cyclase pathway in non-vascular smooth muscle is not as clearly understood.

Pulmonary immune responses result in the liberation of cytokines and inflammatory mediators which contribute to the narrowing of airway smooth muscle. As part of this process, pulmonary endothelial cells, macrophages and polymorphonuclear leukocytes are believed to induce nitric oxide synthetase, thus serving as a source of NO. The consequences of NO production in the lung are not known. However, the potential beneficial effects of NO through bronchodilation may be counterbalanced by generation of toxic nitrogen oxides that form readily under the high ambient concentration of oxygen and other reactive oxygen species.

Likewise, introduction of NO into the lungs also results in significant adverse effects, which occur as a direct result of the particular chemical reactivity of the uncharged NO radical (NO•). These adverse effects create impediments to NO therapy which generally involves administration of NO•. For example, the reaction between NO•, and O_2 or reactive O_2 species which are present in high concentrations in the lung, generates highly toxic products, such as NO_2 and peroxynitrite. These reactions also result in the rapid inactivation of NO, thus eliminating any beneficial pharmacological effect. (Furchgott R.F. *et al.*, *I. Endothelium-Derived Relaxing Factors and Nitric Oxide*; eds. Rubanyi G.M., pp. 8-21 (1990); Gryglewski, R.J. *et al.*, *Nature 320*:454-456 (1986)). Furthermore, NO• reacts with the redox metal site on hemoglobin to form methemoglobin, which inhibits oxygen-hemoglobin binding, thereby significantly reducing the oxygen-carrying capacity of the blood.

Non-vascular smooth muscle is present in numerous organ systems throughout the body, and has a vital role in the physiological function of these systems. For example, airway smooth muscle plays a critical role in constriction and dilation of bronchi. In the gastrointestinal tract, the sphincter of Oddi, a smooth muscle connection between the bile duct and duodenum, provides tonic contraction which serves to prevent reflux of duodenal contents into the pancreatic and bile ducts, and promotes filling of the gall bladder. In

addition, esophageal (sphincters and body), intestinal and colonic motility is regulated by smooth muscle. Smooth muscle of the bladder body, bladder base, and proximal urethra plays an important role in urological function, and erectile function is mediated by relaxation of corpus cavernosal smooth muscle.

In summary, the relaxation kinetics of non-vascular smooth muscle are very important in numerous physiological systems. Moreover, a variety of significant clinical disorders occur, which involve contraction, spasm, or failure to achieve the necessary relaxation of smooth muscle. Examples of such disorders include airway obstruction (i.e., asthma, bronchitis and emphysema), bladder dysfunction, gastrointestinal muscle spasm (i.e., irritable bowel syndrome, achalasia, dumping disorders), and impotence. Thus, a clinical need exists for pharmacological agents which can treat or prevent such disorders by inducing relaxation of the affected smooth muscle.

SUMMARY OF THE INVENTION

This invention is based on the discovery by the inventors that S-nitrosothiols exert a potent relaxant effect on non-vascular smooth muscle. This concept lead the inventors to the discovery that S-nitrosothiol compounds may be used as a therapeutic modality in disorders which involve smooth muscle relaxation.

The invention is directed to an S-nitrosothiol compound which has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

The invention is also directed to the use of S-nitrosothiols for the treatment or prevention of disorders associated with relaxation of smooth muscle, such as airway obstruction, gastrointestinal spasm, bladder dysfunction and impotence. The invention is also directed to the use of S-nitrosothiols to alleviate smooth muscle contraction and spasm, and thus facilitate procedures involving diagnostic instrumentation such as endoscopy and bronchoscopy.

In particular, this invention is directed to a method for relaxing airway smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal. The S-nitrosothiol compound may be selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril. The S-nitrosothiol compound may be selected from the group consisting of a compound having the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

The invention is also directed to a method for treatment or prevention of respiratory disorders by administering a therapeutically effective amount of S-nitrosothiol compound to an animal. Respiratory disorders include obstructive lung disease, emphysema, asthma, bronchitis, fibrosis, excessive mucus secretion, obstruction of air flow, and lung disorders resulting from post-surgical complications.

The invention is also directed to a method for relaxing gastrointestinal smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for ameliorating contraction or spasm of gastrointestinal smooth muscle associated with endoscopic procedures, by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for relaxing corpus cavernosum smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is directed to a method for the treatment or prevention of impotence by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for relaxing bladder smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for relaxing uterine smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to the administration of said S-nitrosothiol compounds for the methods of the invention, as part of the pharmaceutical composition comprising a pharmaceutically acceptable carrier.

The invention is also directed to the methods of the invention wherein the pharmaceutical composition containing the S-nitrosothiol compound is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or intranasal delivery.

The invention is also directed to a method for increasing the capacity of hemoglobin to bind oxygen, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

The invention is also directed to a method for increasing oxygen transport to bodily tissues, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

The invention is also directed to a method for the treatment or prevention of disorders associated with insufficient oxygen supply to bodily tissues, comprising administering a therapeutically effective amount of an S-nitrosothiol to an animal in need thereof.

Brief Description of the Figures

FIGURE 1: Inhibition of the Sphincter of Oddi by administration of S-nitroso-N-acetylcysteine.

FIGURE 2: Inhibition of duodenal motility by administration of S-nitroso-N-acetylcysteine.

FIGURE 3: Side-by-side comparison of the relaxant effect of specific S-nitrosothiols on guinea pig tracheal muscle.

FIGURE 4: Dose-dependent relaxant effect of specific S-nitrosothiols on guinea pig tracheal muscle contracted with 3 μM, as compared to the reactant and NO.

a: S-nitroso-glutathione
b: S-nitroso-cysteine
c: S-nitroso-homocysteine
d: S-nitroso-N-acetylcysteine
e: S-nitroso-penicillamine
f: S-nitroso-captopril

FIGURE 5: Relaxant activities of S-nitroso-N-acetylcysteine (A) and S-nitroso-captopril (B) determined against contractions induced by leukotriene D_4, histamine and methacholine.

FIGURE 6: The course of relaxation induced by S-nitroso-N-acetylcysteine (5×10^{-6}M) over 60 minutes.

FIGURE 7: The relaxation response to S-nitroso-glutathione in guinea pig airway (A) and rabbit aorta (B).

FIGURE 8: Tracheal relaxant effects of S-nitroso-N-acetylcysteine, isoproterenol, and theophylline.

FIGURE 9: Inhibition of tracheal relaxation to S-nitroso-N-acetylcysteine by hemoglobin and methylene blue.

FIGURE 10: Cyclic GMP determinations in tracheal rings incubated with 100 μM S-nitroso-N-acetylcysteine.

FIGURE 11: Comparison between the relaxant effect of S-nitroso-glutathione and nitrite upon human tracheal smooth muscle.

FIGURE 12: Comparison between the relaxant effect of S-nitroso-glutathione and glutathione upon human tracheal smooth muscle.

FIGURE 13: Comparison between the relaxant effect of S-nitroso-N-acetylcysteine and N-acetylcysteine upon human tracheal smooth muscle.

FIGURE 14: Tracheal relaxant effects of theophylline, isoproterenol, S-nitroso-N-acetylcysteine, and S-nitroso-glutathione.

FIGURE 15: Cyclic GMP response to S-nitroso-N-acetylcysteine in human airways.

FIGURE 16: SNOAC-induced airway relaxation is not inhibited by methylene blue.

FIGURE 17: S-nitrosylation of hemoglobin.

FIGURE 18: UV spectrum of hemoglobin incubated with S-nitroso-N-acetylcysteine.

FIGURE 19: Reaction of nitric oxide at the iron-binding site of hemoglobin.

Description Of The Preferred Embodiments

The invention is based on the discovery by the inventors that S-nitrosothiols relax non-vascular smooth muscle, and possess unique and different relaxant activities, kinetic properties and membrane permeability, and thus, may be used to treat or prevent disorders which involve non-vascular smooth muscle.

In one embodiment, the term "S-nitrosothiol" refers to a compound which is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-pantathoeine derivatives, S-nitroso-penicillamine and S-nitroso-captopril.

In another embodiment the term "S-nitrosothiol" refers to particular novel S-nitrosothiol compounds synthesized by the inventors, for use as smooth muscle relaxants. The compounds represented by the general formula of $CH_3(CH_2)_xSNO$ are long carbon-chain lipophilic nitrosothiols. The compounds represented by the general formula of $HS(CH_2)_xSNO$ are S-nitrosodithiols, possessing an additional thiol group. The compounds represented by the general formula of $ONS(CH_2)_xY$ are S-nitrosothiols which possess other functional groups, in addition to the thiol.

The invention is related to the discovery that S-nitrosothiol compounds relax non-vascular smooth muscle. As a result, these compounds may be used to treat or prevent those pathophysiologic conditions which result from, or involve, constriction of smooth muscle, or those which necessitate therapeutic intervention to achieve smooth muscle relaxation.

One embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol to an animal to relax airway smooth muscle. The term "airway smooth muscle" refers to the smooth muscle lining the bronchi or tracheal region. The inventors have demonstrated that S-nitrosothiols exert a potent relaxant effect upon airway smooth muscle.

As a result of this potent relaxant effect exerted by S-nitrosothiols, these compounds may be administered as therapeutic agents for the treatment or prevention of respiratory disorders.

The term "respiratory disorder" refers to any impairment of lung function which involves constriction of airways and changes in blood gas levels or lung function.

For example, airway obstruction constitutes a respiratory disorder which occurs as a result of acute pulmonary impairment or obstructive lung disease. Severe airway obstruction may ultimately result in life-threatening respiratory failure. Airway obstruction occurs in patients with chronic obstructive lung diseases, such as emphysema and bronchitis. These patients often experience recurrent episodes of respiratory failure as a result of severe airway obstruction. Emphysema can result in significant disability due to dyspnea, extreme restriction of physical activity, and mortality.

Airway obstruction also results from asthma, a disorder characterized by increased responsiveness of the tracheobronchial tree to various stimuli, and which leads to generalized airway constriction manifested by dyspnea, cough and wheezing. Asthma sufferers often experience acute exacerbations of bronchoconstriction, which may be life-threatening.

Another obstructive lung disease, cystic fibrosis, results from abnormal exocrine gland function. Clinical manifestations include excessive mucous secretion, hypertrophy of bronchial glands, infection, and inflammatory and structural changes in the airways which lead to obstruction and ventilation-perfusion imbalance.

Acute respiratory failure may result not only from obstructive disease, but also as a consequence of airway constriction secondary to pneumonia, thromboembolism, left ventricular failure and pneumothorax. Acute respiratory failure may also result from ventilation-perfusion imbalance.

A critical component in the treatment of airway obstruction involves the use of pharmacologic agents to remove secretions and reverse airway constriction. The most commonly used bronchodilatory agents are beta-

agonists, such as isoproterenol, given by inhalation or subcutaneous injection, and methylxanthines, such as theophylline, given orally or by infusion.

The margin of safety for theophylline administration is relatively narrow. The minimum therapeutic concentration in plasma is 6 to 10 μg/ml, and unacceptable symptoms of toxicity usually appear at or above 20 μg/ml. Still higher concentrations can lead to serious central nervous system toxicity, with long-term ingestion of theophylline being a predisposing factor in such toxicity. Moreover, because the clearance of theophylline is influenced by genetic, developmental and environmental factors to a significant degree, it is necessary to titrate the dosage cautiously against clinical observations of beneficial or toxic effects, with periodic determination of the concentration of the drug in plasma (Gilman A.G., *The Pharmacological Basis of Therapeutics,* Pergamon Press, New York, (1990)).

Isoproterenol, a non-selective β-agonist, produces cardiovascular side effects such as palpitations, sinus tachycardia and more serious arrhythmias. In addition, tolerance to this drug may result from overuse (Gilman A.G., *The Pharmacological Basis of Therapeutics,* Pergamon Press, New York, (1990)). This characteristic reduces its usefulness in patients with chronic obstructive disease who rely heavily on frequent use of bronchodilators. It has now been demonstrated that β agonists may have long term deleterious effects which result in aggravation of asthma, and ultimately change the natural history of the disease. Consequently, the American Thoracic Society no longer recommends treatment with β agonists as first line therapy in mild asthma (Expert Panel Recommendation, *New England Journal of Medicine, 325:*425-426 (1991)).

The use of S-nitrosothiols for the treatment of airway obstruction provides significant advantages over current methods of treatment. The use of S-nitrosothiols eliminates the untoward side effects associated with β-agonists and methylxanthines. S-nitrosothiols also potently inhibit platelets and neutrophils which have been implicated in the pathogenesis of asthma.

Furthermore, because all current treatment methods act by way of cAMP, S-nitrosothiols satisfy the need for bronchodilators which act by way

of cGMP. This is important because current evidence provided by the inventors demonstrates a role for cyclic GMP in regulation of airway tone in humans (See Example 1). In addition, cyclic GMP agonists act synergistically with cyclic AMP agonists to provide bronchodilation, not obtainable by individual agents.

The inventors have also demonstrated that S-nitrosothiols also cause relaxation of smooth muscle by a cGMP-independent mechanism. Another mechanism by which bronchodilation is effected provides an opportunity for combination therapy, because the independent mechanisms have potential for synergy.

A significant advantage of S-nitrosothiols is that they deliver NO in its most biologically relevant, and non-toxic form. This is critical, because the pharmacological efficacy of NO, particularly in airways, depends upon the form in which it is delivered. As demonstrated by the inventors, S-nitrosothiols can deliver NO as charged species, nitrosonium (NO^+) or nitroxyl (NO^-), as opposed to the uncharged NO radical (NO•). This is important because the charged species behave in a very different manner from NO• with respect to chemical reactivity.

In contrast to NO•, nitrosonium and nitroxyl do not react with O_2 or O_2 species to produce toxic oxides of nitrogen, and are also resistant to decomposition in the presence of redox metals. Consequently, administration of these NO equivalents does not result in the generation of toxic by-products, or elimination of the active NO moiety. Thus, by delivering nitrosonium or nitroxyl, S-nitrosothiols provide a means for achieving the smooth muscle relaxant effects of NO, and at the same time, alleviate significant adverse effects previously associated with NO therapy. S-nitrosothiols may also be used as a means to deliver free NO in a stable and non-toxic form, for use in free NO therapy.

In addition, to causing bronchodilation, S-nitrosothiols may also be used to increase the oxygen-binding capacity of hemoglobin. Hemoglobin is a globular protein, which binds reversibly to blood oxygen through passive diffusion from entry of air into the lungs. Hemoglobin-oxygen binding greatly

increases the capacity of the blood to transport oxygen to bodily tissues; thus, the binding affinity between hemoglobin and oxygen is a critical factor in determining the level of oxygen transport to the tissues. The inventors have demonstrated that S-nitrosothiols do not react with the iron-binding site of hemoglobin, as does NO•, but instead, bind to the thiol group. Thus, methemoglobin formation is prevented and hemoglobin-oxygen binding is unimpaired.

Furthermore, the inventors have also demonstrated that S-nitrosothiols not only prevent impairment, of binding, but actually increase hemoglobin-oxygen binding. Therefore, S-nitrosothiols may be used to increase the oxygen-carrying capacity of the blood, and oxygen transport to bodily tissues. As a result, these compounds may be useful in the treatment of disorders which are associated with insufficient oxygen transport, or in clinical situations in which increased oxygen transport is needed. Examples of such clinical situations include, but are not limited to, hypoxic disorders resulting from pneumothorax, airway obstruction, paralysis or weakness of the respiratory muscles, inhibition of respiratory centers by drugs or other agents, or other instances of decreased pulmonary ventilation. Additional clinical indications include impaired alveolar gas diffusion such as occurs in interstitial fibrosis, bronchiole constriction, pulmonary edema, pneumonia, hemorrhage, drowning, anemias, arteriovenous shunts.

Finally, the inventors have demonstrated that S-nitrosothiols mediate the activity of vasoactive intestinal peptide (VIP), an important airway relaxant. This reinforces the importance of S-nitrosothiols in regulation of airway tone. Deficiency in the effect of VIP is a causal factor in the pathogenesis of asthma. Administration of S-nitrosothiols replenishes the mediator itself rather than a less biologically active derivative.

S-nitrosothiols are also suitable for direct instillation into the lungs by bronchoscopic means. This topical administration permits titration of dose, eliminates the untoward side effects of systemic therapy, and enables the use of combination therapy, involving a topical S-nitrosothiol in conjunction with a systemic agent, in problematic cases. This topical therapy would also

facilitate endoscopy by suppressing the cough reflex and associated bronchospasm.

An important component in the treatment of airway obstruction is the removal of airway mucous. Thus, airway obstruction often necessitates the administration of a mucolytic agent in conjunction with the bronchodilator. N-acetylcysteine, more commonly known as "Mucomist", is one such agent. S-nitroso-N-acetylcysteine, a particular S-nitrosothiol, is advantageous because it possesses both mucolytic and bronchodilator capabilities.

With respect to combined bronchodilator-mucolytic agents, the mucolytic activity of the compound depends upon the amount of thiol which is preserved after NO delivery. Thus, S-nitrosothiol compounds which contain more than one thiol (dithiol compounds) are particularly suitable for achieving mucolysis. In addition, the long-chain lipophilic S-nitrosothiols which contain more than one thiol are advantageous as mucolytic agents because they have a free thiol, and their lipophilic property facilitates penetration of the compound into the lipid portion responsible for the tenacious viscosity of mucous.

In addition to the treatment or prevention of respiratory disorders, S-nitrosothiols may also be used to facilitate diagnostic and therapeutic bronchoscopy. The term "bronchoscopy" refers to the procedure in which a flexible fiberoptic, or rigid bronchoscope is introduced into the tracheobronchial tree for the purpose of bronchial visualization, lung biopsy or brushings, aspiration of secretions, and delivery of pharmacological agents.

A complication of bronchoscopy, and thus an impediment to the successful completion of the procedure, is bronchospasm. Patients with a prior history of bronchospasm are particularly at risk for acute enhancement of spasm. Thus, S-nitrosothiols may also be used to relax airway smooth muscle and eliminate bronchoscopy-induced bronchospasm.

Another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to an animal to relax gastrointestinal smooth muscle. The term "gastrointestinal smooth muscle" refers to smooth muscle which is contained in all areas of the

gastrointestinal tract. Such areas include, but are not limited to, the esophagus, duodenum, sphincter of Oddi, biliary tract, ileum, sigmoid colon, pancreatic duct and common bile duct. S-nitrosothiols may be used for the treatment or prevention of gastrointestinal disorders. Disorders of the gastrointestinal tract include achalasia (spasm of the lower esophageal sphincter), diarrhea, dumping syndrome, and irritable bowel.

An additional embodiment of the invention relates to the administration of S-nitrosothiols to alleviate contraction or spasm of gastrointestinal smooth muscle, and thus facilitate successful completion of endoscopic procedures. Contraction or spasm of gastrointestinal smooth muscle imposes a technical obstacle which must frequently be overcome in order to enable the clinician to successfully perform endoscopic procedures.

The term "endoscopic procedures" refers to those diagnostic procedures which utilize an instrument which is introduced into the gastrointestinal tract to provide direct visualization of the gastrointestinal tract, for examination and therapeutic purposes. Such purposes include direct visualization, biopsy, access to the common bile duct, fluid aspiration and removal of foreign bodies, polyps, and other lesions. An example of a particular endoscopic procedure is esophagogastro-duodenoscopy, which is utilized for examination of the esophageal lumen, stomach and duodenum. Another example, endoscopic retrograde cholangiopancreatography (ERCP), enables visualization of the pancreatic duct, common bile duct and the entire biliary tract, including the gall bladder. Further examples of endoscopic procedures are colonoscopy and sigmoidoscopy.

Current methods for alleviating gastrointestinal muscle spasm include the administration of intravenous diazepam, anticholinergics and glucagon, as well as sublingual administration of nitroglycerin. However, these methods produce generalized, systemic effects which persist for a much longer duration than the procedure itself. In addition, nitroglycerin is significantly less effective as a smooth muscle relaxant than S-nitrosothiols, and produces systemic side effects, the most significant of which is hypotension. It is therefore, not used clinically. Clearly, a need exists for a topical smooth

muscle relaxant which could be directly instilled into the various regions of the gastrointestinal tract to facilitate both diagnostic and therapeutic endoscopic procedures.

Patient studies, conducted by the inventors, have measured the efficacy of S-nitrosothiols both in facilitating cannulation of the sphincter of Oddi, and in decreasing colon motility to allow for removal of colon polyps. As shown in Figure 1, topical administration of S-nitroso-N-acetylcysteine eliminated duodenal motility. As shown in Figure 2, topical administration of S-nitroso-N-acetylcysteine inhibited the contractile activity of the Sphincter of Oddi, and thus, permitting successful endoscopic cannulation of the sphincter. In addition, administration of S-nitroso-N-acetylcysteine eliminated colon motility to facilitate successful removal of colon polyps. Notably, the relaxant effects were temporary (lasting only for the duration of the procedure), completely reversible and produced no change in systemic blood pressure, heart rate or oxygen saturation. The same type of effects would occur with the use of other cell impermeable S-nitrosothiols, such as S-nitroso-glutathione.

Prior to the present invention, there were no available pharmacological agents which could be applied directly by endoscopic means to exert a direct, immediate, localized, and completely reversible relaxant effect on gastrointestinal smooth muscle. Topical administration of S-nitrosothiols, during endoscopy, eliminates systemic side effects and allows for the use of the lowest effective concentration of the drug.

Administration of S-nitrosothiols obviates the need for sphincterotomy, a procedure which substantially increases the morbidity and mortality of ERCP. In addition, administration of S-nitrosothiols aids in the cannulation and manipulation of the pancreatic duct and biliary tract during therapeutic procedures such as gall bladder cannulation, bile duct stone removal and stint placement, and decreases the incidence of post-ERCP complications, such as pancreatis and cholangitis. Another use of S-nitrosothiols involves the intraoperative injection of these compounds into the gall bladder prior to cholecystectomy to alleviate cystic duct spasm. This would allow for a laparoscopic cholangiogram by providing access to the cystic duct. In addition

to the uses discussed above, S-nitrosothiols may also be administered to treat or prevent any other technical problems associated with endoscopy which are known to those in the medical art.

Another embodiment of the invention relates to administration of a therapeutically effective amount of an S-nitrosothiol compound to relax corpus cavernosum smooth muscle. The term "corpus cavernosum" refers to two areas of smooth muscle which lie side by side on the dorsal aspect of the penis, and together with the corpus spongeosum that surrounds the urethra, constitute erectile tissue. This erectile tissue consists of an irregular sponge-like system of vascular spaces interspersed between arteries and veins. Erection occurs when cavernosa smooth muscle relaxation causes a decease in arterial resistance and resulting increase in arterial blood flow to the penis.

Smooth muscle has a critical role in erectile function Thus, another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound for the treatment of impotence. "Impotence" refers to a condition of male sexual dysfunction which is characterized by the inability to obtain or maintain an erection.

Organic causes of erectile impotence, may include endocrine, drug-induced, local injury, neurologic, and vascular. In particular, impotence may result from neurologic blockade caused by such drugs as antihistamines, antihypertensives, psychogenic agents, and anticholinergics. Impotence may also result from neurologic disorders such as interior temporal lobe lesions, spinal cord disorders, and insufficiency of sensory input resulting from diabetic neuropathy. An additional cause of impotence is insufficient blood flow into the vascular network resulting from an intrinsic defect, or from penile trauma.

Currently available methods for treating impotence consist largely of surgical techniques and intracavernosal injections of pharmacological agents. One surgical technique involves the implantation of a penile prosthesis by inserting within the corpora, a small silastic rod. However, this method does not produce full erection and the complication rate is high. Alternatively, an inflatable prosthetic device may be implanted on either side of the corpora,

with a connecting reservoir of material placed in the perivascular space. Erection is achieved through the use of pumps which are located in the scrotum.

Intracavernous injection of the smooth muscle relaxant, papaverine has been used to induce erections. However, a significant disadvantage of this treatment method is the need for a painful injection each time an erection is desired. In addition, numerous side effects and complications result from the chronic use of drugs such as papaverine. Clinical reports indicate that a significant proportion of potential candidates refuse these injections from the onset of treatment. A larger number of patients, even after favorable initial response to the treatment, become increasingly unresponsive or unwilling to continue injections as a means of treatment (Morales *et al.*, *World J. Urol.* 8:80-83 (1990)).

In general, a significant number of patients who are potential candidates for current methods of impotence treatment refuse initially because of the invasive nature of the treatment, or reject further treatment because of pain, fibrosis, or dissatisfaction with results.

As demonstrated by the discussion above, prior to the present invention, there was a significant need for a less invasive approach to the treatment of impotence. Because they exert a relaxant effect on corpus cavernosal smooth muscle, S-nitrosothiols are particularly well suited for the treatment of impotence.

Administration of S-nitrosothiols results in relaxation of corpus cavernosum smooth muscle, which leads to dilation of the cavernosal arteries and a concommittent increase in blood flow. S-nitrosothiols provide significant advantages in the treatment of impotence over current treatment methods, because they can be administered topically, thereby eliminating the systemic side effects, significant discomfort, fibrosis, and ineffectiveness associated with the currently available, invasive methods of treatment.

Another embodiment of the claimed invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to relax bladder smooth muscle. Bladder smooth muscle includes

that of the bladder base, bladder body and proximal urethra. In addition, S-nitrosothiols may be used for the treatment of bladder dysfunction disorders which involve relaxation of bladder smooth muscle. Such disorders include, but are not limited to, problems with bladder filling, volume and continence.

In addition, S-nitrosothiols may be administered to cause relaxation of urethral and bladder base smooth muscle, and thus, facilitate cystoscopic examination of the urinary tract. The term "cystoscopic examination" refers to the introduction of a fiberoptic instrument through the urethra and into the bladder, to achieve visualization of the interior of the urethra and bladder for diagnostic and therapeutic purposes.

Another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to relax uterine smooth muscle. Increased contractility of uterine smooth muscle precipitates premature labor. Thus, an additional embodiment of the invention relates to the administration of S-nitrosothiol compounds for the treatment or prevention of premature labor.

S-nitrosothiols may also be used to relax fallopian tube smooth muscle. Fallopian tube smooth muscle plays a role in the transport of the egg to the uterus. Thus, S-nitrosothiols may be used to regulate ovum transport, or to facilitate laparoscopic examination of the fallopian tubes, or to facilitate fertilization procedures.

The long-chain lipophilic compounds have unique potential for NO delivery by incorporation into cell membranes, and for accessing the central nervous system (CNS). In the CNS, nitric oxide has been shown to inhibit cell death resulting from ischemic injury, as well as to possess neurotransmitter functions. Membrane permeability achieved by these compounds also provides the unique potential for NO delivery in every organ system. In addition, NO delivery can be regulated by the incorporation of additional functional groups into the molecule. Each functional group, including but not limited to nitrite, nitrate, redox metal, amine, aromatic, and basic amino acids, has its own unique functional aspects which will affect (a)

a targeted site of delivery (b) rate of NO release (c) lipophilicity (d) cell permeability (e) duration of action (f) bioactivity and (g) nitrosation potential.

An additional embodiment of the invention relates to the administration of an S-nitrosothiol compound as part of a pharmaceutical composition, comprising a pharmaceutically acceptable carrier, to achieve the physiological effects discussed above.

The pharmaceutical compositions utilized in this invention can be administered by intranasal, oral, enteral, topical, sublingual, rectal, intramuscular, intravenous, or subcutaneous means. The compositions may be administered by medical instrumentation including, but not limited to, bronchoscopic, endoscopic, laporascopic, and cystoscopic means. With respect to the administration of these composition for the treatment of impotence, the term "topical" includes administration in the form of a condom which contains the pharmaceutical composition. Certain S-nitrosothiols, such as lipophilic S-nitrosothiols, are especially suitable for (i.e. lipophilic) incorporation into the condom itself, to provide sustained release of the compound.

The compounds of this invention can be employed in combination with conventional excipients; i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

For parenteral application, particularly suitable vehicles consist of solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees or capsules having talc and/or a carbohydrate carrier binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or the like can be used wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active component is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

It will be appreciated that the actually preferred amounts of active compounds used will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application and the particular site of administration. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art, using conventional dosage determination tests conducted with regard to the foregoing guidelines.

According to the present invention, a "therapeutically effective amount" of a pharmaceutical composition is an amount which is sufficient to achieve the desired pharmacological effect. Generally, the dosage required to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art, will vary, depending upon the age, health, physical condition, sex, weight and extent of disease, of the recipient. Additionally, the dosage may be determined by the frequency of treatment and the nature and scope of the desired effect.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire text of all publications cited above and below are hereby incorporated by reference.

EXAMPLES

Example 1. Airway Smooth Muscle Relaxation by S-nitrosothiols

A. Methods

1. Materials

Glutathione, L-cysteine, DL-homocysteine, D-penicillin, hemoglobin (bovine), methylene blue and Medium 199 sets were purchased from Sigma Chemical Co., St. Louis, MO. N-acetylcysteine was obtained from Aldrich Chemical Co., Milwaukee, WI. Captopril was kindly provided by Dr Victor Dzau. Sodium nitrite, histamine and methacholine were purchased from Fisher Scientific, Fairlawn, NJ. Leukotriene D_4 was purchased from Anaquest, BOC Inc., Madison, WI. Antibiotic/antimycotic mixture (10,000 U/ml penicillin G sodium, 10,000 mcg/ml, streptomycin sulfate, 25 mcg/ml amphotericin B) was purchased from Gibco Laboratories, Grand Island, NY. Radioimmunoassay kits for the determination of cyclic GMP were purchased from New England Nuclear, Boston, MA.

2. Preparation of Airways

Male Hartley guinea pigs (500-600g) were anesthetized by inhalation of enflurane to achieve a surgical plane of anesthesia. The trachea were excised and placed in Kreb's-Henseleit buffer (mM): NaCl 118, CKl 5.4, NaH_2PO_4 1.01, glucose 11.1, $NaHCO_3$ 25.0, $MgSO_4$ 0.69, CaCl 2.32, pH 7.4. The airways were then dissected free from surrounding fat and connective tissue and cut into rings 2-4 mm in diameter. The trachea rings were placed in sterile Medium 199 containing 1% antibiotic/antimycotic

mixture in an atmosphere of 5% CO_2, 45% O_2, 55% N_2, and kept for up to 48 hours in tissue culture. The experiments were also performed on human airway smooth muscle, isolated by the same method.

3. Preparation of Blood Vessels

New Zealand White female rabbits weighing 3-4 kg were anesthetized with 30 mg/kg IV sodium pentobarbital. Descending thoracic aortic were isolated and placed immediately in a cold physiologic salt solution (Kreb's) (mM): NaCl 118, CCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 12.5, and D-glucose 11.0, pH 7.4. The vessels were cleaned of adherent connective tissue, and the endothelium removed by gentle rubbing with a cotton tipped applicator inserted into the lumen, and cut into 5 mm rings.

4. Preparation of S-nitrosothiols

S-nitrosothiols were prepared at 25°C by reacting equimolar (100 μM) concentrations of reduced thiols with $NaNO_2$ in 0.5 N HCl (acidified $NaNO_2$) as described previously (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)). Solutions turned from clear to various shades of red instantaneously upon product formation, with the notable exception of S-nitroso-penicillamine, which is green.

In this method of synthesis, the reaction of thiols with NO (generated from sodium nitrite) is complete and stoichiometric (Aldred *et al., J. Chem. Soc. Perkin Trans. II*:777-782 (1982); Byler *et al., J. Agric. Food Chem. 31*:523-527 (1983)).

The long-carbon chain lipophilic nitrosothiols, long and short chain S-nitrosodithiols, and S-nitrosothiols with additional functional groups were synthesized by one or more of the following methods: (a) exposure to equimolar N_2O_3 or N_2O_4 in CCl4; (b) exposure to equimolar acidified nitrite;

(c) exposure to equimolar bubbled NO gas; (d) exposure to excess cold bubbled NO_2 gas; and (e) exposure to metherolic acid or equimolar $NaNO_2$ diluted in methersol.

The synthesis of S-nitroso-homocysteine has not been previously characterized. This compound displayed the distinct absorption maxima of other S-nitrosothiols at approximately 340 nm and 550 nm (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)). The molar absorptivity of S-nitroso-homocysteine at 547 nm is 16.7 $cm^{-1}M^{-1}$ and correlates well with published values of 16.6 and 16.1, for S-nitro-cysteine and S-nitroso-glutathione, respectively (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990)).

Owing to the modest decay of S-nitrosothiols over time, fresh examples were made at hourly intervals and kept at 4°C until use. Solutions were diluted as necessary into physiologic buffer immediately prior to each experiment.

5. Bioassay

Trachea and aortic rings were mounted on stirrups and connected to transducers (model FOT3C Grass) with which changes in isometric tension were measured. Rings were then suspended in 10 cc of oxygenated (95% O_2, 5% CO_2) buffer. Conditions for both the vessel and airway bioassays were established according to standard methodologies as described in Cooke *et al., Am. J. Physiol. 259*(3):H804-H812 (1990).

In airway experiments, the rings were equilibrated for 60 minutes under a load of 1 gm and then primed twice by exposure to 100 μM methacholine. Tissues were contracted with various agonists at concentrations determined to generate 50% (± 16% S.D.) of maximum tone, after which the effects of different thiols and their S-nitrosylated derivatives were assessed. In selected experiments, relaxation responses were determined in the presence

of hemoglobin, or after rings had been preexposed to methylene blue for 30 minutes.

In vessel experiments, aortic rings were contracted with 1 μM epinephrine and relaxations were induced with S-nitrosothiols.

6. Cyclic Nucleotide Assays

The mechanism by which S-nitrosothiols relax vascular smooth muscle is felt to be through activation of guanylate cyclase with consequent increase in intracellular cyclic GMP (Ignarro *et al., Circ. Res. 65*:1-21 (1989); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989)). In order to assess this mechanism in airways, tracheal rings in Kreb's-Henseleit solution were exposed to 100 μM S-nitroso-N-acetylcysteine (SNOAC) for 90 seconds. Reactions were terminated by the addition of ice cold 10% trichloracetic acid and rapid freezing in ethanol-saturated dry ice.

In selected experiments, rings were preexposed to the guanylate cyclase inhibitor, methylene blue (10^{-4} M) for 30 minutes. Tissues were then individually pulverized with a glass (s) homogenizer and centrifuged at 8000 g for 5 minutes. The clear supernatant was extracted with water-saturated ether and assayed for cyclic GMP by radioimmunoassay. Acetylation of samples with acetic anhydride was used to increase the sensitivity of the assay and the determination of recoveries was aided by the use of [^{3}H] cyclic GMP.

Dose-response relationships to SNOAC were obtained in airways contracted with 3 uM histamine, and repeated in the presence of 10^{-4} M hemoglobin, 10^{-5} M methylene blue, and 10^{-4} M methylene blue. Relaxation responses to SNOAC are inhibited by hemoglobin and methylene blue, with the latter in a dose-dependent manner. Cyclic GMP determinations were performed in duplicate for each experiment.

7. Statistics

All results are presented as means ± SEM. Paired samples were compared by the Student's t-test. Dose-response curves were compared by two-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered significant.

Table 1

Inhibitory Concentrations Inducing 50% Relaxation (IC50)

RS-NO	IC50 mean ± S.D.; x 10^{-6} M)
S-nitroso-glutathione	0.99 ± 2.0
S-nitroso-cysteine	3.2 ± 0.2
S-nitroso-homocysteine	2.1 ± 0.3
S-nitroso-N-acetylcysteine	2.1 ± 0.8
S-nitroso-penicillamine	1.8 ± 0.8
S-nitroso-captopril	20.0 ± 0.7

B. Results and Discussion

The mammalian fraction of sulfur that exists as free sulfhydryl is contained largely in the form of glutathione, cysteine, and homocysteine (Jocelyn, P.C., In *Biochemistry of the SH Group*, Academic Press, London/New York pp. 1-46 (1972)). N-acetylcysteine is a minor metabolite of cysteine that is used for its mucolytic properties in the treatment of airway obstruction. N-acetylcysteine has also received attention within the context of nitrate metabolism and undergoes S-nitrosylation in plasma upon treatment with nitroglycerin (Fung *et al., J. Pharmacol. Exp. Ther. 245(2)*:524-531 (1988)). The S-nitrosylated derivatives of these four sulfhydryls comprise the group of biological S-nitrosothiols under investigation.

Captopril and penicillamine are examples of nonbiological low molecular weight thiols, and their S-nitrosylated derivatives have been well characterized (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981).

An initial examination of the relaxant activity of each of the biological and nonbiological S-nitrosothiols in guinea pig tracheal rings was conducted. The results are shown in Figures 3 and 4(a)-(f). As demonstrated by dose-response relationships, these compounds are potent airway smooth muscle relaxants, with relaxant effects that are unmatched by equimolar amounts of reactant thiol or NO (generated from $NaNO_2$ alone).

In every case, the dose-response curves for the S-nitrosothiols were significantly different from the dose-response curves for NO and for the individual thiols by two-way ANOVA to $p < 0.001$. Results are presented as mean ± SEM, ($n = 5$).

With the exception of S-nitroso-captopril (SNOCAP), the S-nitrosothiols revealed comparable bioactivity with IC50s in the range of 1×10^{-6} M (Table 1). SNOAC and SNOCAP were then selected as representative biological and nonbiological S-nitrosothiols for further detailed investigation.

Dose-effect relationships were obtained for SNOAC and SNOCAP using tracheal rings induced to constrict with leukotriene D_4, histamine, and methacholine. As shown in Figure 5, airways exhibited agonist specificity toward S-nitrosothiol-mediated relaxations: S-nitrosothiols were most active for relaxation of leukotriene D_4-induced contractions and progressively less effective with contractions induced by histamine and methacholine. In every case, SNOAC was approximately 10-fold more active in relaxation of airways than SNOCAP. Results are presented as mean ± SEM (n=3-5).

The time course of relaxation to SNOAC is shown in Figure 6. Using a concentration (5×10^{-6} M) selected to induce approximately 50% of the maximal response, maximal relaxation occurred by five minutes and a

significant residual loss of tone was still evident at one hour. In control experiments, airway contractions remained stable over the study period.

These relaxation responses contrast markedly with those generally ascribed to low-molecular-weight S-nitrosothiols. Figure 7 illustrates the notable difference in relaxation kinetics between these tissues. In vascular smooth muscle, the relaxations are rapid and transient, whereas in tracheal smooth muscle, relaxations occur more slowly and persist for a much longer duration.

Figure 8 shows a comparison between the efficacy of SNOAC and isoproterenol or theophylline under identical study conditions. Of the drugs evaluated, isoproterenol was the most active relaxant, however, SNOAC was approximately 50 times more active in relaxation than theophylline. The dose response curves for these agents are each significantly different from each other by two-way ANOVA to $p < 0.01$. Results are expressed as mean ± SEM (n=3-5).

Hemoglobin and methylene blue are established inhibitors of NO-induced relaxations in vascular smooth muscle (Ignarro *et al., Circ. Res.* 65:1-21 (1989)). When their effects were examined in guinea pig airways, hemoglobin and methylene blue each partially attenuated (only 10-20% attenuation)the actions of SNOAC, as evidenced by rightward shifts in the dose-effect relationships to SNOAC in their presence (Figure 9). In human airways, neither hemoglobin or methylene blue attenuated the relaxation effect. The dose-response curves for SNOAC were significantly different from each of the curves derived in the presence of hemoglobin and methylene blue by two-way ANOVA to $p=0.05$. Results are presented as mean ± SEM (n=3-5).

The biochemical mechanism of action of S-nitrosothiols was further investigated in isolated tracheal rings. As shown in Figure 10, tracheal rings incubated with SNOAC exhibited 4-fold increases in cyclic GMP over basal levels (control). Increases in cyclic GMP were attenuated by pretreatment of tissues with the guanylate cyclase inhibitor, methylene blue (10^{-4}M). Cyclic GMP levels in the presence of SNOAC were significantly greater than control

values ($p < 0.0005$) and levels determined in the presence of methylene blue ($p = 0.05$). Results are presented as mean ± SEM ($n = 4$-8).

An examination of the relaxant activity of S-nitrosothiols in human tracheal rings was also conducted. The results are shown in Figures 11-15. In particular, Figure 11 shows that S-nitroso-glutathione has a relaxant effect upon human trachea which is significantly greater than nitrite (NO_2). Figure 12 demonstrates that the relaxant effect of S-nitrosoglutathione upon human trachea is significantly greater than glutathione alone. This data underscores the fact that the bioactivity of nitric oxide in airways depends upon the form in which it is delivered. S-nitrosothiols provide efficient delivery of NO in its most bioactive and non-toxic form.

Figure 13 demonstrates that the relaxant effect of SNOAC upon human trachea is significantly greater than that of N-acetylcysteine. As shown in Figure 13, NAC caused significant constriction of the tracheal smooth muscle, which is consistent with the fact that NAC, when given as a mucolytic agent, causes the untoward side effect of bronchospasm. SNOAC not only causes relaxation of airway tissue, but also eliminates bronchospasm.

Figure 14 demonstrates that SNOAC and SNOGSH exert a relaxant effect on airway smooth muscle which is significantly more potent than that of theophylline, and compares favorably with that exerted by isoproterenol.

Experiments were also conducted to assess the cGMP response to SNOAC in human airways. As shown in Figure 15, tracheal rings incubated with SNOAC exhibited 4-fold increases in cyclic GMP over basal levels (control).

Unexpectedly, the relaxation response to low molecular weight S-nitrosothiols in airways differs markedly from that observed in blood vessels. In the latter case, relaxations occur slowly and persist for a much longer duration. This is most likely attributed to the inherent differences between vascular and nonvascular smooth muscle. There may be additional contributing factors responsible for this heterogeneity. Finally, any disparity among smooth muscle cells in redox state, availability of reducing equivalents,

pH, oxygen tension, or any other factor that might influence the stability of the S-NO bond would be predicted to influence relaxation kinetics.

The results also suggests that, in addition to the primary site of obstruction in the lung, the efficacy of nitro(so)-bronchodilators may be determined by the nature of the chemical mediators contributing to bronchoconstriction. In particular, S-nitrosothiols were most effective in this study against leukotriene D_4-mediated bronchoconstriction and progressively less effective against histamine and methacholine-mediated constriction. Thus, regional variation in guanylate cyclase content or activity, the site of obstruction, the form in which the active species of NO is administered, and the nature of the bronchoconstrictor stimuli are all variables which may influence the determination of nitro(so)-bronchodilator efficacy and the importance of guanylate cyclase in regulating airway tone.

Example 2. Guanylate Cyclase Inhibitors Do Not Inhibit S-nitrosothiol Induced Relaxation in Human Airways

The effect of guanylate cyclase inhibitors upon S-nitrosothiol-induced airway relaxation and cGMP increase was assessed, using the previously described bioassay and cyclic nucleotide assay procedures. Bronchodilator effects of S-NOGSH and SNOAC were examined in human airways (5-12 mm outer diameter). Concentration-response relationships for rings contracted with methacholine (7μM) resulted in IC50 values of 22 μM, approximately two orders of magnitude greater than theophylline.

SNOAC (100 mm) induced 4-fold increases ($P < 0.02$), over control airway cGMP levels, as shown in Table 3. However, as shown in Figure 16, SNOAC-induced airway relaxation was not significantly inhibited by methylene blue (10^{-4}) or LY83583 (5×10^{-5}). Similarly, hemoglobin (100 μM) had little effect on S-nitrosothiol-induced relaxation (P = NS).

These results demonstrate that the mechanism by which S-nitrosothiols cause airway relaxation is not due solely to increases in cGMP. Thus, S-

nitrosothiols cause airway relaxation through both increase in cyclic GMP, as well as a cGMP-independent pathway.

Example 3. S-nitrosothiols Resist Decomposition In The Presence of Redox Metals

The stability of SNOAC and SNOGSH in the presence of oxygen and redox metals was assessed. When subjected to conditions consisting of 95% 0_2, pH 7.4, the half lives of these compounds were shown to be on the order of hours, and significantly greater than that of NO, or NO•, which, under similar conditions, are on the order of seconds.

In addition, S-nitrosothiol stability was assessed in the presence of various redox metals or chelating agents. These compounds were resistant to decomposition when Cu^+, Fe^{2+}, or Cu^{2+} (50 μM) or defuroxamine or EDTA (10μM) were added. Thus, these experiments demonstrate that, unlike NO•, S-nitrosothiols are not rapidly inactivated in the presence of oxygen, nor do they decompose in the presence of redox metals.

Example 4. S-nitrosothiols Increase Hemoglobin-oxygen Binding

Additional experiments were conducted to evaluate the reaction between S-nitrosothiols and hemoglobin. S-nitrosylation of hemoglobin was accomplished by reacting 12.5 μMol hemoglobin with 12.5 μM SNOAC for 5 and 20 minute intervals (pH 6.9). S-nitrosylation was verified, using standard methods for detection of S-nitrosothiols (Saville, *Analyst 83*:670-672 (1958)). The Saville method, which assays free NO_x in solution, involves a diazotization reaction with sulfanilamide and subsequent coupling with the chromophore N-(1-naphthyl)ethylenediamine. The specificity for S-nitrosothiols derives from assay determinations performed in the presence and absence of $HgCl_2$, the latter reagent catalyzing the hydrolysis of the S-NO bond. Confirmatory evidence for S-nitrosothiol bond formation was obtained by spectrophotometry, demonstrated by the absorption maximum of 450 nm,

as shown in Figure 17. This was demonstrated using NO^+ equivalents in the form of SNOAC.

As demonstrated by Figure 18, the UV spectrum of hemoglobin incubated with SNOAC shows no reaction at the redox metal (iron-binding site) of hemoglobin, over 15 minutes. For the purposes of comparison, equimolar concentrations of hemoglobin and $NaNO_2$ were reacted in 0.5 N HCl, to form nitrosyl-hemoglobin, and the UV spectrum was obtained. As shown in Figure 19, NO reacted instantaneously with the redox metal site on hemoglobin. The fact that the S-nitrosothiol did not react with the redox metal site of hemoglobin, but with its thiol group instead, indicates that the reactive NO species donated by the S-nitrosothiol is nitrosonium or nitroxyl.

S-nitrosylation of hemoglobin does not result in the formation of methemoglobin and consequent impairment in hemoglobin-oxygen binding,. Furthermore, an additional experiment demonstrated that S-nitrosylation of hemoglobin causes a leftward shift in the hemoglobin-oxygen association curve, indicating an increase in oxygen binding. Thus, the reaction between S-nitrosothiols and hemoglobin not only eliminates the inhibition of oxygen binding which occurs from the reaction with NO•, but actually increases binding and oxygenation of the blood.

In summary, S-nitrosothiols are important intermediates in the metabolism of organic nitrates and endogenously-derived NO. Furthermore, these compounds provide greater stability, a longer half life than NO, and retain its cyclic GMP-dependent bioactivity in blood vessels.

In the present invention, the inventors have demonstrated that S-nitrosothiols are also potent airway smooth muscle relaxants and mediate their effects through both activation of guanylate cyclase, and a cGMP-independent mechanism. The results indicate that there are a number of important mediators of airway tone, including cGMP. The results also demonstrate a mechanism by which the bioactivity of NO is preserved in the presence of high ambient concentrations of oxygen and reactive oxygen species and redox metals.

In addition to the relaxant effect exerted upon airways, S-nitrosothiols also increase hemoglobin-oxygen binding, thus providing a means for enhancing oxygenation of the blood and oxygen transport to tissues. As a result of the potent effects exerted by S-nitrosothiols on airway relaxation and blood oxygenation, these compounds have significant pharmacological utility for the treatment of airway obstruction, or other disorders resulting in insufficient blood oxygenation.

TABLE 3

cGMP LEVELS IN HUMAN AIRWAYS

	cGMP (Pmol/gm)
Control	12 ± 8
SNOAC	46 ± 17*
SNOACC + M.B.	17 ± 5
$p < 0.05$ c/w control and MB (methylene blue)	

WHAT IS CLAIMED IS:

1. A compound having the formula:

$CH_3(CH_2)_2SNO$

wherein:

X equals 2 to 20.

2. A compound having the formula:

$HS(CH_2)_xSNO$

wherein:

X equals 2 to 20.

3. A compound having the formula:

$ONS(CH_2)_xY$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

4. A method for relaxing airway smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

5. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$CH_3(CH_2)_xSNO$

wherein:

X equals 2 to 20.

6. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

7. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

8. The method of claim 4 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

9. The method of claim 4 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

10. The method of claim 9 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, intramuscular, aerosol, topical or bronchoscopic delivery.

11. A method for treatment or prevention of respiratory disorders, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

12. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

13. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

14. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

15. The method of claim 11 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

16. The method of claim 11 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

17. The method of claim 16 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, intramuscular, aerosol topical or bronchoscopic delivery.

18. The method of claim 11 wherein said respiratory disorder is in the group comprised of all subsets of obstructive lung disease, including emphysema, asthma, bronchitis, fibrosis, excessive mucous secretion, obstruction of air flow, and lung disorders resulting from post-surgical complications.

19. A method for relaxing gastrointestinal smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

20. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

21. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

22. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

23. The method of claim 19 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

24. The method of claim 19 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

25. The method of claim 24 wherein said pharmaceutical composition is administered to a patient by a route comprising oral, sublingual, intravenous, topical, intramuscular, aerosol or endoscopic delivery.

26. A method for alleviating contraction or spasm of gastrointestinal smooth muscle associated with endoscopic procedures comprising, administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

27. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$CH_3(CH_2)_xSNO$

wherein:

X equals 2 to 20.

28. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$HS(CH_2)_xSNO$

wherein:

X equals 2 to 20.

29. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$ONS(CH_2)_xY$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

30. The method of claim 26 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

31. The method of claim 26 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

32. The method of claim 31 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular, aerosol or endoscopic delivery.

33. A method for relaxing corpus cavernosum smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

34. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

35. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

36. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

37. The method of claim 33 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

38. The method of claim 33 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

39. The method of claim 38 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

40. A method for the treatment or prevention of impotence, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

41. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

42. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

43. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

44. The method of claim 40 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

45. The method of claim 40 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

46. The method of claim 45 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

47. A method for relaxing bladder smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

48. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

49. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

50. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

51. The method of claim 47 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

52. The method of claim 47 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

53. The method of claim 52 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

54. A method for relaxing uterine smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

55. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

56. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

57. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

58. The method of claim 54 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

59. The method of claim 54 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

60. The method of claim 59 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

61. A method for increasing the capacity of hemoglobin to bind oxygen, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

62. A method for increasing oxygen transport to bodily tissues, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

63. A method for the treatment or prevention of disorders associated with insufficient oxygen supply to bodily tissues, comprising administering a therapeutically effective amount of an S-nitrosothiol to an animal in need thereof.

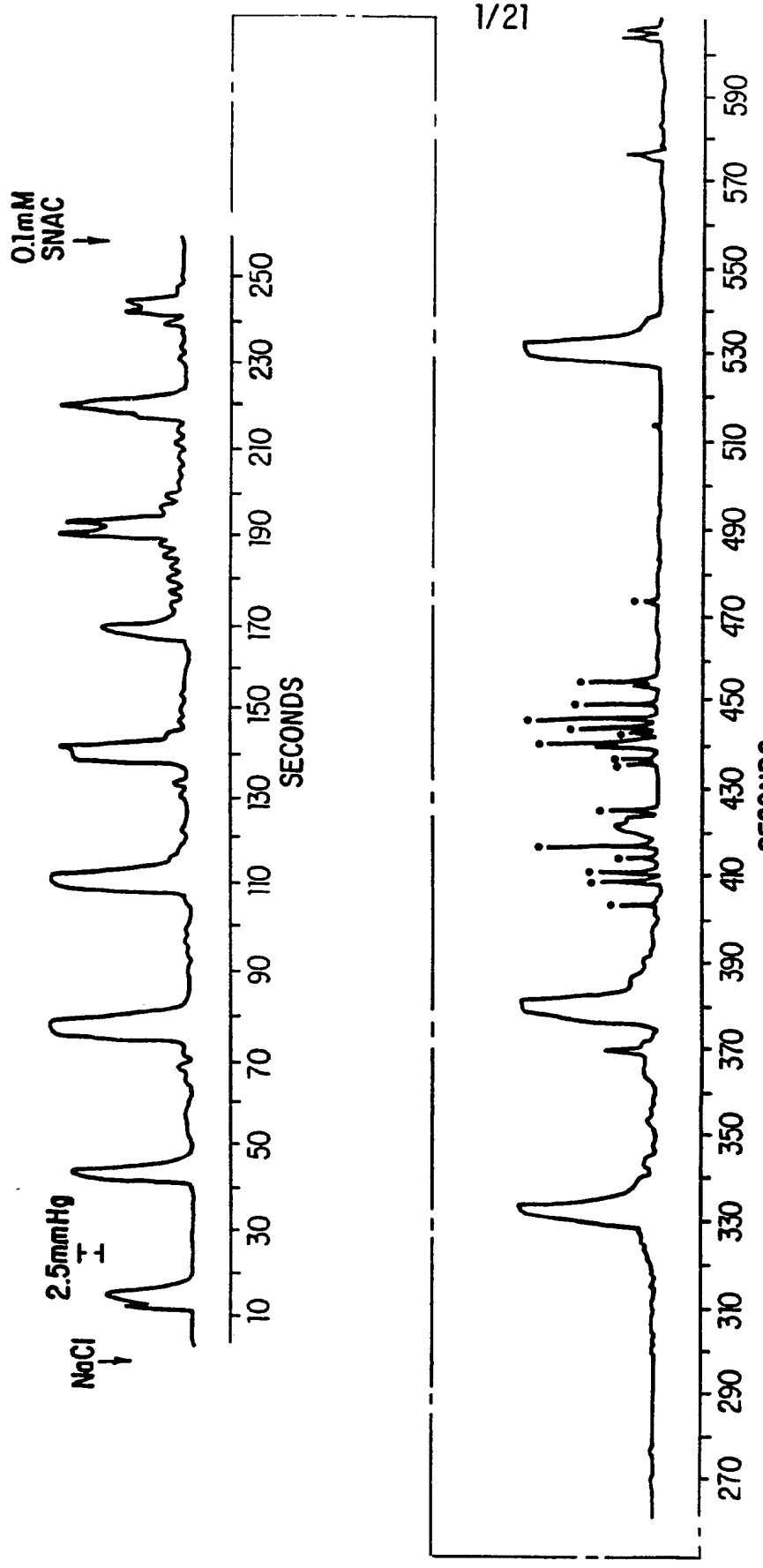
0.1mM
SNAC
2.5mmHg
NaCl
10 30 50 70 90 110 130 150 170 190 210 230 250
SECONDS
270 290 310 330 350 370 390 410 430 450 470 490 510 530 550 570 590
SECONDS
•=ARTIFACT

FIG. 1

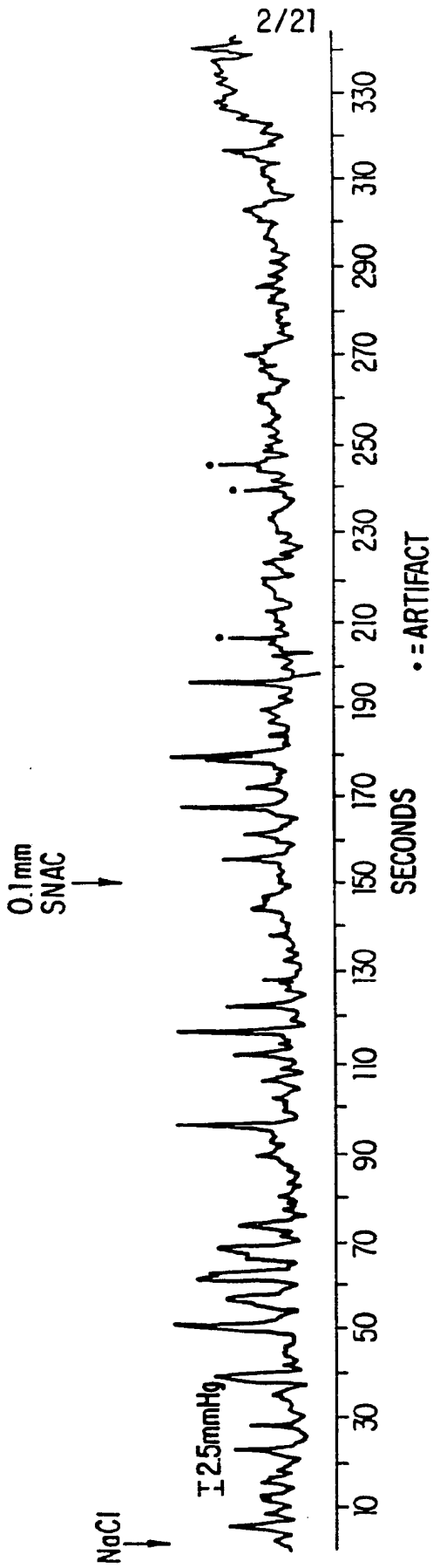
2/21
NaCl
I 25mmHg
0.1mm
SNAC
10
30
50
70
90
110
130
150
170
190
210
230
250
270
290
310
330
SECONDS
• = ARTIFACT

FIG. 2

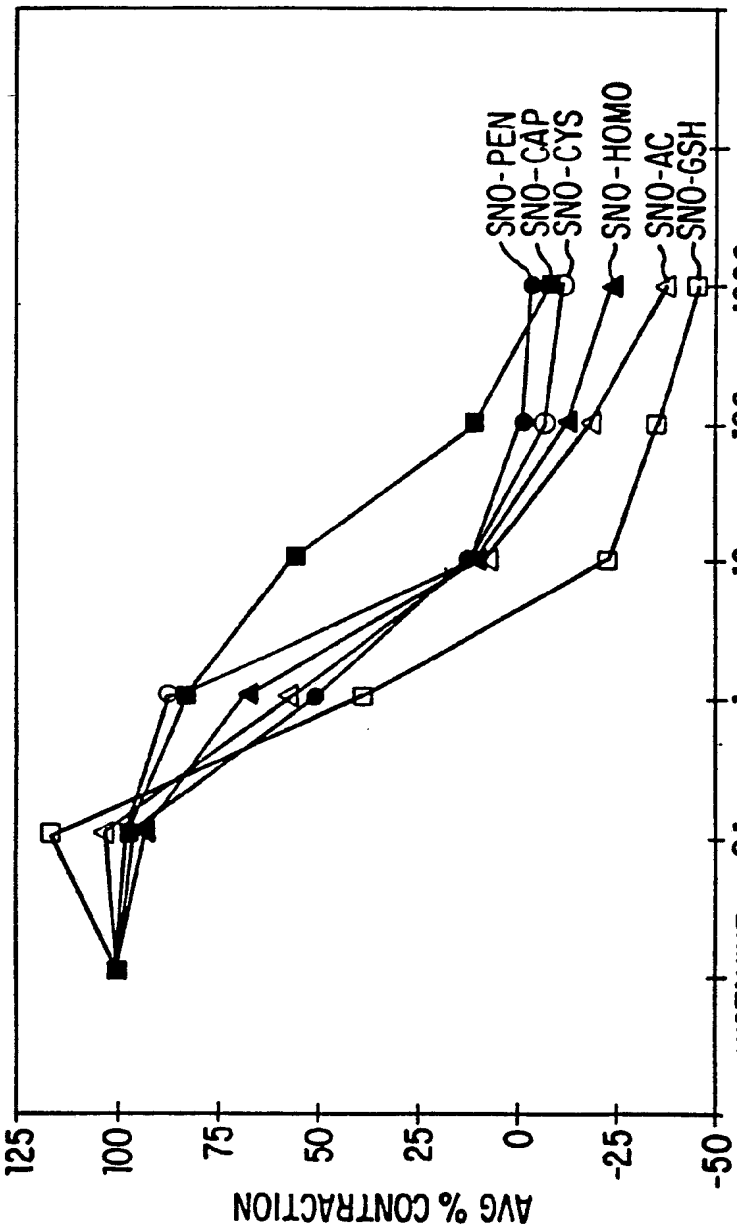
SNO-PEN
SNO-CAP
SNO-CYS
SNO-HOMO
SNO-AC
SNO-GSH
125
100
75
50
25
0
-25
-50
AVG % CONTRACTION
0.1
1
10
100
1000
HISTAMINE CONTRACTION CONCENTRATION IN MICROMOLAR

FIG. 3

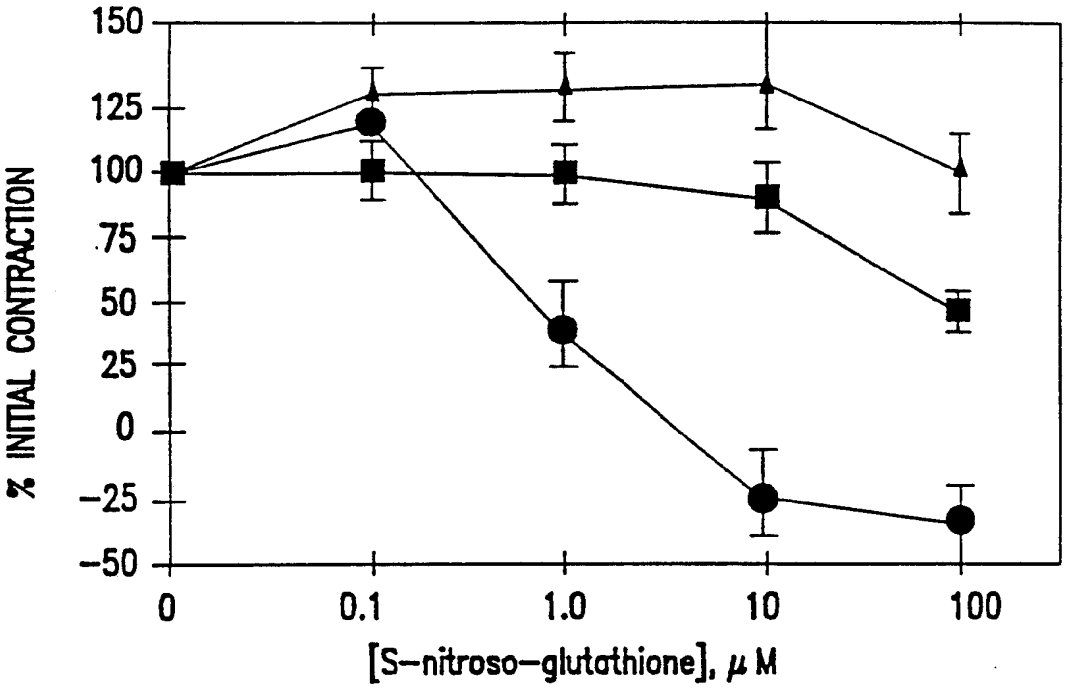
150
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−glutathione], μ M

FIG.4A

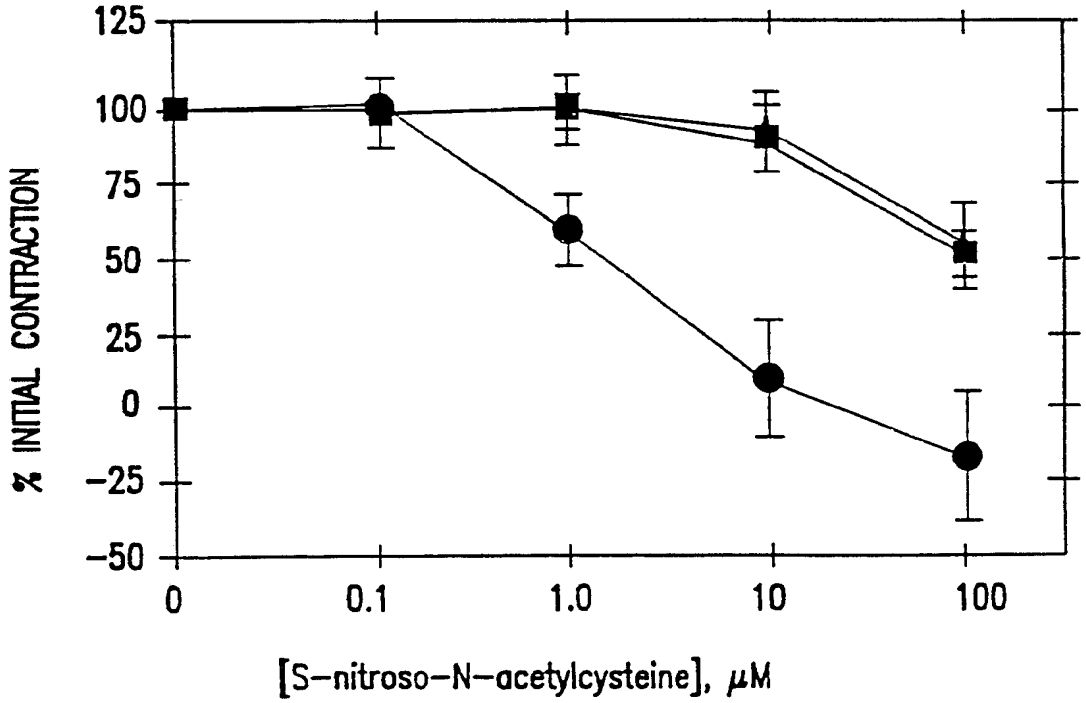
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−N−acetylcysteine], μM

FIG.4B

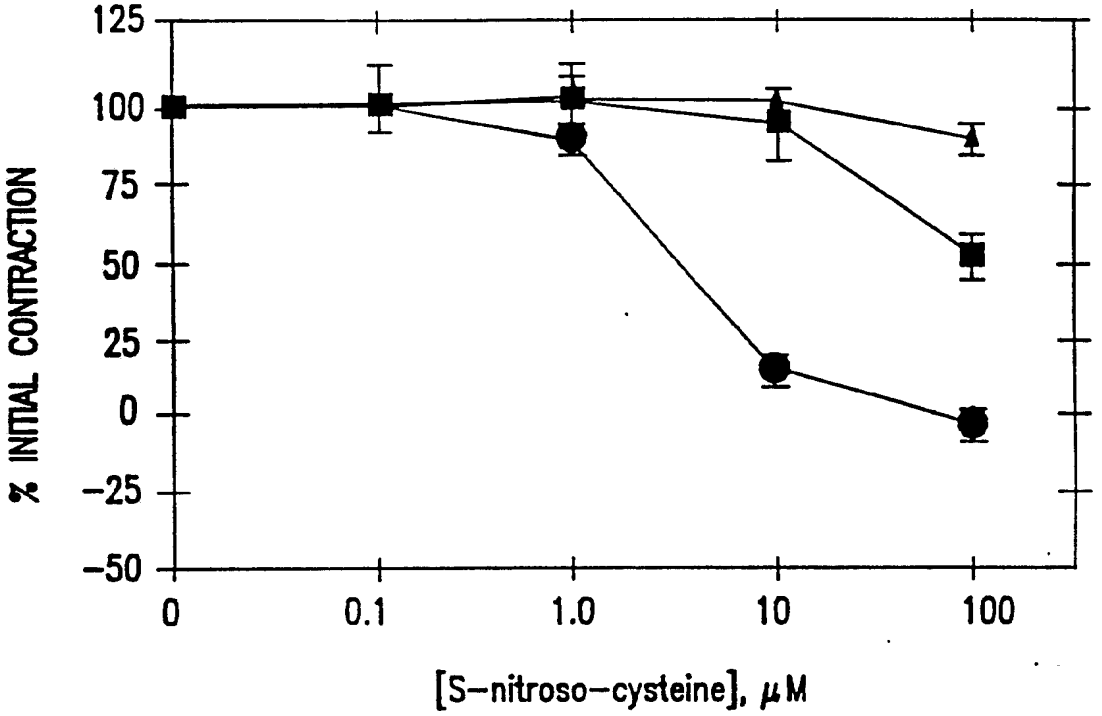
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-cysteine], μM

FIG.4C

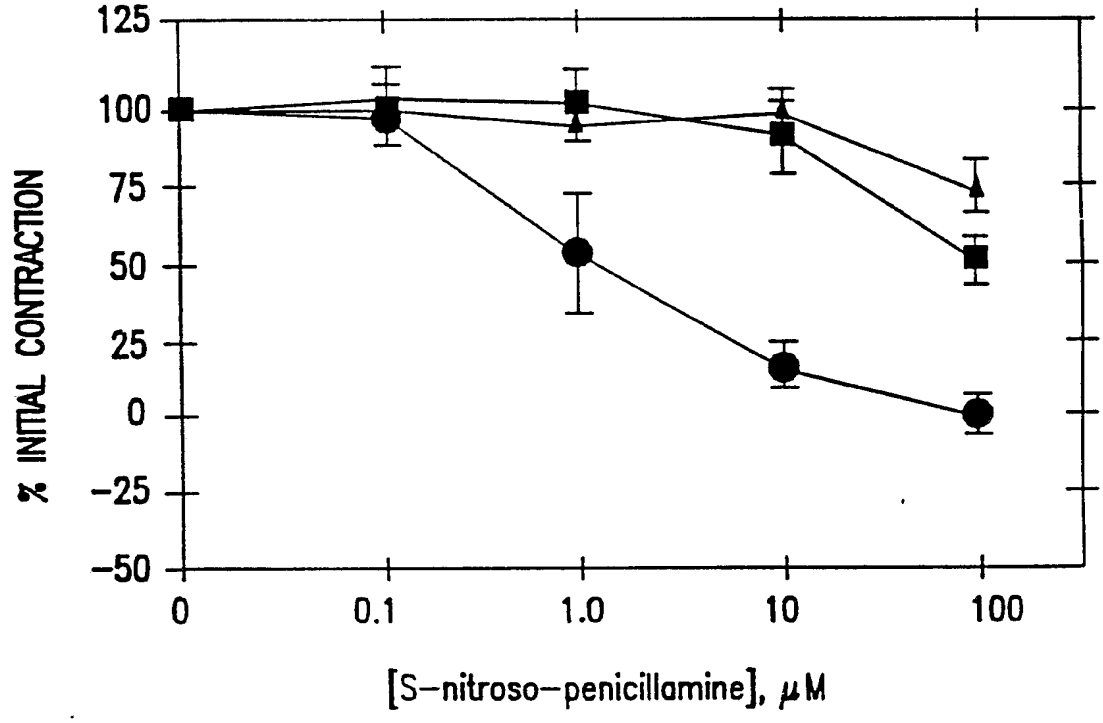
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-penicillamine], μM

FIG.4D

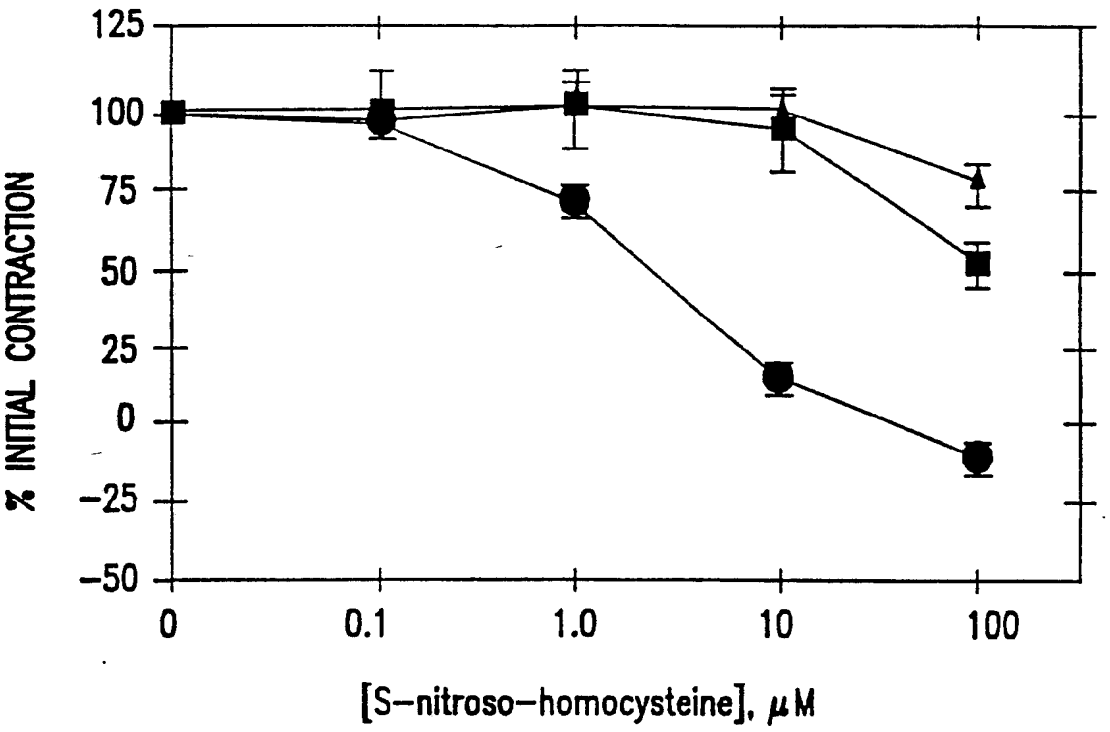
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-homocysteine], μM

FIG.4E

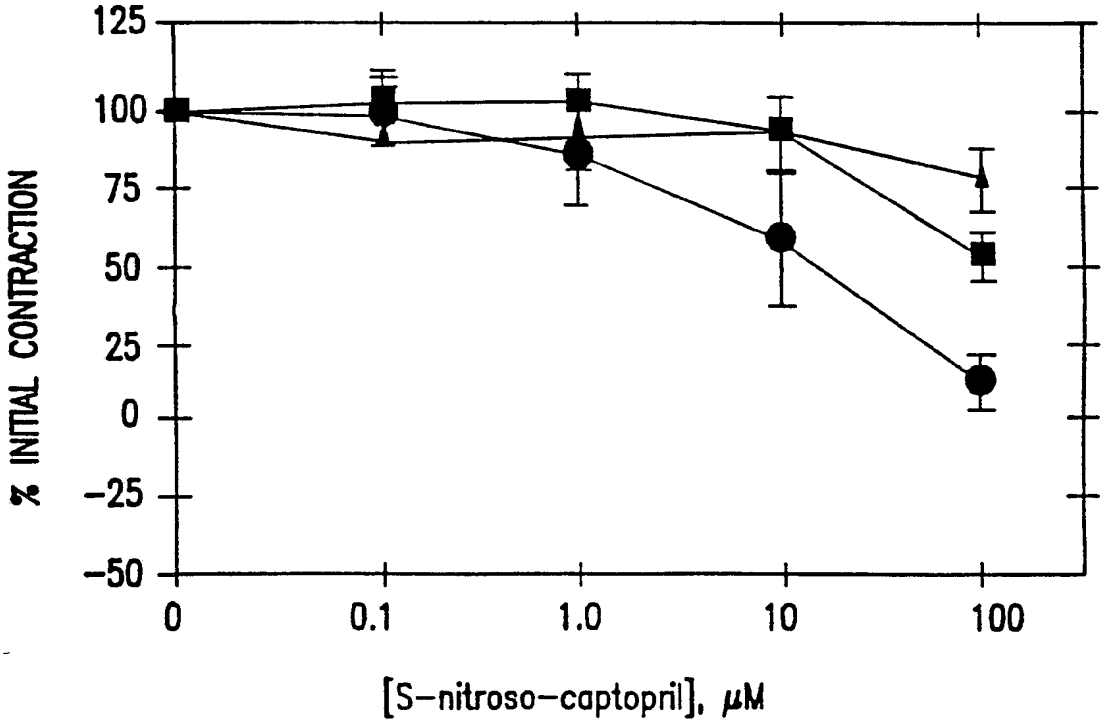
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-captopril], μM

FIG.4F

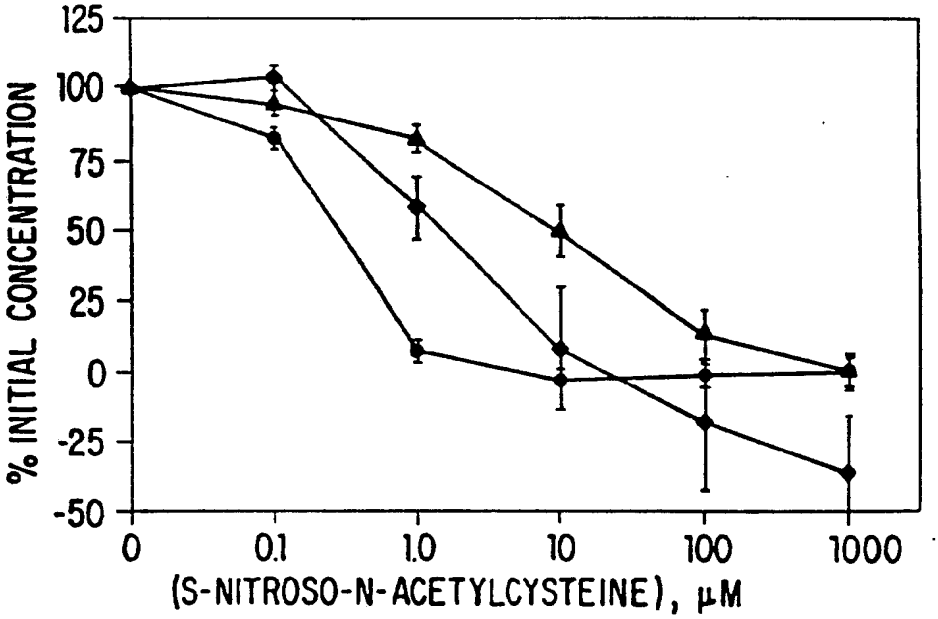
125
100
75
50
25
0
-25
-50
% INITIAL CONCENTRATION
0
0.1
1.0
10
100
1000
(S-NITROSO-N-ACETYLCYSTEINE), μM

FIG. 5A

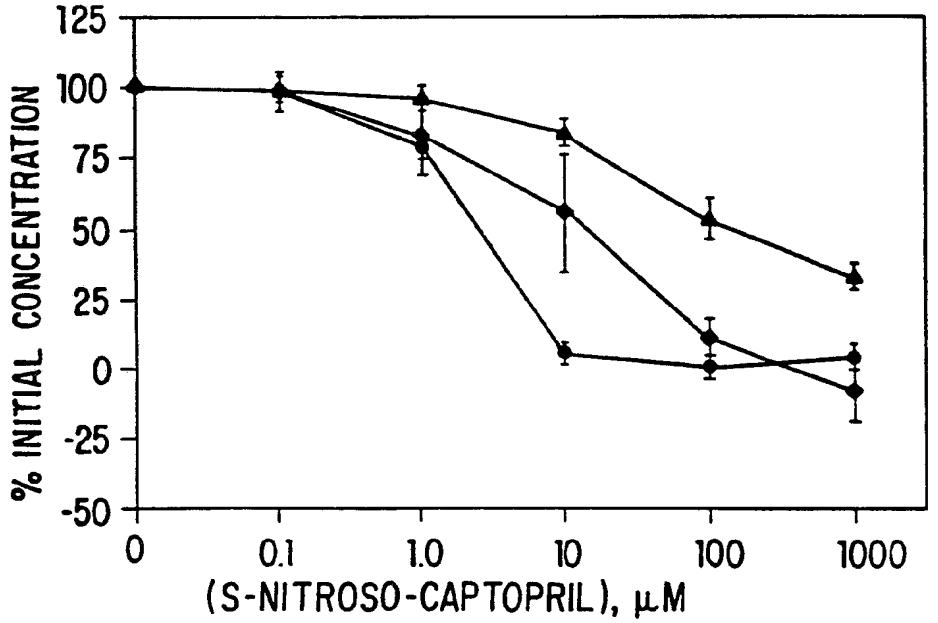
125
100
75
50
25
0
-25
-50
% INITIAL CONCENTRATION
0
0.1
1.0
10
100
1000
(S-NITROSO-CAPTOPRIL), μM

FIG. 5B

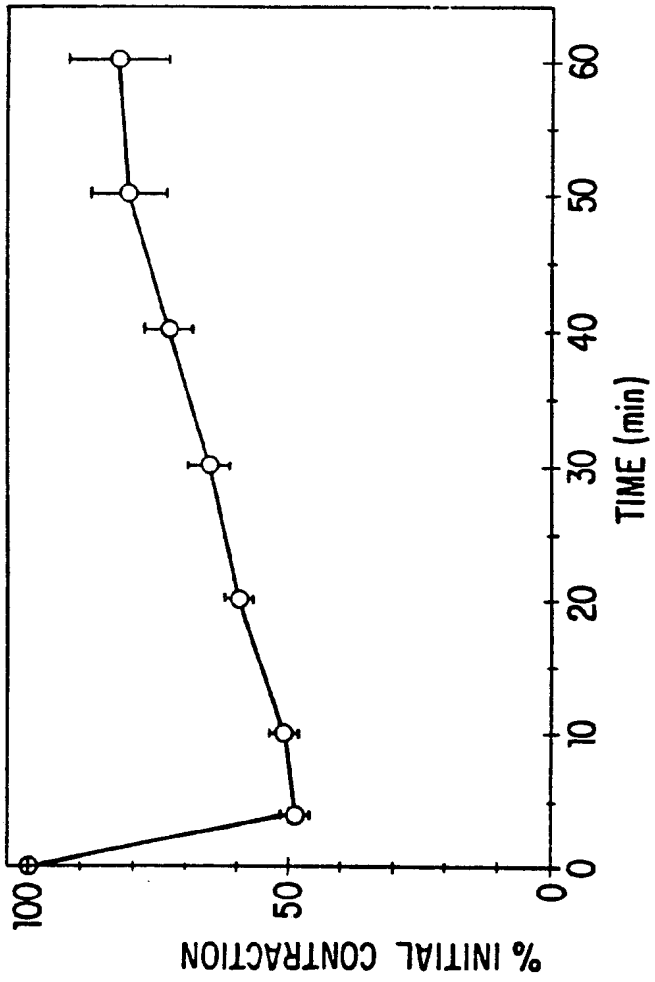

FIG. 6

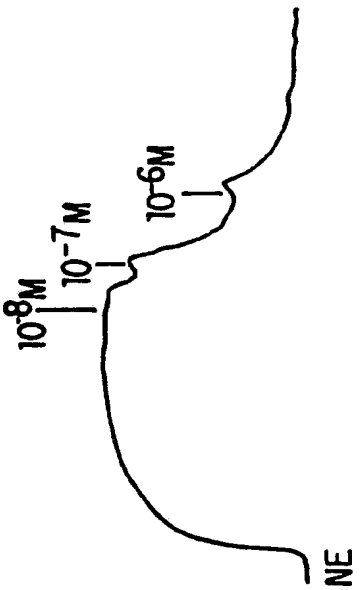

FIG. 7A

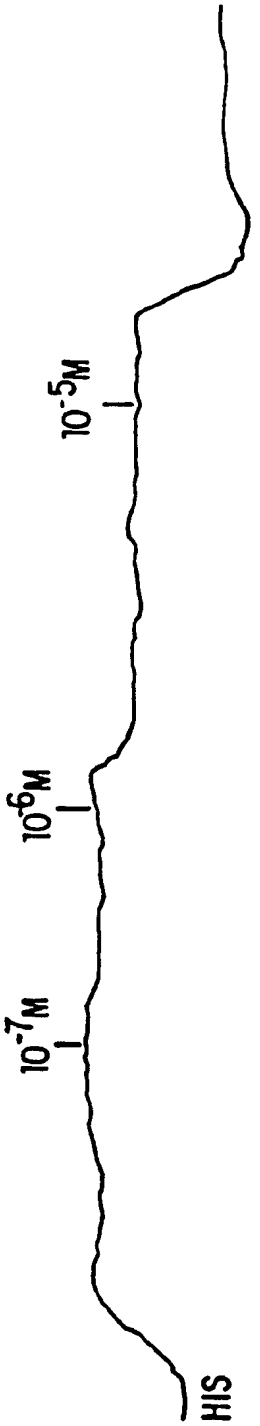

FIG. 7B

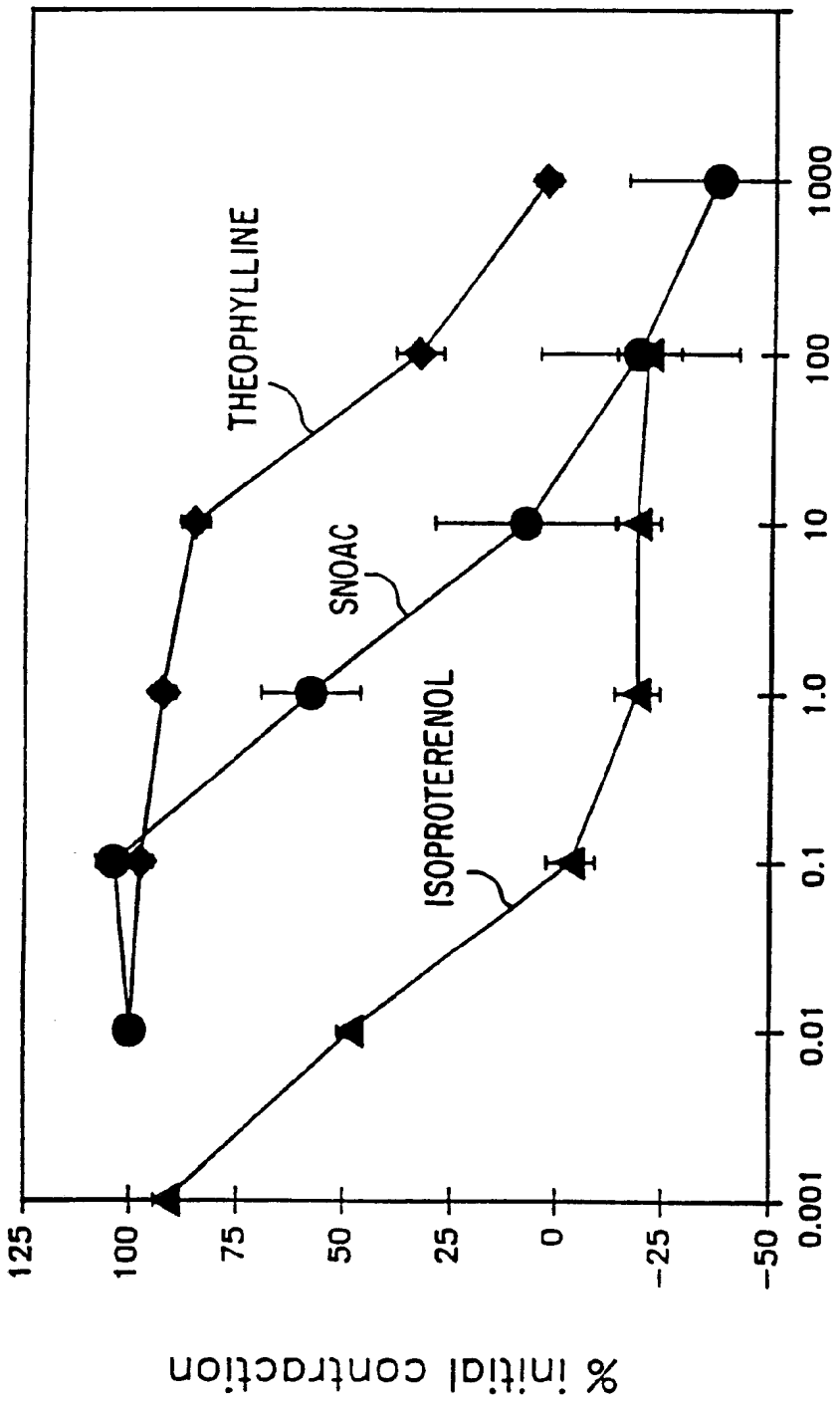
THEOPHYLLINE
SNOAC
ISOPROTERENOL
% initial contraction
125
100
75
50
25
0
-25
-50
0.001
0.01
0.1
1.0
10
100
1000
[Relaxant], μM

FIG. 8

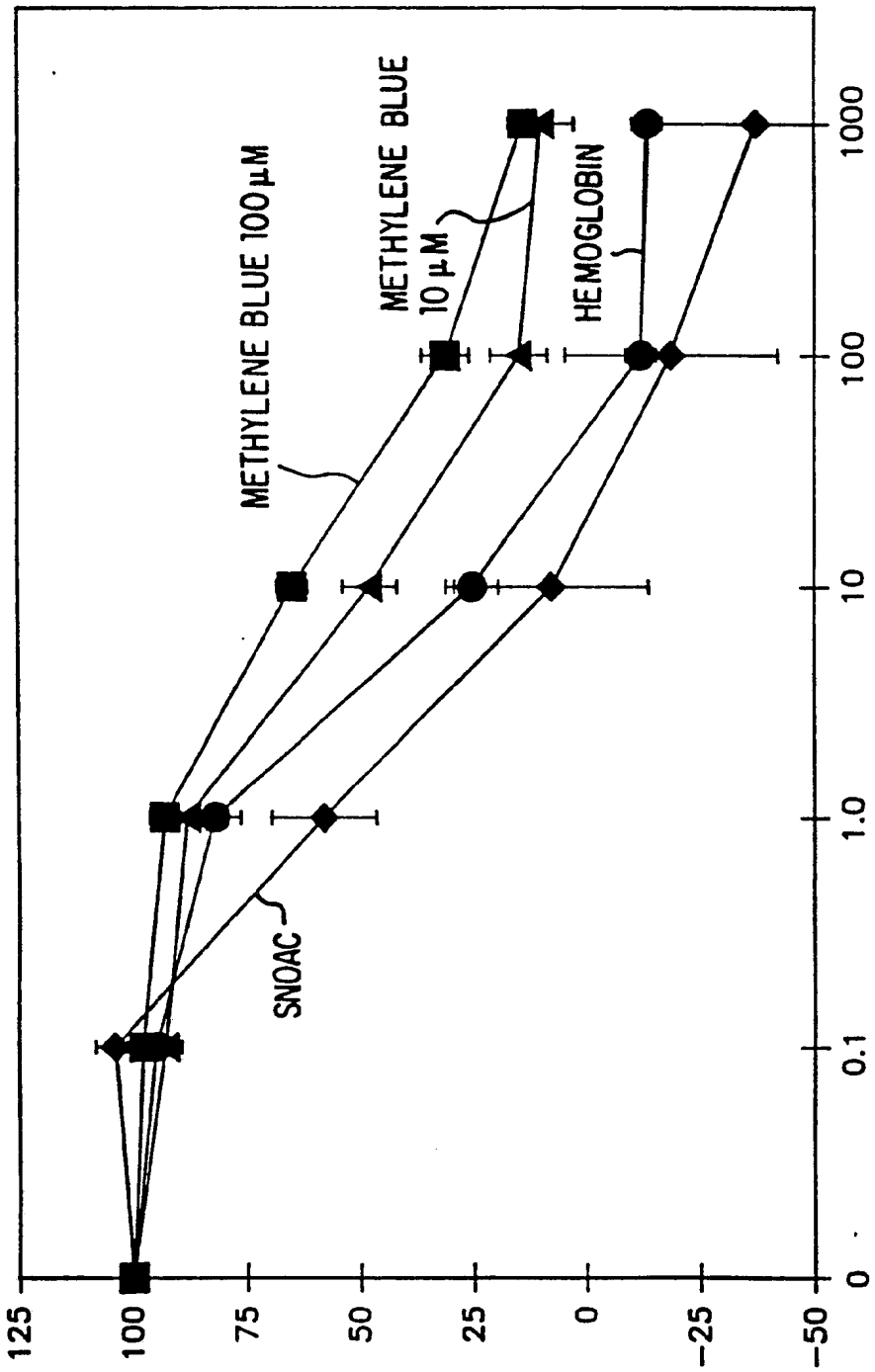
METHYLENE BLUE 100μM
METHYLENE BLUE 10μM
HEMOGLOBIN
SNOAC
125
100
75
50
25
0
−25
−50
0
0.1
1.0
10
100
1000
% initial contraction
[S−Nitroso−N−acetylcysteine], μM

FIG. 9

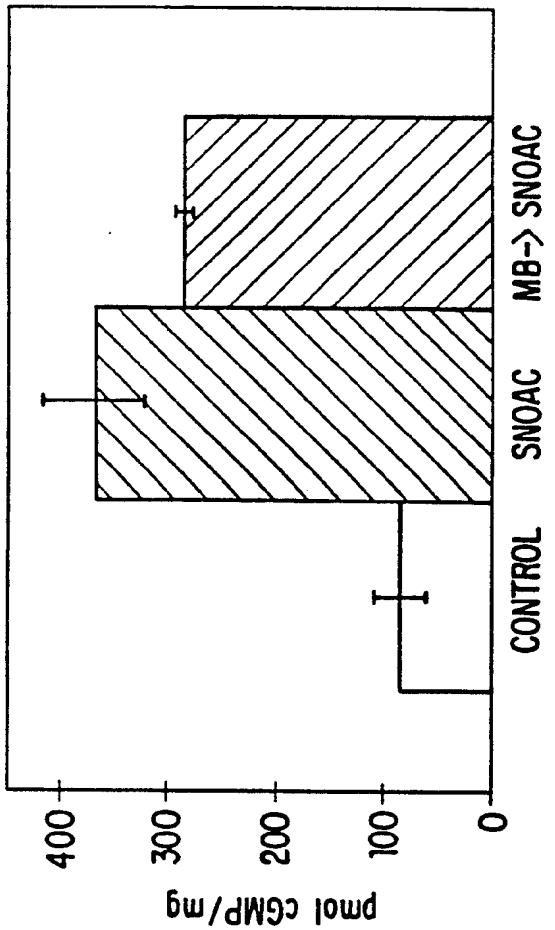

FIG. 10

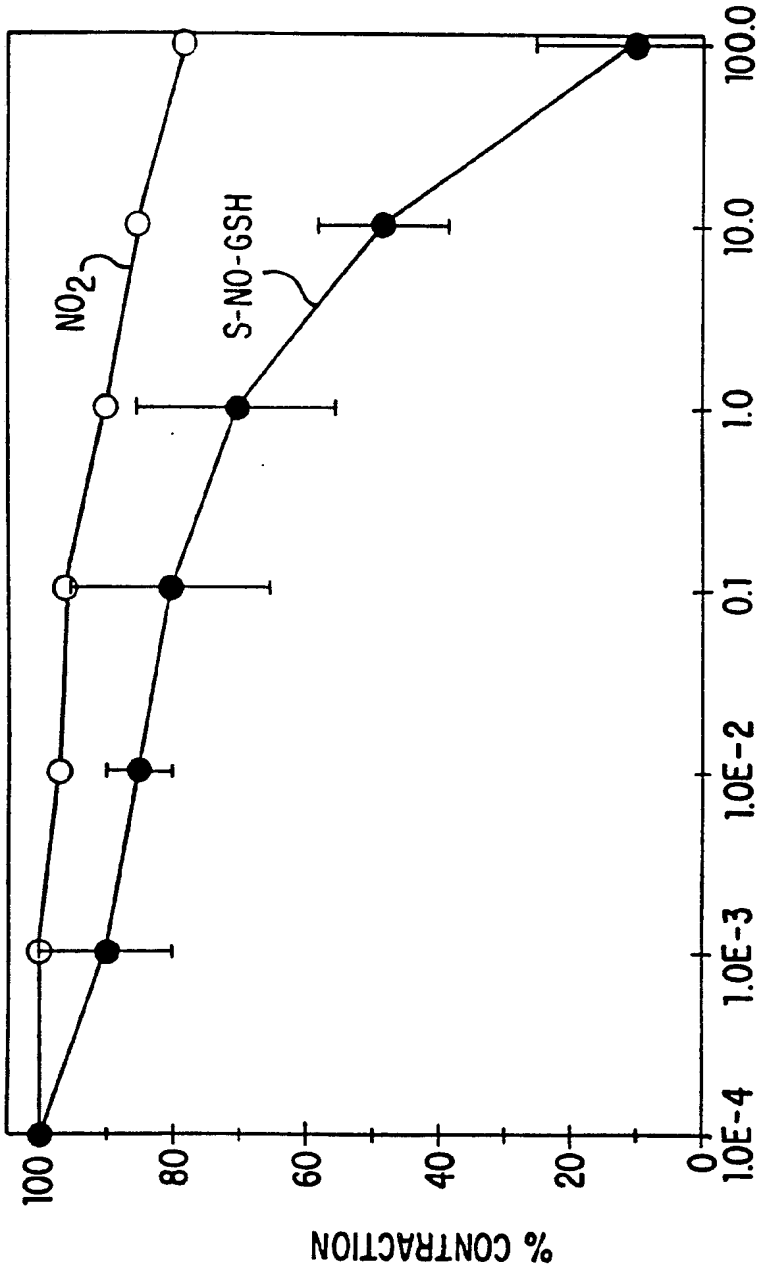
% CONTRACTION
100
80
60
40
20
0
1.0E-4
1.0E-3
1.0E-2
0.1
1.0
10.0
100.0
(RELAXANT), μM
NO_2
S-NO-GSH

FIG. 11

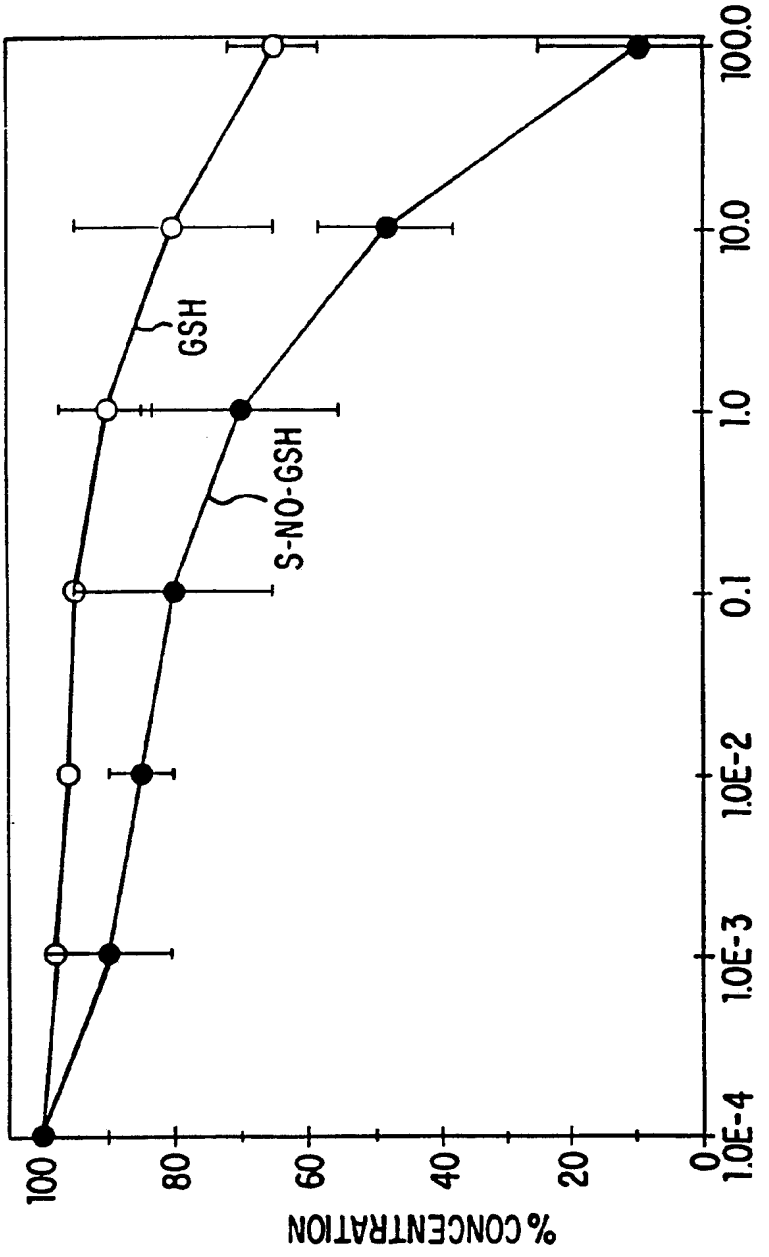
% CONCENTRATION
100
80
60
40
20
0
1.0E-4
1.0E-3
1.0E-2
0.1
1.0
10.0
100.0
(RELAXANT), μM
GSH
S-NO-GSH

FIG. 12

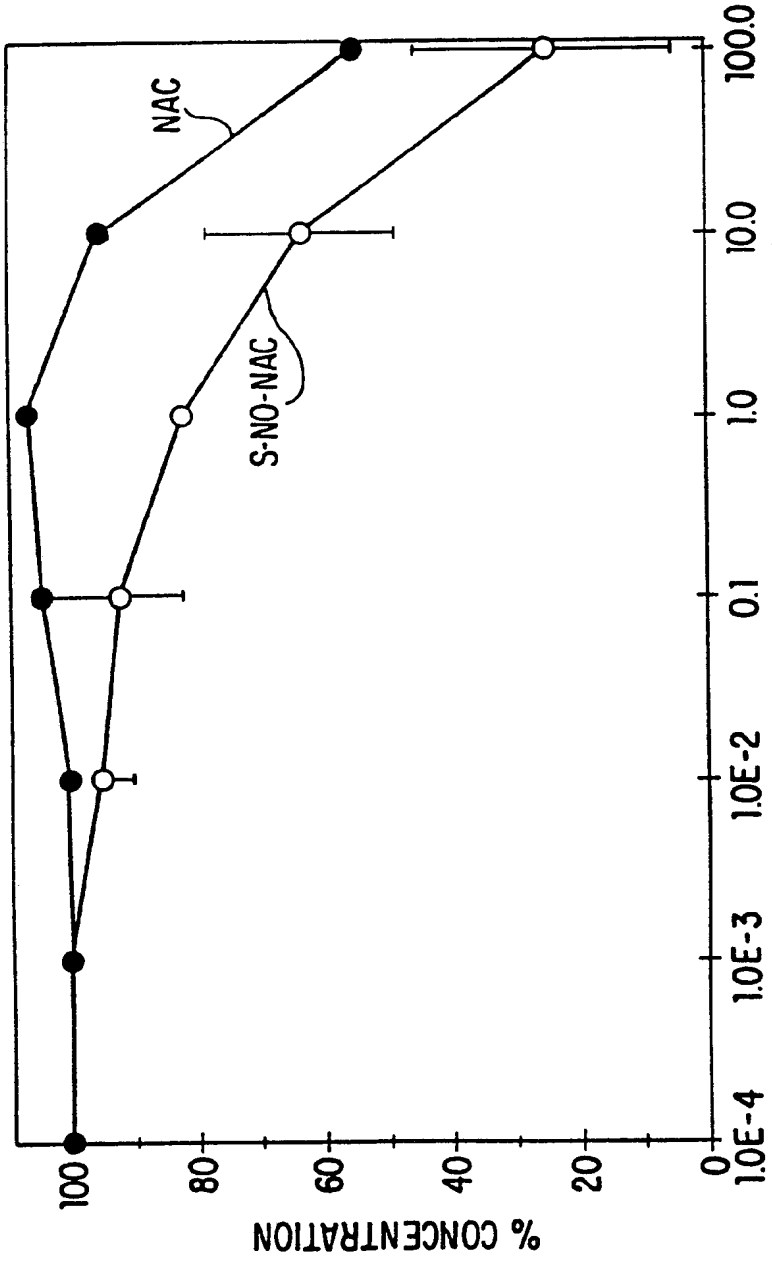
NAC
S-NO-NAC
% CONCENTRATION
100
80
60
40
20
0
1.0E-4
1.0E-3
1.0E-2
0.1
1.0
10.0
100.0
(RELAXANT), μM

FIG. 13

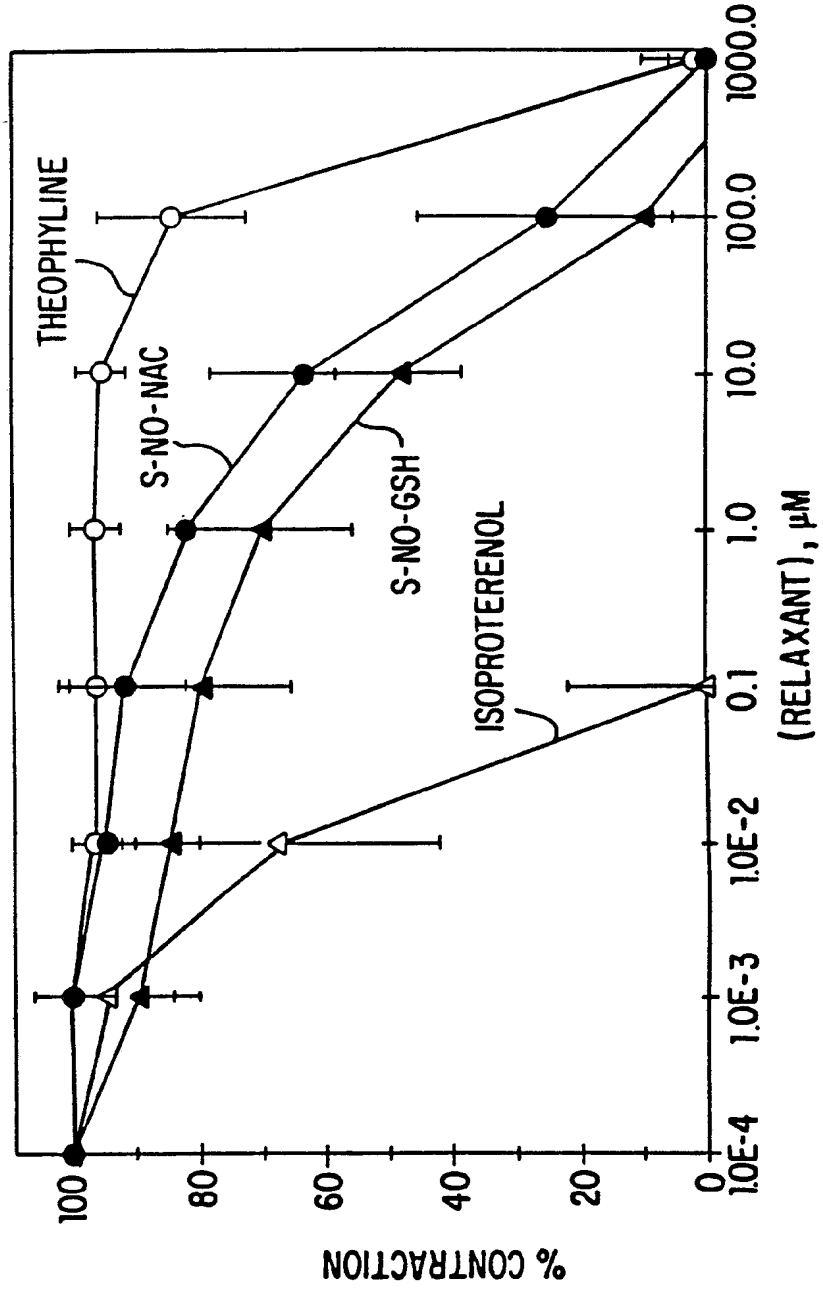
% CONTRACTION
100
80
60
40
20
0
1.0E-4
1.0E-3
1.0E-2
0.1
1.0
10.0
100.0
1000.0
(RELAXANT), μM
THEOPHYLINE
S-NO-NAC
S-NO-GSH
ISOPROTERENOL

FIG. 14

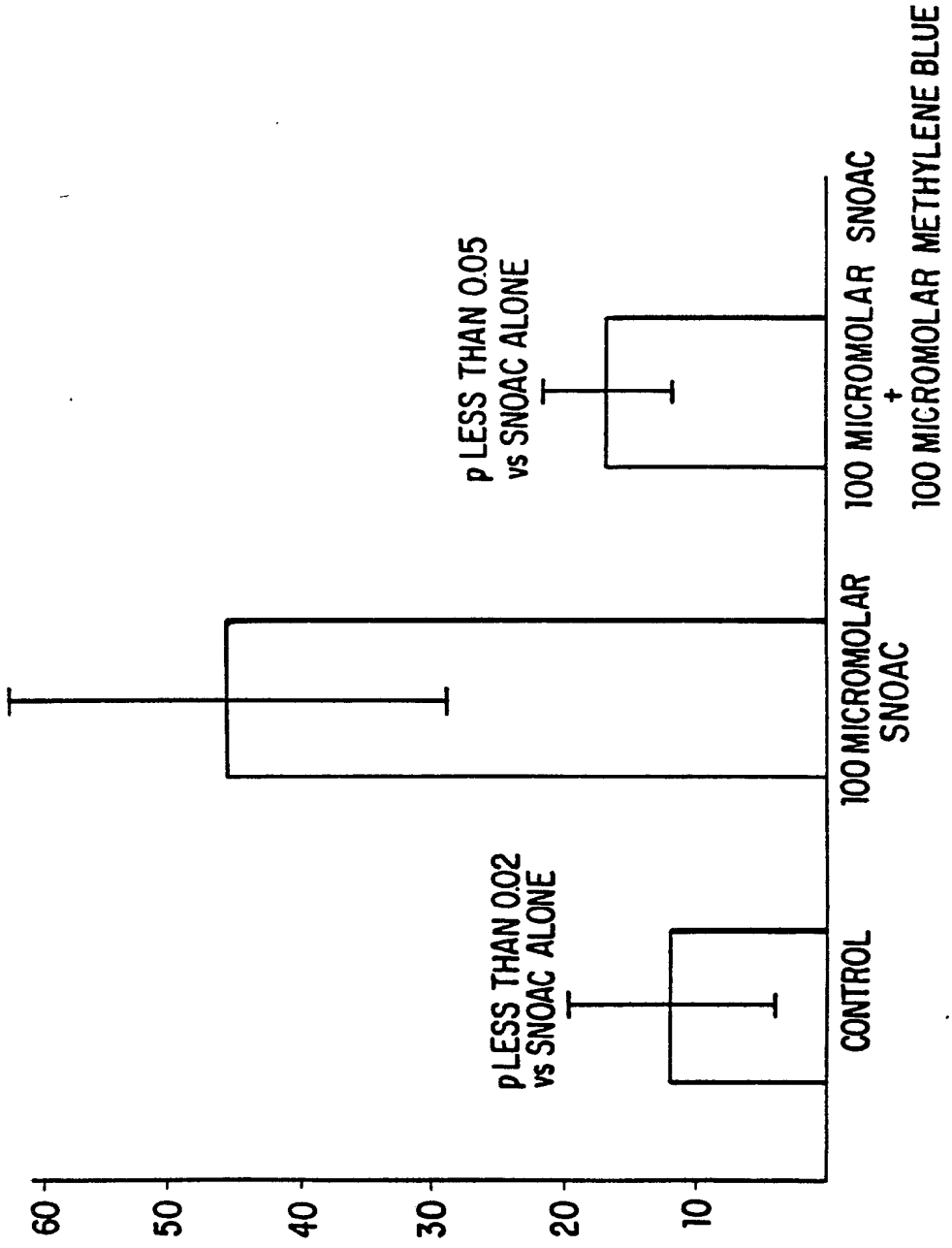
60
50
40
30
20
10
p LESS THAN 0.02
vs SNOAC ALONE
p LESS THAN 0.05
vs SNOAC ALONE
CONTROL
100 MICROMOLAR
SNOAC
100 MICROMOLAR SNOAC
+
100 MICROMOLAR METHYLENE BLUE

FIG. 15

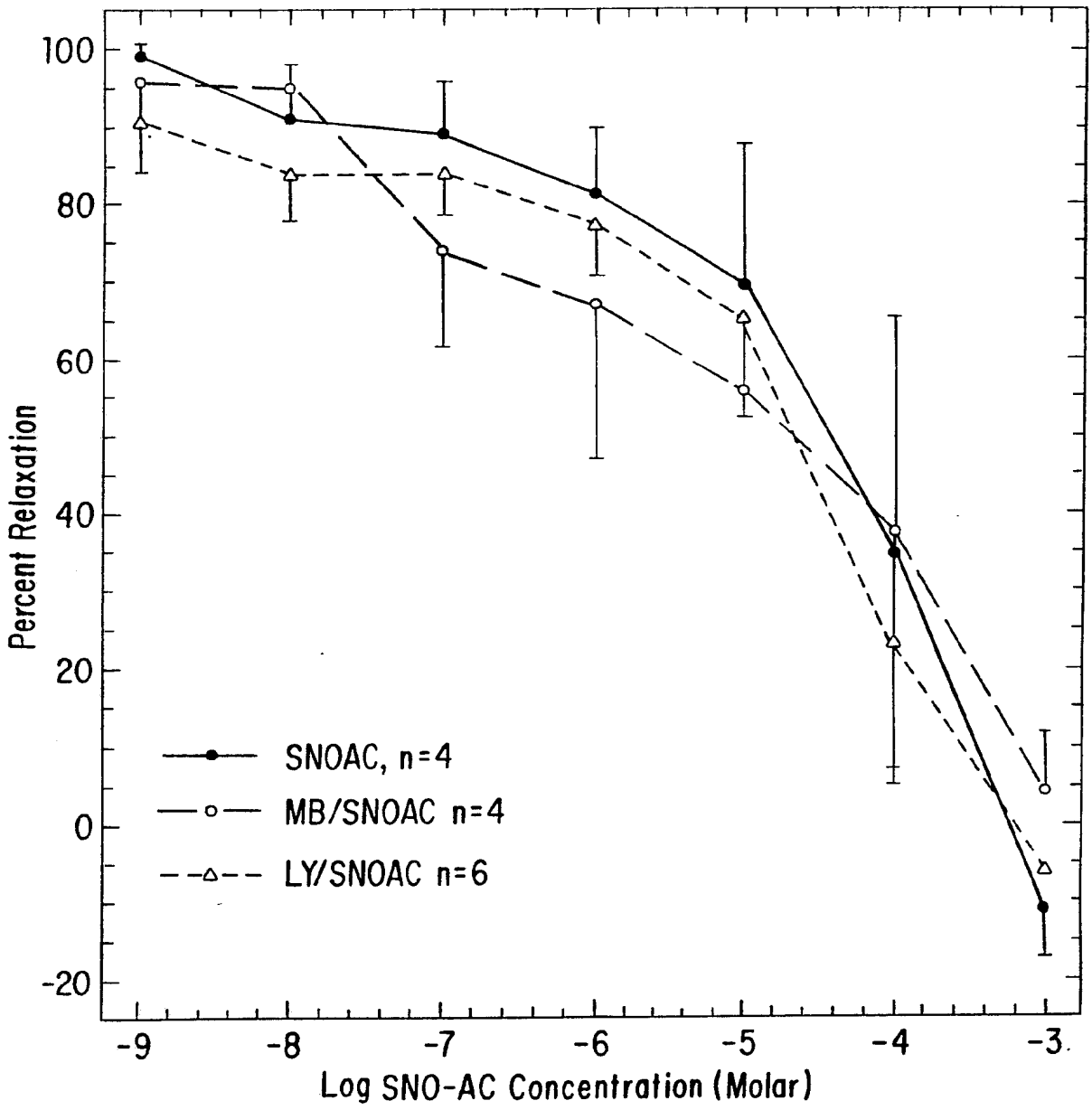
100
80
60
40
20
0
-20
Percent Relaxation
SNOAC, n=4
MB/SNOAC n=4
LY/SNOAC n=6
-9
-8
-7
-6
-5
-4
-3
Log SNO-AC Concentration (Molar)

FIG. 16

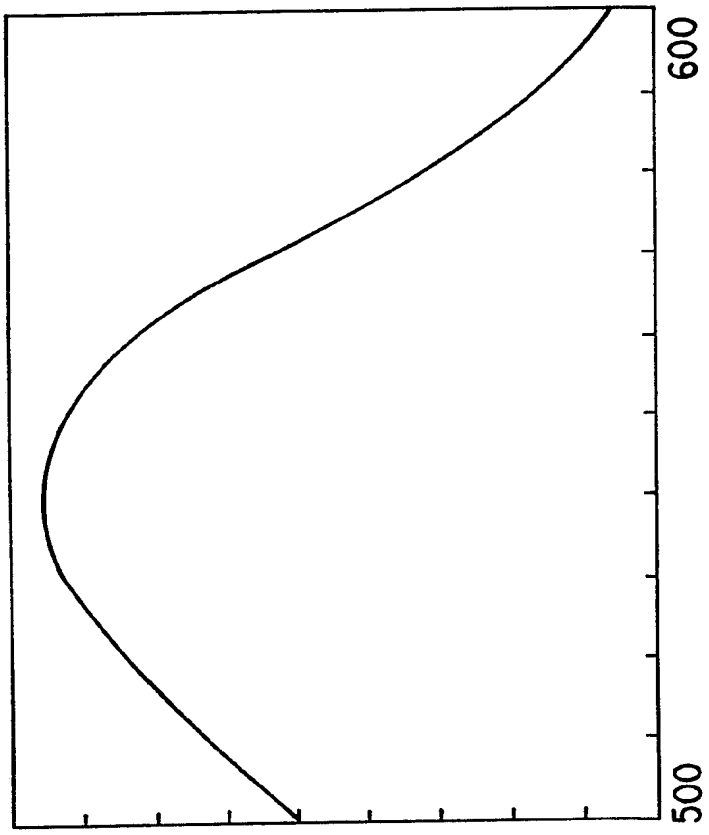

FIG.17

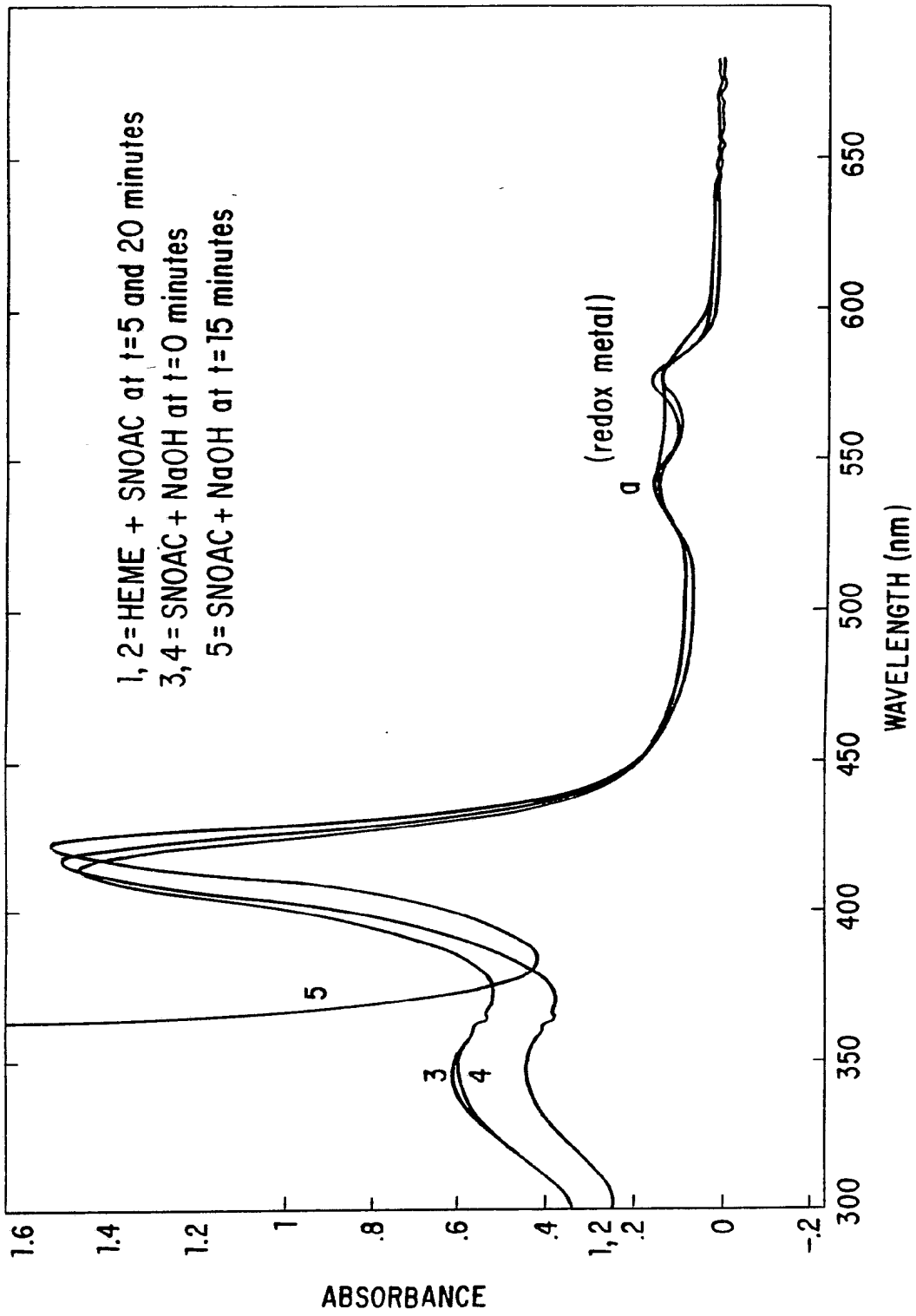
1, 2=HEME + SNOAC at t=5 and 20 minutes
3, 4=SNOAC + NaOH at t=0 minutes
5=SNOAC + NaOH at t=15 minutes
(redox metal)
a
5
3
4
1,2
ABSORBANCE
WAVELENGTH (nm)
1.6
1.4
1.2
1
.8
.6
.4
.2
0
-.2
300
350
400
450
500
550
600
650

FIG. 18

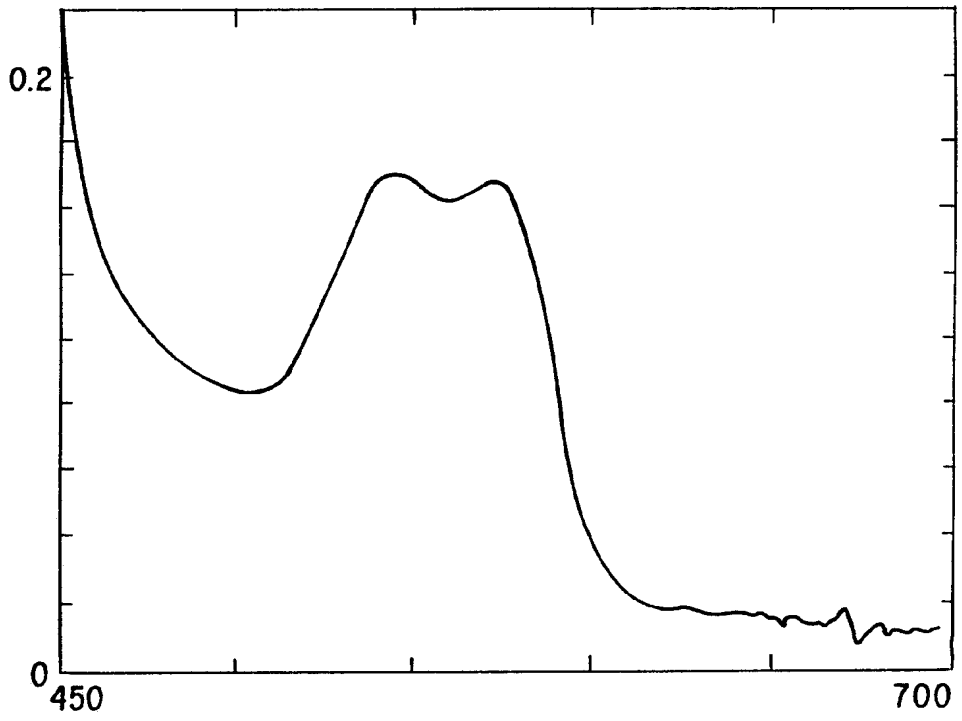

FIG. 19

INTERNATIONAL SEARCH REPORT

PCT/US92/10447

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07C 203/00, 331/00, 381/00; A01N 37/00; A61K 31/21
US CL :558/488; 514/506
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 558/488; 514/506

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS- structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A, 2,328,709 (Crandall et al.) 07 September 1943 See entire document.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 MARCH 1993	Date of mailing of the international search report 26 APR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer REBECCA COOK Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10447

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Henry et al., Br.J. Pharmacol. (1989), 98, 757-766 See entire document.	4(in part) 5,9-11 in part), 12,16-19 (in part), 20, 24-27, 31-33 (in part) 34,38-40 (in part) 41,45-47 (in part) 48,52-54 (in part)
Y	Kowaluk et al. J. Pharmacology and Experimental Therapy, 255(3) 1256-1264 See entire document.	4 (in part) 5,9-11 (in part), 12, 16-19 (in part), 20, 24-27, 31-33 (in part), 34, 38-40 (in part), 41, 45-47 (in part), 48, 52-54 (in part)

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10447

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1,4 (in part) 5,9-11 (in part), 12,16-19 (in part), 20,24-27,31-33 (in part), 34,38-40 (in part), 41,45-47 (in part), 48,52-54

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1, 4 (in part), 5, 9-11 (in part), 12,16-19 (in part), 20,24-27,31-33 (in part), 34,38-40 (in part), 41, 45-47 (in part), 48, 52-54 (in part) 55,59-60 (in part) drawn to a compound having the formula CH_3 $(CH_2)_2$ SNO and method of use, classified in 568/44, 514/706.

II. Claims 2,4 (in part), 6,9-11 (in part) 13, 16-19 (in part), 21,24-26 (in part) 28,31-33 (in part), 35, 38-40 (in part), 42, 45-47 (in part), 52-54 (in part), 49,56,59-60 (in part) drawn to a compound having the formula HS (CH_2), SNO of use, classified in 568/61, 514/706.

III. Claims 3,4 (in part), 7,9-11 (in part), 14,16-19 (in part), 22,24-26 (in part) 29, 31-33 (in part), 36, 38-40 (in part), 43,45-47 (in part), 50,52-54 (in part), 57, drawn to compound having the formula ONS $(CH_2)_xY$ and method of use classified in 564/123, 514/706.

IV. Claim 4 (in part), 8,11 (in part), 15,19 (in part), 23,26 (in part) 30,33 (in part), 37,40 (in part), 44,47 (in part), 51,54 (in part), 58, drawn to a method of using S-nitroso-amino acids.

V. Claims 61-63, drawn to a method of use of 8-nitrosothiols to treat or prevent disorder associated with insufficient oxygen supply classified in 514/706 among others.

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The claims of Group I-IV are drawn to methods of use employing distinct compounds which have separate status in the art as shown by their different classification Groups I-IV and Group V are drawn to distinct methods of use; Group I-IV are to a method of treating smooth muscle and Group V is to a method of binding and delivering oxygen in the body. PCT Rules 13.1 and 13.2 do not provide for multiple distinct compounds and/or methods within a single general inventive concept.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61F 9/007

A1

(11) International Publication Number: **WO 99/26567**

(43) International Publication Date: 3 June 1999 (03.06.99)

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(71) Applicant *(for BB only)*: WERNER, Mary, C. [US/US]; 0–155 Blue Hill Avenue, Fair Lawn, NJ 07410 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: YARON, Ira [IL/IL]; Har Adar 215, 90836 (IL). YARDEN, Orit [IL/IL]; Rahavat Ilan 22, 54056 Givat Shmuel (IL).

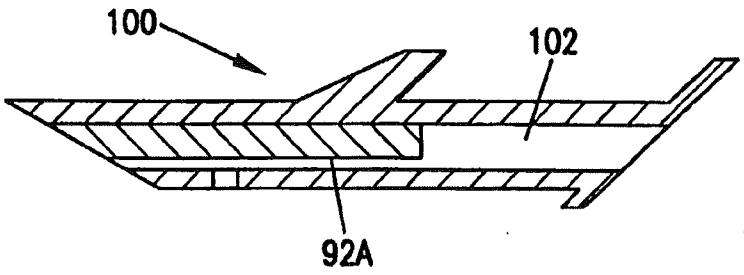
(74) Agents: BRAINARD, Charles, R. et al.; Kenyon & Kenyon, One Broadway, New York, NY 10004 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: FLOW REGULATING IMPLANT, METHOD OF MANUFACTURING, AND DELIVERY DEVICE

(57) Abstract



An implant includes a tube for permitting fluid flow. A flow controlling rod may be inserted within the tube passage. One or more holes around the circumference of the tube may be selectively permanently or temporarily occluded to give desired flow characteristics. A delivery device for implanting the implant may include a central bore in which a retractable wire is located. The retractable wire penetrates a tube passage of the implant when the implant is attached to the delivery device. A hook on the delivery device prevents the implant from moving down the wire. After the implant is in position in the eye, the retention wire is retracted out of the implant. With the retention wire retracted, the implant is then free to slide away from the hook, allowing the delivery device to be withdrawn, leaving the implant in place. In a method for manufacturing an implant, two tubes of different diameters are utilized. The smaller tube fits inside the longitudinal bore of the larger tube. When the tubes are cut, the smaller tube forms the tube of the implant and the remaining portions of the larger tube form the retention projection and/or disk of the implant.

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FLOW REGULATING IMPLANT, METHOD OF MANUFACTURE, AND DELIVERY DEVICE

FIELD OF THE INVENTION

The invention relates generally to medical implants used to regulate the flow of fluids within the body. The invention may be applied, for example, to ophthalmic implants for treatment of glaucoma. The invention also relates to methods of manufacturing such implants and to delivery devices for implanting such implants.

BACKGROUND OF THE INVENTION

Medical implants used to regulate the flow of fluids within the human body are known and used.

One application for the use of such implants is in the treatment of glaucoma. Glaucoma is an eye condition characterized by an increase in the intraocular pressure (IOP) of the eye to an abnormal level. A normal eye maintains a proper IOP by the circulation within the eye of aqueous humor -- aqueous humor is secreted from the ciliary body, passes through the pupil into the anterior chamber of the eyeball, and is filtered out of the eyeball via the trabeculum and the Canal of Schlemm. With glaucoma, the aqueous humor excretory pathway is blocked, the aqueous humor cannot pass out of the eyeball at an adequate rate, the IOP rises, the eyeball becomes harder, and the optic nerve atrophies by the pressure applied on its fibers leaving the retina. A characteristic optic neuropathy develops, resulting in progressive death of the ganglion cells in the retina,

restriction of the visual field, and eventual blindness. Advanced stages of the disease are characterized also by significant pain.

Glaucoma treatment, if initiated early in the course of the disease, can prevent further deterioration and preserve most of the ocular functions. The goal of glaucoma treatment is to reduce the IOP to a level which is considered safe for a particular eye, but which is not so low as to cause ocular malfunction or retinal complications.

One type of glaucoma treatment is filtration surgery, which provides an alternate route for aqueous humor to exit the anterior chamber of the eyeball and enter the sub-conjunctival space, thereby lowering the IOP. In full thickness operations a fistula is created through the limbal sclera, connecting directly the anterior chamber of the eyeball and the sub-conjunctival space. Full thickness operations provide long-lasting control of IOP; however, excessive loss of aqueous humor from the eyeball during the early postoperative period frequently leads to hypotony.

In guarded filtration surgery (trabeculectomy), a fistula created through the limbal sclera is protected by an overlying partial thickness sutured scleral flap. The scleral flap provides additional resistance to excessive loss of aqueous humor from the eyeball, thereby reducing the risk of early postoperative hypotony. However, trabeculectomy may result in higher eventual IOP

and increased risk of late failure of filtration, compared with full thickness operations.

In accordance with one recently introduced procedure, a full thickness filtering fistula may be created by a holmium laser probe, with minimal surgically induced trauma. After retrobulbar anesthesia, a conjunctival incision (approximately 1 mm) is made about 12-15 mm posterior to the intended sclerostomy site, and a laser probe is advanced through the sub-conjunctival space to the limbus. Then, multiple laser pulses are applied until a full thickness fistula is created. This technique has sometimes resulted in early hypotony on account of a difficulty in controlling the sclerostomy size. In addition, early and late iris prolapse into the sclerostomy has resulted in abrupt closure of the fistula and eventual surgical failure. Further, despite its relative simplicity, the technique still necessitates the use of retrobulbar anesthesia to avoid pain caused by the laser applications. The injection of anesthetic material close to the already damaged optic nerve may sometimes lead to further visual damage. A further disadvantage of this procedure, as well as other types of glaucoma filtration surgery, is the propensity of the fistula to be sealed by scarring.

Various attempts have been made to overcome the problems of filtration surgery, for example, by using ophthalmic implant devices. Typical ophthalmic implants utilize drainage tubes so as to maintain the integrity of

the openings formed in the eyeball for the relief of the IOP.

Typical ophthalmic implants suffer from several disadvantages. For example, the implants typically utilize a valve mechanism for regulating the flow of aqueous humor from the eyeball; defects in and/or failure of such valve mechanisms could lead to excessive loss of aqueous humor from the eyeball and possible hypotony. The implants also tend to clog over time, either from the inside by tissue, such as the iris, being sucked into the inlet, or from the outside by the proliferation of cells, for example by scarring. Additionally, the typical implant insertion operation is complicated, costly and takes a long time.

United States Patent No. 3,788,327 to Donowitz et al. shows a prior art implant utilizing a valve mechanism for regulating the flow of agueous humor from the eyeball. As stated above, defects in and/or failure of such a valve mechanism could lead to excessive loss of aqueous humor from the eyeball and possible hypotony. Additionally, both the inlet opening and the outlet opening in the implant shown in United States Patent No. 3,788,327 may be susceptible to clogging -- the inlet opening by the iris and the outlet opening by scarring. Finally, implantation of an implant according to United States Patent No. 3,788,327 may involve the separate steps of first providing a tract for receiving the implant and/or suturing the implant once it is in place,

which add time and possible complications to the operation.

SUMMARY OF THE INVENTION

It is an object of the invention to provide an improved implant to regulate the flow of fluids within the body. The invention may be applied, for example, to an ophthalmic implant which may be implanted into the eyeball for the treatment of glaucoma. It is a further object of the invention to provide a method of manufacturing such an implant and a delivery device for implanting such an implant.

In one embodiment of an improved implant in accordance with the invention, an intraocular implant is provided to be implanted in the eyeball. The implant includes a tube having an inlet end, an outlet end, and a tube passage therebetween for permitting aqueous humor to flow out of the eyeball, and a disk connected to the tube at the outlet end of the tube. The tube passage may have a cross-sectional area sufficiently small to inhibit the flow of aqueous humor through the tube passage. A flow controlling wire or rod may be inserted within the tube passage to provide further control over the flow. The configuration of the flow controlling rod may be selected in accordance with the desired flow characteristics. The configuration may be chosen to prevent flow when the IOP is below a threshold amount.

The disk, which is designed to be located underneath the conjunctiva, may have an outer rim for forming a reservoir having an enlarged cross-sectional area relative to the cross-sectional area of the tube passage. When aqueous humor flows through the tube passage, a bleb of aqueous humor forms under the conjunctiva so that the bleb and the elasticity of the conjunctiva assist in regulating the flow of aqueous humor through the tube as a function of the IOP.

To prevent clogging of the implant, the tube at its inlet end may be provided with a beveled surface which faces away from the iris when the implant is inserted. Additionally, one or more circumferential holes may be provided along the tube for allowing aqueous humor to flow into the tube passage even if the axial inlet opening is blocked. The hole or holes may be selectively permanently or temporarily occluded to give desired flow characteristics.

To prevent clogging at the outlet end, the disk may have an outer rim as described above which raises the conjunctiva away from the axial outlet of the tube passage to allow outflow. One or more inner uprights (which may be in the form of an inner rim) may also be provided on the disk for this purpose. Clogging is further avoided by implanting the implant under the conjunctiva at a distance away from an insertion slit in the conjunctiva, such that healing of the slit does not cause scar tissue to form in the area of the axial outlet

opening of the implant.

Implantation may be facilitated by further features of the implant. For example, the implant may have one or more retention projections (for example, in the form of a spur, flange, or plate). The retention projection may be rigid, or it may be made of an elastic material such that it is able to be flexed inward against the tube during penetration through the sclera. Alternatively, the retention projection may be designed to lie initially relatively flat against the tube for easier penetration through the sclera and to prevent tearing of the sclera, with a mechanism for extending the retention projection outwardly when the implant is implanted in the eyeball. For example, the retention projection may be extended outwardly by a separate expansion tool or may be constructed of a shape memory material, such as PMMA or nitinol, so that it is extended outwardly when subjected to the heat of the eyeball. One or more such retention projections are sufficient to reliably anchor the implant in the eyeball without the need for sutures, saving time and costs.

Implantation may also be facilitated by the provision of one or more markers on the implant visible through the cornea upon passing through the sclera. For example, a circumferential hole as described above may serve as a marker; alternatively, the marker may be some other suitable visible mechanism, such as a scratch or colored mark on the tube. The visibility of the marker

lets the doctor know that the marker has passed through the sclera, indicating that the implant is in place.

Implantation of an implant may be performed by use of a delivery device comprising a handle and a rodlike instrument, for example a needle or probe, for carrying the implant for insertion. The delivery device has a tip for insertion into the tube passage of the implant and a suitable retention mechanism for preventing the implant from moving up the delivery device during implantation. The retention mechanism may also be constructed to prevent the implant from rotating during implantation to insure proper orientation of the implant. The delivery device may additionally have a suitable expansion tool for extending one or more retention projections of the implant outwardly once the projection or projections have penetrated through the desired tissue.

In an embodiment of a delivery device according to the invention, the rodlike instrument has a central bore in which is located a retractable wire. The retractable wire penetrates a tube passage of the implant when the implant is attached to the delivery device. A hook on the delivery device prevents the implant from moving down the wire. After the implant is in position in the desired implantation site, the retention wire is retracted out of the implant. With the retention wire retracted, the implant is then free to slide away from the hook, allowing the delivery device to be withdrawn,

leaving the implant in place.

In one method of implanting an implant according to the invention, a small slit is cut in a portion of the conjunctiva which normally lies at a distance away from the intended implantation site. As the implant itself is very small, the slit also may be very small, for example about 2 mm in length or less. The small size of the slit as well as its positioning at a distance away from the implantation site, for example about 10 mm, helps prevent contamination of the sclerostomy site and reduces the risk of infection.

The implant is placed through the slit, directed to the implantation site, and inserted into the sclera at the implantation site. The sclera may be pierced either by a needle-like tip of the tube of the implant formed by a beveled surface at the inlet end of the tube as described above or by the tip of a needle of the delivery device which carries the implant. Thus, the implant may be inserted directly into the eyeball without the need for any separate piercing step, resulting in cost and time savings.

In a method for manufacturing an intraocular implant according to the invention, two tubes of different diameters are utilized. The smaller tube is able to fit inside the longitudinal bore of the larger tube. When the tubes are cut, the smaller tube forms the tube of the implant and the remaining portions of the larger tube form the retention projection and disk of the

implant.

An intraocular implant according to the invention provide the advantages of a full thickness fistula, while avoiding the limitations of the standard trabeculectomy. An implant according to the invention may be very small and implantable without surgery. No surgery room or hospitalization is necessary, thereby reducing costs. Implantation is minimally invasive, simple and quick, requiring only local anesthesia. Retrobulbar anaesthesia is not necessary, and thus iatrogenic damage to the optic nerve is avoided. There is no need to perform an iridectomy, and thus aqueous flow is maintained, lens nourishment is unaffected, and the likelihood of cataracts developing as a result of the procedure is reduced.

An implant according to the invention has other applications aside from the field of intraocular implants. For example, the implant may be used for drainage of a hydrocele sac, regulating flow between the hydrocele sac and the subcutaneous scrotum. As will be appreciated by persons of ordinary skill in the art, other applications of an implant in accordance with the invention are possible.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic cross-sectional view of a first embodiment of an intraocular implant shown inserted in an eyeball;

Figure 2 is an enlarged perspective view of the intraocular implant of Figure 1;

Figure 3 is a view similar to Figure 2, with part of the intraocular implant cut away to show a sectional view thereof;

Figure 4 is an enlarged perspective view of a disk portion of the intraocular implant of Figure 1;

Figures 5 through 7 illustrate the action of the conjunctiva during operation of the intraocular implant of Figure 1, with Figure 5 showing a stage of operation without a bleb formed, Figure 6 showing a formation of the bleb, and Figure 7 showing further formation of the bleb;

Figures 8 through 10 illustrate a delivery device and insertion of the intraocular implant of Figure 1 into an eyeball, with Figure 8 showing the delivery device and implant before insertion, Figure 9 showing the delivery device and implant being placed through a slit in the conjunctiva, and Figure 10 showing the implant after insertion with the delivery device withdrawn;

Figure 11 is an enlarged perspective view of a second embodiment of an intraocular implant with part of the intraocular implant cut away to show a sectional view thereof;

Figure 12 is a top view of the intraocular implant of Figure 11, showing a disk portion of the implant;

Figure 13 illustrates a delivery device and insertion of the intraocular implant of Figure 11 into an eyeball;

Figure 14 is a schematic cross-sectional view of the intraocular implant of Figure 11, shown inserted in an eyeball;

Figures 15 and 16 illustrate a third embodiment of an intraocular implant with Figure 15 showing the implant prior to attachment of a retention plate and Figure 16 showing the implant after attachment of the retention plate;

Figures 17 through 19 illustrate successive steps in a method of manufacturing an intraocular implant according to an embodiment of the invention, with Figure 17 showing an outer tube cut in an initial phase of the manufacturing process,

Figure 18 showing the outer tube joined to an inner tube, and Figure 19 showing the finished intraocular implant;

Figure 20 illustrates an intraocular implant according to the invention with a flow controlling wire or rod in the tube passage;

Figures 21A through 21D illustrate four variations of cross-sections for a flow controlling rod;

Figure 22 illustrates an intraocular implant with a threaded flow controlling rod;

Figure 23 illustrates an intraocular implant with a tapered flow controlling rod;

Figure 24 illustrates an intraocular implant with an adjustable flow controlling rod;

Figure 25 illustrates an intraocular implant with selectively occluded side holes;

Figure 26 illustrates an intraocular implant with a flexible flow controlling rod;

Figure 27 illustrates an intraocular implant with a flow controlling rod biased against a spring;

Figure 28 illustrates the end of an embodiment of a delivery device according to the invention and an implant attached to the delivery device; and

Figure 29 illustrates a view similar to that of Figure 28, with a retention wire of the delivery device retracted from the implant.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates an intraocular implant 30, implanted in an eyeball 10. The implant 30 comprises a needle-like tube 32 and a disk 34. The plane of the disk 34 forms an angle with the tube 32 that corresponds to the angle between the surface of the sclera 12 and the axis of insertion of the implant 30. The implant 30 is inserted in the sclera 12 of the eyeball 10, in the limbal area 14 adjacent to the cornea 16, and protrudes into the anterior chamber 20 adjacent the iris 22. The implant 30 is inserted so that the disk 34 is placed on a surface of the sclera 12 underneath the conjunctiva 18. The implant 30 may be placed above or below the Tenon's capsule (not shown). It will be appreciated by persons of ordinary skill in the art that the exact location for inserting the implant 30 is not restricted to that shown, and may be any other suitable position, such as behind the iris 22.

Figure 2 shows an enlarged perspective view of the implant 30 of Figure 1, and Figure 3 shows a similar

view, with part of the implant 30 cut away. The tube 32, which may take the form of a modified standard retrobulbar tip, has an inlet end 40, an outlet end 50, and a tube passage 38 extending therebetween, with the tube passage 38 having an axial inlet 41 and an axial outlet 51. The disk 34 is connected to the tube 32 at its outlet end 50. The entire implant is very small; for example the tube 32 may have a length of about 2 mm and a width of about 0.5 mm, and the disk 34 may have a diameter of about 1 mm and a thickness of less than 0.1 mm.

The tube passage 38 has a cross-sectional area sufficiently small to inhibit the flow of aqueous humor through the tube passage. In one embodiment, for example, the cylindrical tube passage 38 has a diameter of about 300 micrometers. By using a specified internal cross-sectional area for the tube passage, excessive loss of aqueous humor from the eyeball is prevented.

When the IOP is above a threshold amount, for example about 5 mm Hg, aqueous humor drains from the anterior chamber 20 of the eyeball 10 through the axial inlet 41 and one or more circumferential holes 42, through the tube passage 38, and into the space under the conjunctiva 18. The circumferential holes 42 may take any suitable form; for example, they may be in the form of circular openings whose combined cross-sectional area is equal to the cross-sectional area of the tube passage 38. The circumferential holes 42 prevent the tube

passage 38 from becoming clogged at its inlet end because, even if the iris 22 obstructs the axial inlet 41, aqueous humor can still pass through the circumferential holes 42. In the event the axial inlet 41 is obstructed, the circumferential holes 42 also serve to cause a back pressure in the tube passage 38 to unclog the axial inlet 41. The circumferential holes 42 serve the additional purpose of insuring a proper insertion depth of the implant 30, as the upper hole is visible during implantation after penetration through the sclera and thus can be used as a marker. To serve this function, any other suitable marker (such as a scratch or colored mark) may be used.

The inlet end 40 of the tube 32 has a needle-like tip formed by a beveled surface 36, angled sharply for easy insertion into the eyeball. The beveled surface 36 increases the area of the axial inlet 41 to enlarge the entrance to the tube passage 38. The beveled surface 36 is designed to face away from the iris 22 to reduce the possibility of obstruction of the axial inlet 41. Because the disk 34 is designed to rest against the sclera 14 and the beveled surface 36 is designed to face away from the iris 22, the beveled surface 36 lies in a plane which is angled opposite to the plane in which the disk 34 lies.

The tube 32 may have one or more retention projections in the form of one or more spurs 52 provided integrally with it for retaining the implant 30 in the

eyeball 10 after insertion. Alternatively, the retention spur 52 may be made as a separate part connected to the tube 32 by, for example, welding or brazing. The retention spur 52 may be rigid, or it may be flexible such that it bends toward the tube 32 during penetration of the sclera and springs outward to its original shape after passing through the sclera. Alternatively, the retention spur 52 may be designed for plastic deformation by a separate expansion tool (for example, a balloon) once it is in the eyeball 10, or the retention spur 52 may be constructed of a shape memory material, such as PMMA or nitinol, such that the spur is flat against the tube when cool but expands to its final shape when subjected to the heat of the eyeball 10.

The disk 34, shown enlarged in Figure 4, comprises a base 44, an outer rim 46, and a plurality of inner uprights 48. The areas between the uprights 48 constitute passageways 56 for the transverse flow of aqueous humor. The base 44 and outer rim 46 define a reservoir 54 such that, in operation, the aqueous humor flows out of the axial outlet 51 of the tube passage 38, between the uprights 48, and into the reservoir 54. The passageways 56 may be considered as part of the reservoir 54. The enlarged cross-sectional area of the reservoir 54 as compared to the cross-sectional area of the tube passage 38 provides a larger area for absorption of the aqueous humor by the conjunctiva 18 and also acts in conjunction with the elasticity of the conjunctiva 18 to

assist in regulating the flow of aqueous humor through the implant 30 as a function of the IOP.

Figures 5 through 7 illustrate the action of the conjunctiva 18 during operation of the implant 30, in which it can be seen that the aqueous humor which flows out of the tube passage forms a "bleb" 24 below the conjunctiva 18. It will be appreciated by persons having ordinary skill in the art that a higher IOP results in a higher flow rate through the implant 30, and a greater force of the aqueous humor on the conjunctiva 18.

In addition to defining the reservoir 54, the outer rim 46 of the disk 34 serves the additional purpose of raising the conjunctiva 18 away from the axial outlet 51 to prevent clogging of the tube passage 38. The inner uprights 48 also serve this purpose.

The shape of the disk 34 may be, but is not limited to, an ellipse, and it will be appreciated by persons having ordinary skill in the art that it may conform to any shape which allows the implant to fit under the conjunctiva 18 and which regulates the IOP. The size and/or shape of the disk 34 and/or the angle between the disk 34 and the tube 32 can also be changed in order to use different implants for different persons' eyes.

Figures 8 through 10 illustrate a delivery device 60 and a method of inserting the intraocular implant 30 into an eyeball. The implant 30 is first attached to the delivery device 60, having a handle 62

and a suitable rodlike instrument 64 such as a needle or probe. The rodlike instrument 64 has a tip 70 for penetrating a tube passage of the implant 30 and a retention mechanism for preventing the implant from moving up the delivery device during implantation, for example in the form of an abutment surface 68 having an angle generally corresponding to that of the disk 34. This configuration also prevents rotation of the implant 30 on the delivery device 60, thereby insuring proper orientation of the implant in the eyeball. The retention mechanism may also include one or more projections for extending inside the outer rim and/or between the inner uprights on the disk 34. In an alternative embodiment, the retention mechanism may be the tip of the rodlike instrument, constructed to engage the inside of the tube passage of the implant with a friction fit, thereby preventing the implant from moving up the delivery device during implantation.

A delivery device 60 in which the rodlike instrument is a needle 65 is illustrated in Figure 9. In that illustrated embodiment, the delivery device 60 is similar to a standard medical syringe having a housing and a needle 65 with a bore 67. The front tip 69 of the needle 65 is configured as an abutment surface having an angle generally corresponding to that of the disk 34. The bore 67 of the needle 65 has a tip in the form of a plug 71 which is configured to have a cross-sectional shape corresponding to that of the tube passage 38. The

implant 30 is placed over the plug 71, with the end of the plug 71 projecting into the tube passage 38, and with the front tip 69 of the needle 65 abutting against the disk 34. The plug 71 blocks the tube passage 38 during implantation.

To insert the implant 30 into the eyeball 10, a small slit 26 is cut in a portion of the conjunctiva 18 which normally lies at a distance away from a portion 28 of the conjunctiva 18 which normally covers the intended implantation site. A small slit distanced away from the implantation site, for example a 1-2 mm slit about 5-15 mm away from the implantation site, reduces the possibility of aqueous humor flowing out of the conjunctiva through the slit, reduces the possibility of infection, reduces the possibility of scarring over the axial outlet of the implant, and facilitates closing and healing.

The implant 30, by delivery device 60, is passed through the slit 26, under the conjunctiva 18, to the implantation site in the sclera 14. Figure 9 shows the advancement of the implant only schematically; it will be appreciated that in practice the implant is directed from the slit to the implantation site generally along the surface of the sclera, such that the longitudinal axis of the implant is generally parallel to the surface of the sclera. Upon reaching the implantation site, the implant is tilted for penetration into the sclera. The acute angle of the needle-like tip

formed by the beveled surface 36 of the implant 30 ensures that the implant 30 enters the sclera 14 easily. The needle-like tip penetrates through the sclera 14 into the anterior chamber 20 of the eyeball 10, while the disk 34 is pushed up against the sclera 14.

When the implant 30 is in place, as shown in Figure 10, the retentien spur (or spurs) 52 anchors the implant 30 in the eyeball 10 and prevents the implant 30 from sliding out as the delivery device 60 is withdrawn. The retention spur 52 also prevents the implant 30 from slipping out once in place.

It will be appreciated by persons having ordinary skill in the art that the insertion of the implant is not restricted to the method described above, and it may be inserted by any of several methods known in the art. The delivery device may comprise an 'internal' or 'external' needle. A straight or twisted guide wire, known in the art, may also be used to guide the delivery device to its precise position. To ease insertion, the delivery device may be vibrated, or a lubricant, such as medical paste or gel, can be spread onto the delivery device. Additionally, after implantation of the implant a suitable fibrosis inhibiting compound (e.g. 5FU, mitomycin) may be applied to the implantation site.

Figure 11 shows an alternative embodiment of an intraocular implant 130. The implant 130 comprises a tube 132 attached to an elliptical disk 134. The tube 132 has an inlet end 140, an outlet end 150, and a tube

passage 138, with the tube passage 138 having an axial inlet 141, an axial outlet 151, and circumferential holes 142 to drain the aqueous humor from the anterior chamber 20 of the eyeball 10 into the space under the conjunctiva 18.

The distal end 152 of the tube 132 has a partially conical shape. A plurality of retention projections in the form of retention flanges 158 are formed on the outer circumference of the tube 132, approximately parallel to the disk 134, to act as anchors to retain the implant 130 in the eyeball.

As shown in the enlarged view in Figure 12, the disk 134 comprises an elliptical base 144, an outer rim 146, and an inner upright curved to form an inner rim 148, defining therebetween a reservoir 154. A plurality of "U"-shaped passageways 156 are formed in the inner rim 148 for allowing aqueous humor to flow from the axial outlet 151 into the reservoir 154. The outer rim 146 and the inner rim 148 prevent the conjunctiva 18 from clogging the axial outlet 151.

As shown in Figure 12, the disk 134 is elliptical in shape. The longer axis of the disk 134 is approximately twice the diameter of the tube 132, and the disk 134 is eccentrically displaced relative to the tube 132. The elliptical shape and placement of the disk 134 allows a wide anchoring area for the implant 130 and maximizes the outlet drainage area on the longer axis of the ellipse. The shorter axis of the ellipse enables the

implant 130 to fit within the narrow space under the conjunctiva 18.

Figure 13 illustrates a delivery device 160 and a method of inserting the intraocular implant 130 into an eyeball. The implant 130 is slidably fixed over a needle 164 of the delivery device 160, which, similar to a standard medical syringe, has needle 164 attached to a housing 162. The tip 174 of needle 164, which passes through the implant 130, is acutely angled so that the tip 174 is generally in line with the angle of the lower part of the implant 130.

A front surface of the delivery device 160 is formed as an abutment surface angled to match the angle of the disk 134 and further comprises an indent 172 to hold the implant 130 in place during implantation. The shape of the delivery device 160 and the angled surface of the disk 134 prevent the implant 130 from rotating during implantation.

The delivery device 160 shown in Figure 13 is used in a manner similar to that described above with reference to Figures 8 through 10. In this embodiment, however, the acute angle of the needle tip 174 pierces the sclera. The angled inlet end of the implant device 130 follows the needle tip 174 through the sclera 14, into the anterior chamber 20 of the eyeball. As shown in Figure 14, the retention flanges 158 anchor the implant 130 in position and prevent the implant 130 from sliding out as the delivery device 160 is withdrawn. The

anchorage of the retention flanges 158 also prevents the implant 130 from slipping out once in place.

Figures 15 and 16 illustrate a third embodiment of an intraocular implant. This embodiment is similar to that shown in Figures 1 through 10, with the exception that a separately attached retention projection in the form of a retention plate 252 is used for anchoring instead of the retention spur 52. The retention plate is inserted into a groove 253 in the tube of the implant 230 and may be fastened by any suitable means, for example by welding in the case of an implant 230 constructed of stainless steel.

An implant constructed in accordance with the invention may be manufactured entirely from or covered with any suitable material such as stainless steel, silicon, gold, nitinol, Teflon, tantalum, PMMA, or any other suitable plastic or other material. The implant may also be coated with heparin or any other suitable biology active compound.

Manufacture of an implant in accordance with the invention may be carried out according to the following process. The tube may be formed from the tip of a standard stainless steel hypodermic needle. Using an EDM machine, small holes are drilled proximate the tip of the needle to form the circumferential holes. At a distance from the tip corresponding to the desired length of the tube, the needle is cut at the appropriate angle to correspond to the desired angle of the disk. The side

of the needle is then undercut to form a projection which can be later bent outwardly to form the spur.

The disk may be chemically etched from a stainless steel sheet according to the following process. A pattern of the disk is drawn on a computer aided design (CAD) system and plotted on a transparent film using a laser plotter. Plottings are made of both the upper side and the lower side of the disk. The plotting for the upper side, for example, includes the outer rim and the inner uprights; the plotting for the lower side, for example, includes the base of the disk.

A layer of photoresist is adhered to both surfaces of the stainless steel sheet. The photoresist is then exposed to UV light through the film on which the plottings are made. The areas of the sheet which are blocked by the plottings are not exposed. The photoresist which has been exposed to UV light is then chemically removed.

Using an etching chemical, the stainless steel sheet is then etched, so that the chemical eats away the areas of the sheet from which the photoresist has been removed. The etching is time-controlled such that the chemical takes away material only to a predetermined depth.

By use of a plotting for the upper side which includes the outer rim and the uprights, the chemical on the upper surface of the sheet takes away material on the outside of the disk, in the reservoir including between

the uprights, and in the center of the disk which is to receive the tube. Because the etching is time-controlled, the chemical acting on the top of the sheet takes away material only part way through the thickness of the sheet. By use of a plotting for the lower side which includes the base of the disk, the chemical on the lower surface of the sheet takes away material on the outside of the disk and in the center of the disk which is to receive the tube. The chemical acting on the bottom of the sheet takes away material part way through the thickness of the sheet. Because of action from both the top and the bottom, the material on the outside of the disk and in the center of the disk which is to receive the tube is completely taken away by the etching process through the entire thickness of the sheet. A small projection may be left on the outside of the disk during the etching process to prevent the disk from being dislodged from the sheet.

An alternative method for manufacturing an implant according to the invention is illustrated in Figures 17 through 19. Figure 17 shows an initial step of the process in which an outer tube 74 having a longitudinal bore is cut into the illustrated pattern. The outer tube 74 may have, for example, an outer diameter of about 1 mm and an inner diameter (i.e., a diameter for its longitudinal bore) of about 400 micrometers. In the illustration, the outer tube 74 has been cut into two pieces 76 and 78; however, it should be

recognized by persons skilled in the art that the two pieces 76 and 78 need not be completely separated. For example, the bottom half of the tube 74 could be left intact between the two pieces, leaving a connection piece in the form of a half-cylinder between the piece 76 and the piece 78.

In a next step of the process, illustrated in Figure 18, a smaller inner tube 90 is placed inside the longitudinal bore of the remaining portion or portions of the outer tube 74. The inner tube 90 has an outer diameter that generally corresponds to the inner diameter of the outer tube 74. For example, the inner tube may have an outer diameter of about 400 micrometers. The inner tube also has a longitudinal bore, which may have a diameter, for example, of about 200 micrometers. When the inner tube 90 is placed inside the outer tube 74, the two tubes may be secured together, for example by welding the tubes together at the areas identified by reference numerals 86 and 88.

After the two tubes are joined together, further cuts are made to form the implant as shown in Figure 19. This step includes simultaneously cutting the outer tube and inner tube along an angled plane at the outlet end of the implant to form the upper surface of the disk 84 and to cut away the unwanted portion of the inner tube 90 that would otherwise have projected beyond that upper surface of the disk 84. The portion of the inner tube 90 that remains after these final cuts forms

the implant shaft. The portions of the outer tube 74 that remain after these final cuts form the retention projection 82 and the disk 84.

It will be appreciated by persons having ordinary skill in the art that variations on this manufacturing process and other manufacturing processes are possible. For example, an implant made of plastic may be manufactured by a suitable molding operation.

Various mechanisms may be used, if desired, for giving different flow characteristics to the implant. It may be desirable to use implants with different flow characteristics for different patients and/or to have an implant in which the flow characteristics may be changed after implantation in a particular patient.

Figures 20 through 25 illustrate various mechanisms for assisting in controlling the flow of fluid, e.g. aqueous humor, through an implant 100 according to the invention. In Figure 20, the implant 100 has a flow controlling wire or rod 92A in the tube passage 102. The flow controlling rod 92A may be spot welded on one side to the inside of the tube passage 102.

The effect of the flow controlling rod 92A is to reduce the cross-sectional area through which the fluid flows for a particular length inside the tube passage 102 of the implant 100. Because the flow is a function of the cross-section and length of the lumen through which it passes, the interposition of the flow controlling rod 92A serves to increase the resistance to

flow. In an intraocular implant, for example, this assists in reducing the risk of hypotony.

The diameter of the flow controlling rod 92A may be selected in accordance with the flow characteristics that are desired. For example, an internal tube passage of the implant having a diameter of 200 micrometers may be fitted with a flow controlling rod 92A having a diameter that is, for example, between 175 micrometers and 195 micrometers. A larger diameter for the flow controlling rod 92A provides more resistance to flow.

The length and cross-sectional shape of the flow controlling rod may similarly be selected to achieve the flow characteristics that are desired. Figures 21A through 21D show four possible cross-sectional shapes for the flow controlling rod. Flow controlling rod 92A has a circular cross-section. Flow controlling rod 92B is similar to flow controlling rod 92A with the addition of grooves 94B. Flow controlling rod 92C has a flat surface 96C. Flow controlling rod 92D has a longitudinal bore 98D.

Figures 22 and 23 illustrate further possible modifications to the flow controlling rod to modify the flow characteristics. As shown in Figure 22, the flow controlling rod 92E may have an external helical groove 99E giving it a threaded appearance. If the diameter of the flow controlling rod 92E is large such that most or all of the flow occurs through the helical groove 99E,

this embodiment provides a longer path for the fluid to travel and thus a greater resistance to flow. Additionally or alternatively, as shown in Figure 23, the flow controlling rod 92F may be tapered or partially conical in shape. This embodiment provides less resistance to flow toward the outlet end of the implant. Persons skilled in the art will appreciate that numerous other variations are possible for the shape and size of the flow controlling rod.

With the use of a flow controlling rod that is adjustable, the flow characteristics of the implant may similarly be adjustable. Thus, for example, the flow controlling rod may be mounted within the tube passage by only a friction fit, so that its position within the tube passage may be adjusted. As illustrated schematically in Figure 24, the longitudinal position of the flow controlling rod 92 may be adjusted to provide a longer or shorter distance d for the fluid to travel from the inlet side hole(s) 104 to the end of the flow controlling rod 92. A longer distance d for the fluid to travel provides a higher resistance to flow. Another way to adjust the flow when using a flow controlling rod with a non-circular cross-section, as in Figures 21B and 21C, is to rotate the rod within the tube passage. This rotation changes the orientation of the rod with respect to the side holes 104, giving different flow characteristics to the implant.

The flow characteristics of the implant may be adjusted before implantation in accordance with the patient's needs, or, if desired, the implant may be constructed to allow for the flow characteristics through the implant to be varied after the implant has been implanted. After the implant has been implanted, the flow controlling rod 92 may be pushed forward toward the inlet end of the implant, for example by a tool with a wire. This reduces the distance d that the fluid must travel from the inlet side hole(s) 104 to the end of the flow controlling rod 92, and thus reduces the resistance to flow through the implant. Alternatively, a rod with a non-circular cross-section may be rotated after implantation.

Another way to have different flow characteristics is to have different locations or configurations of the side holes 104. Thus, different models of the implant may have side holes in different locations and/or with different configurations. Alternatively, a single implant may have side holes which can be changed, for example by temporary occlusion of one or more of the side holes. Figure 25 illustrates an implant with occluded side holes 104. The occlusion may be permanent or temporary. Temporary occlusion may be with an absorbable material or with a material that may be removed after implantation, for example by a tool or laser probe. In this way, the resistance to flow can be reduced after implantation.

The implant may additionally or alternatively be designed to give different flow characteristics as a function of the fluid pressure. The flow controlling rod or wire may itself be flexible or movable and designed to flex or move in response to the fluid pressure. For example, as shown in Figure 26, the flow controlling rod 92G may be fixed at one end 122 to a front end of the implant 100 with the other end 124 of the rod 92G unattached and free to bend. Before implantation, the rod 92G extends essentially parallel to the axis of the tube passage. When implanted, pressure from the fluid through the side holes 104 causes the rod 92G to flex, as indicated by the dashed lines. In this way, when the fluid pressure rises at the inlet end of the implant, the rod 92G bends to allow greater flow.

Another related example is shown in Figure 27. In that embodiment, the tube passage 102A is tapered and the flow controlling rod 92H is biased within the tube passage 102A by a spring 126. The flow controlling rod 92H is illustrated as tapered, but it will be appreciated that other shapes are possible. The spring 126 is shown as braced against a flange 128 near the outlet end of the tube passage 102A, but it will be appreciated that it also may be attached on the opposite side of the rod 92H near the inlet end of the tube passage 102A. When the fluid pressure increases at the inlet end, the force on the rod 92H causes the spring 126 to compress (or, if the spring is positioned on the opposite side of the rod, the

force on the rod causes the spring to extend). The rod 92H is thus displaced longitudinally toward the outlet end of the implant, to a position at which the cross-section of the tube passage 102A is greater. Thus, the area through which fluid is allowed to flow is increased, allowing greater flow. As persons skilled in the art will appreciate, other variations are possible in which the rod moves or flexes to increase flow in response to increased pressure at the inlet end of the implant.

Figure 26 illustrates an end portion of an alternative embodiment of a delivery device 110 according to the invention. The delivery device 110 has a handle (not shown) and a rodlike instrument 112. In this case, the rodlike instrument 112 has central bore 114 in which is located a retractable wire 116. The retractable wire 116 is positioned for penetrating a tube passage 102 of the implant 100 when the implant 100 is attached to the delivery device 110. The delivery device 110 has a retention mechanism including an abutment surface 118 having an angle generally corresponding to that of the disk 106 of the implant 100 for preventing the implant 100 from moving up the delivery device 110 during implantation and a hook 120 for preventing the implant 100 from moving down the wire 116.

For implantation, the implant 100 is placed over the wire 116 with the wire 116 projecting into the tube passage 102 and with the abutment surface 118 abutting against the disk 106 with the hook 120 retaining

the disk 106 around the opposite side. Figure 26 illustrates the end of the delivery device 110 in this condition, with the retention wire 116 in its forward position.

After the implant is in position, the retention wire 116 is retracted out of the implant 100. Figure 27 illustrates the end of the delivery device 110 with the retention wire retracted. With the retention wire retracted, the implant is free to slide away from the hook 120, allowing the delivery device 110 to be withdrawn, leaving the implant in place.

As will also be appreciated by persons having ordinary skill in the art, the various embodiments of implants, methods of manufacture, delivery devices, and methods for implantation described hereinabove are given by way of example only. Various changes, modifications and variations may be applied to the described embodiments without departing from the scope of the invention, defined by the appended claims.

What is Claimed is:

1. A method for manufacturing an implant comprising the steps of:

selecting a first tube having a longitudinal bore and a second tube having a longitudinal bore and dimensioned to fit inside the longitudinal bore of the first tube;

placing the second tube inside the longitudinal bore of the outer tube;

cutting the first tube and the second tube to form the implant.

2. A method for manufacturing an implant as described in claim 1 wherein at least some of the cutting of the first tube is performed before the second tube is placed inside the longitudinal bore of the first tube.

3. A method for manufacturing an implant as described in claim 1 wherein at least some of the cutting of the first tube is performed after the second tube is placed inside the longitudinal bore of the first tube.

4. A method for manufacturing an implant as described in claim 3 wherein the cutting includes simultaneously cutting the outer tube and inner tube

along an angled plane at the outlet end of the implant.

5. A method for manufacturing an implant as described in claim 1 further comprising the step of welding the second tube to the first tube after it is placed in the longitudinal bore of the first tube.

6. A method for manufacturing an implant as described in claim 1 wherein the portion of the inner tube that remains after completion of the cutting forms an implant shaft and the portions of the outer tube that remain after completion of the cutting form a retention projection and a disk of the implant.

7. An implant for regulating fluid flow comprising:

a tube comprising an inlet end, an outlet end, and a tube passage extending between the inlet end and the outlet end for permitting fluid to flow through the tube passage;

wherein the implant has a flow controlling rod located in the tube passage.

8. An implant according to claim 7 wherein the flow controlling rod has a circular cross-section.

9. An implant according to claim 7 wherein the flow controlling rod has a noncircular cross-section.

10. An implant according to claim 7 wherein the flow controlling rod has an external groove on its outer surface.

11. An implant according to claim 10 wherein the external groove is helical.

12. An implant according to claim 7 wherein the flow controlling rod is tapered.

13. An implant according to claim 7 wherein one of the location or angular orientation of the flow controlling rod within the tube passage is adjustable.

14. An implant according to claim 7 wherein the flow controlling rod is bendable within the tube passage to regulate flow in accordance with the fluid pressure.

15. An implant according to claim 7 wherein the flow controlling rod is movable within the tube passage to regulate flow in accordance with the fluid pressure.

16. An implant according to claim 15 wherein the flow controlling rod is biased by a spring within the tube passage.

17. An implant for regulating fluid flow comprising:

a tube comprising an inlet end, an outlet end, and a tube passage extending between the inlet end and the outlet end for permitting fluid to flow through the tube passage;

wherein the tube has at least one circumferential hole which opens into the tube passage and one or more of the circumferential holes is occluded.

18. An implant according to claim 17 wherein the occlusion of at least one of the circumferential holes is temporary.

19. An implant according to claim 18 wherein the temporary occlusion is caused by material in the hole which is later removed.

20. An implant according to claim 19 wherein the temporary occlusion is caused by an absorbable material located in the hole.

21. An implant according to claim 19 wherein the temporary occlusion is caused by a material located in the hole which is later removed by one of a tool or laser.

22. An implant according to claim 17 comprising at least two circumferential holes which opens into the tube passage, wherein at least two of the circumferential holes are located at different distances from the inlet end of the implant and wherein the circumferential holes may be occluded or open to provide the implant with different flow characteristics.

23. A delivery device for implanting an implant, the delivery device comprising:

a handle;

a rodlike instrument having a bore;

a retractable wire located in the bore of the rodlike instrument; and

a retention mechanism including an abutment surface for preventing the implant from moving up the delivery device during implantation and a hook for preventing the implant from moving down the wire during implantation.

24. A delivery device according to claim 23 wherein the hook prevents movement of the implant in a direction parallel to the wire but permits movement in a direction transverse to the wire, such that when the wire is retracted from a tube passage of the implant, the implant is permitted to slide away from the hook to separate the implant from the delivery

device.

25. A delivery device according to claim 23 wherein the abutment surface has an angle generally corresponding to that of a disk of the implant.

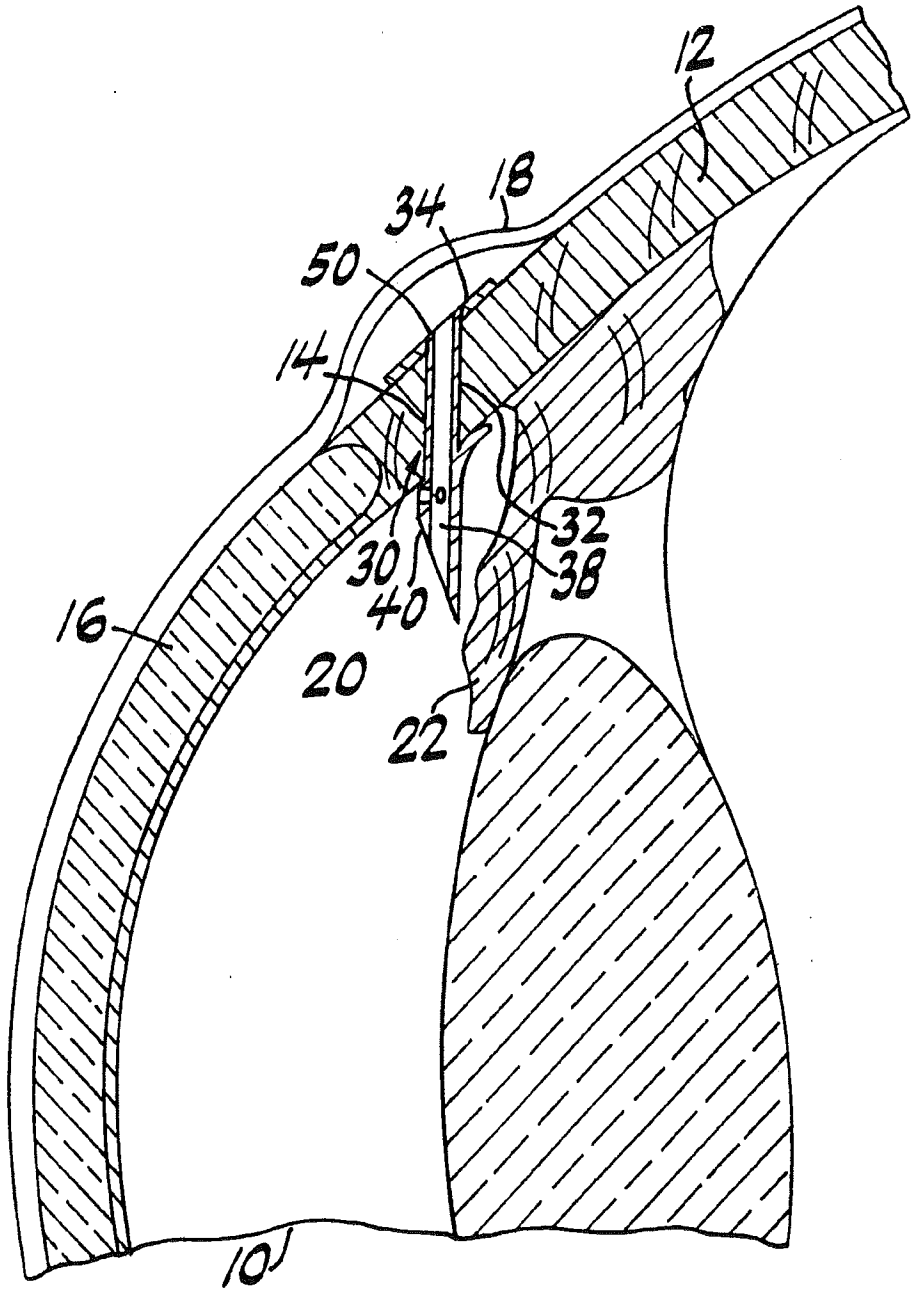


FIG. 1

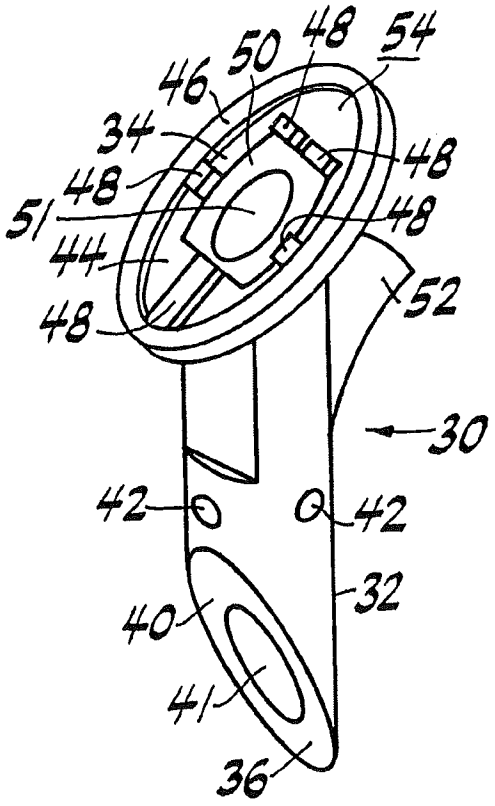


FIG. 2

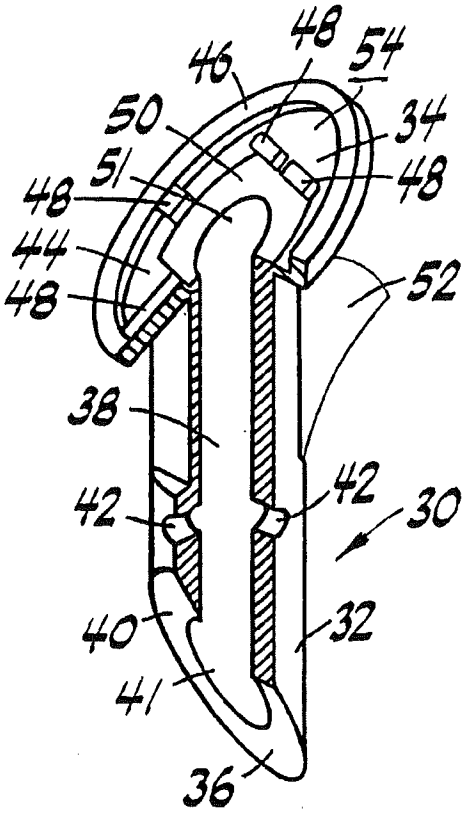


FIG. 3

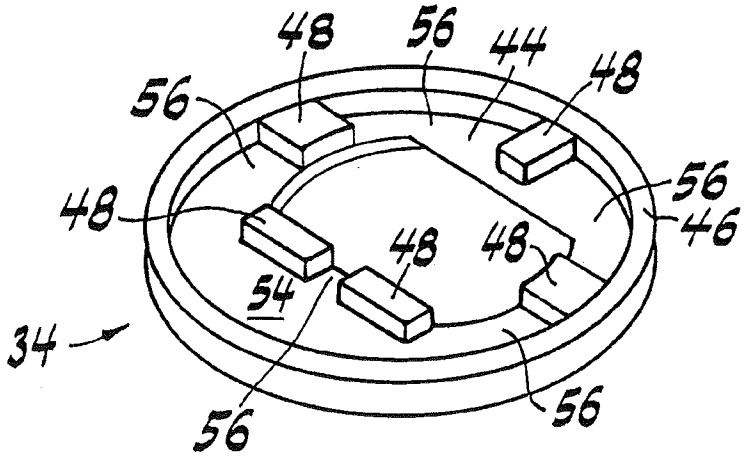


FIG. 4

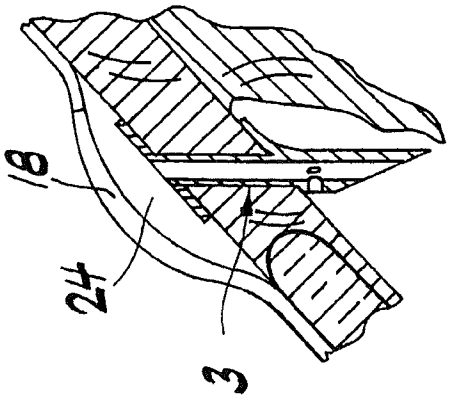


FIG. 7

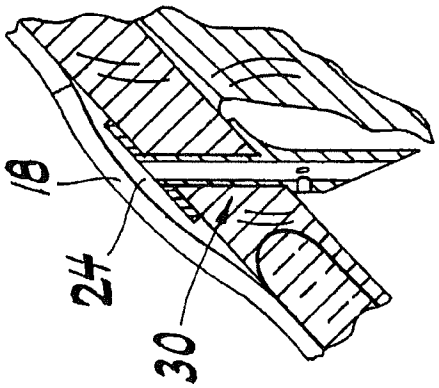


FIG. 6

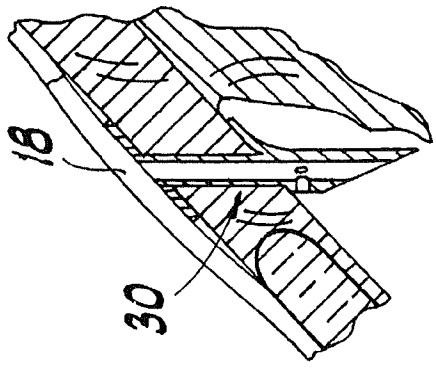


FIG. 5

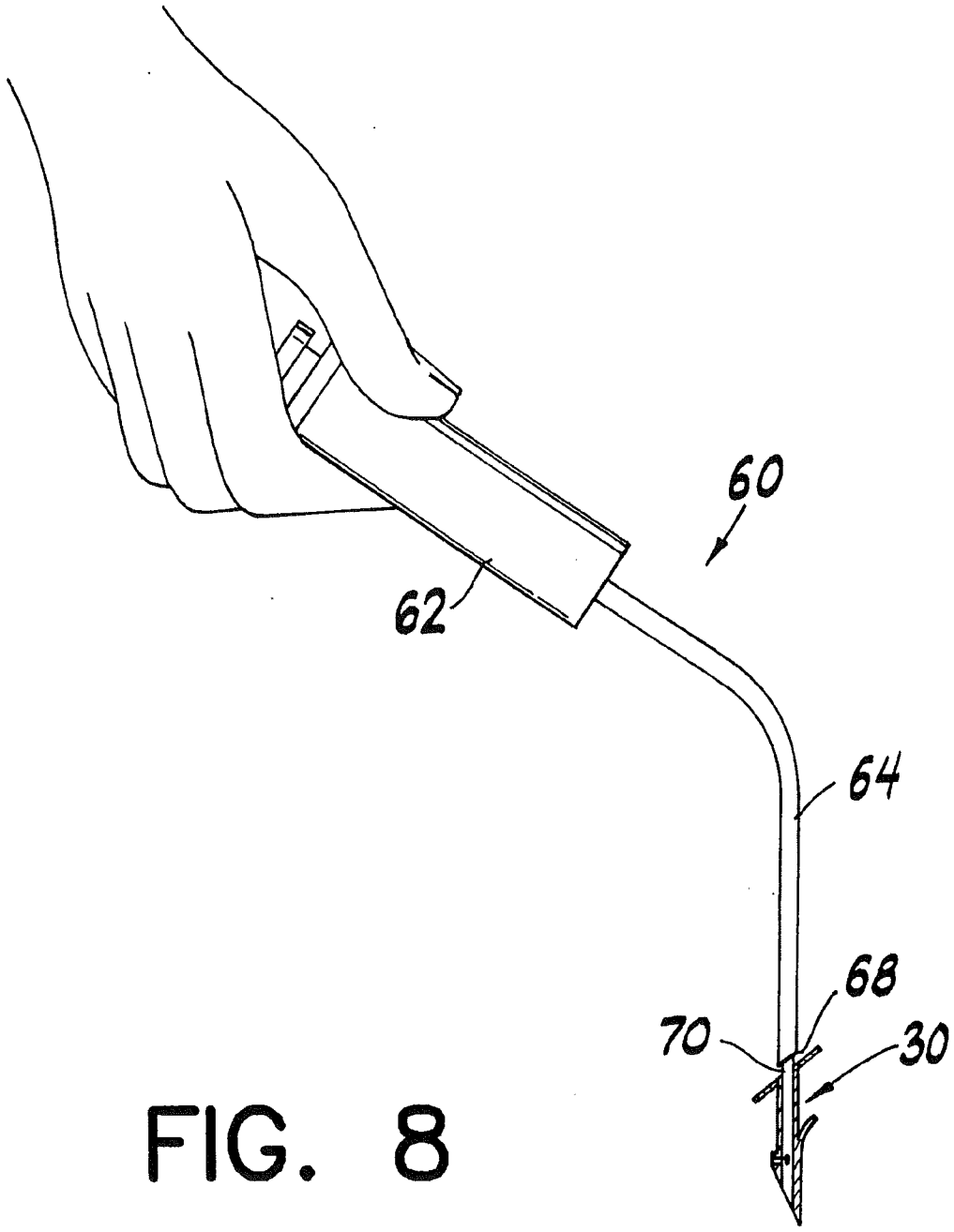


FIG. 8

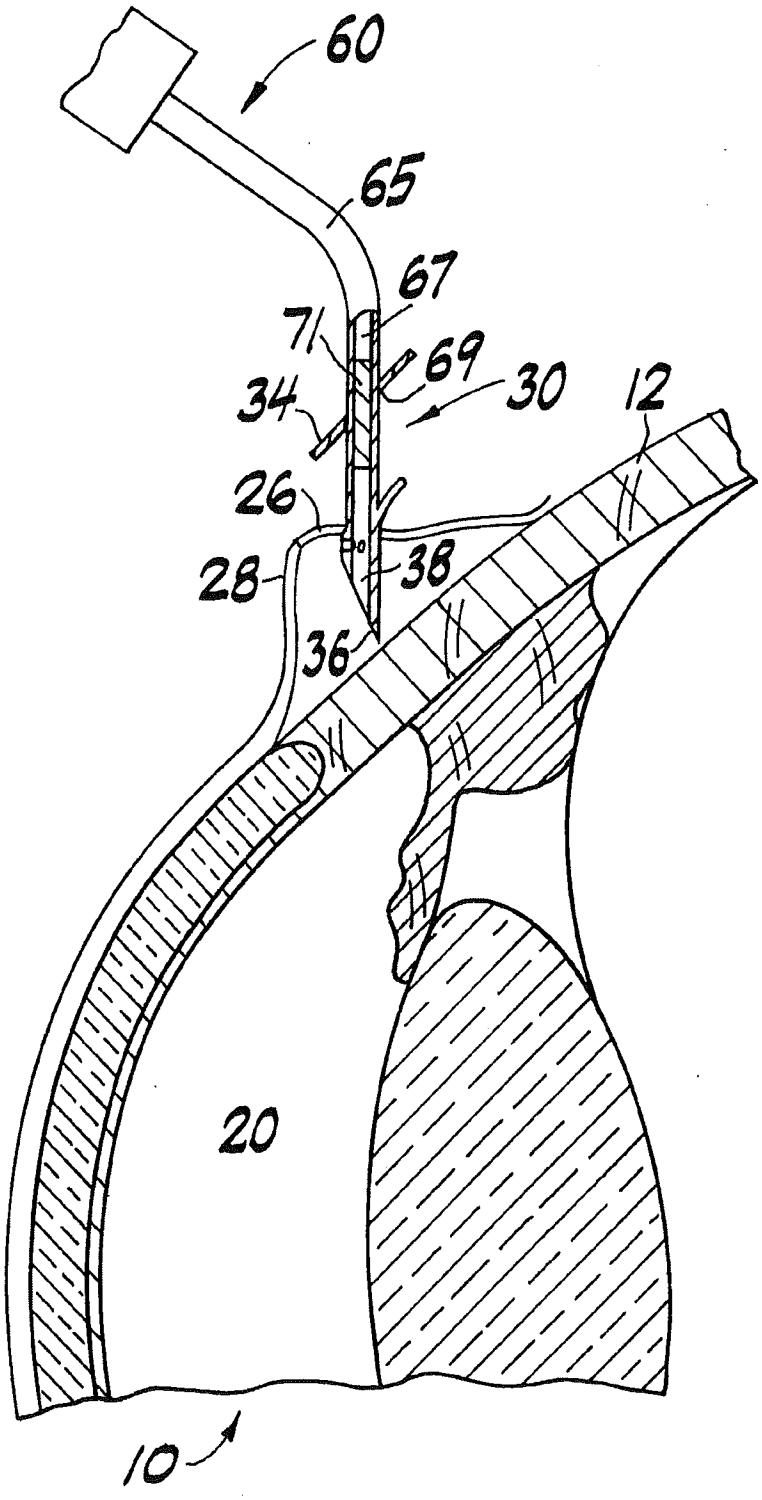
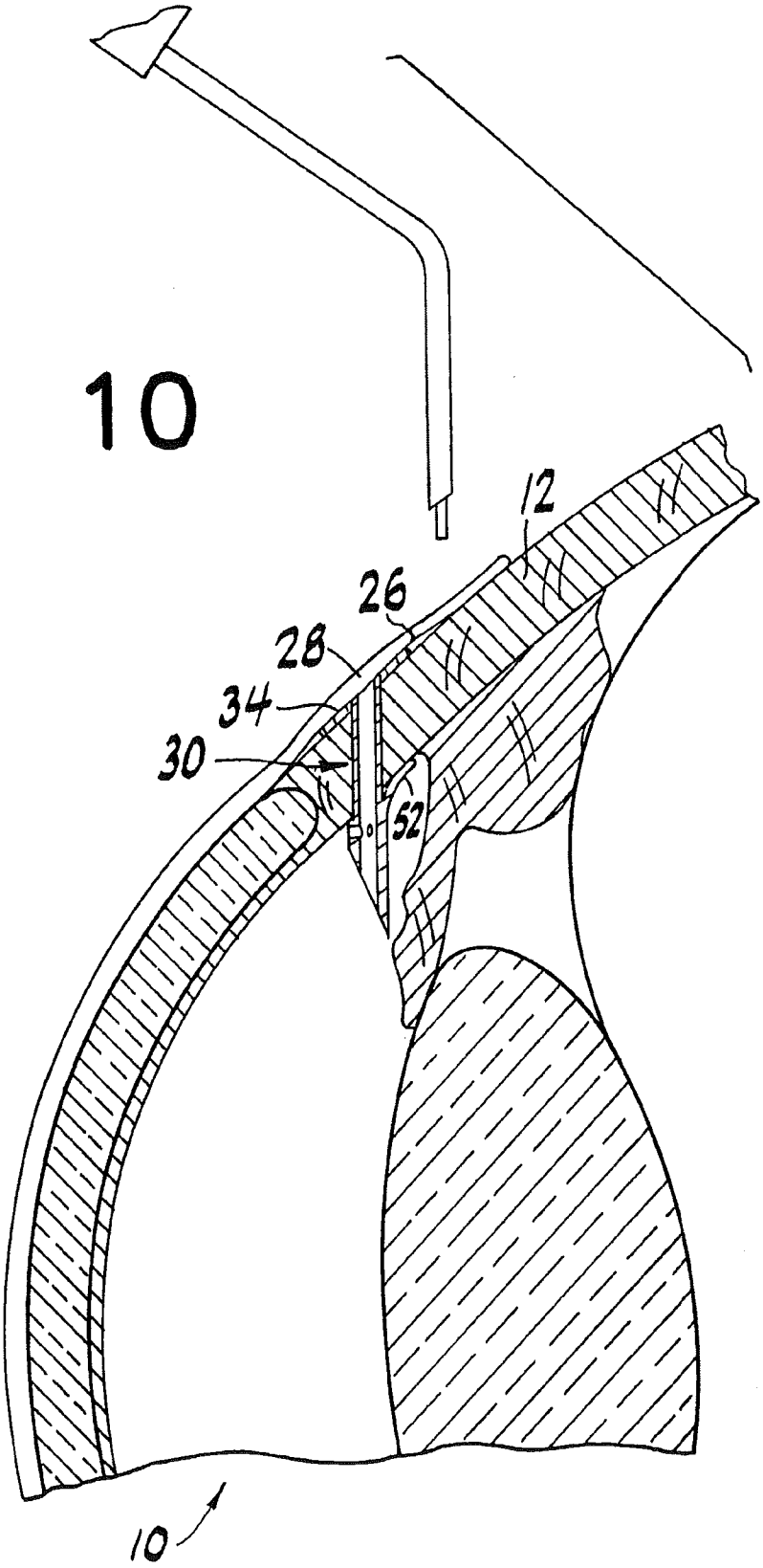


FIG. 9

FIG. 10



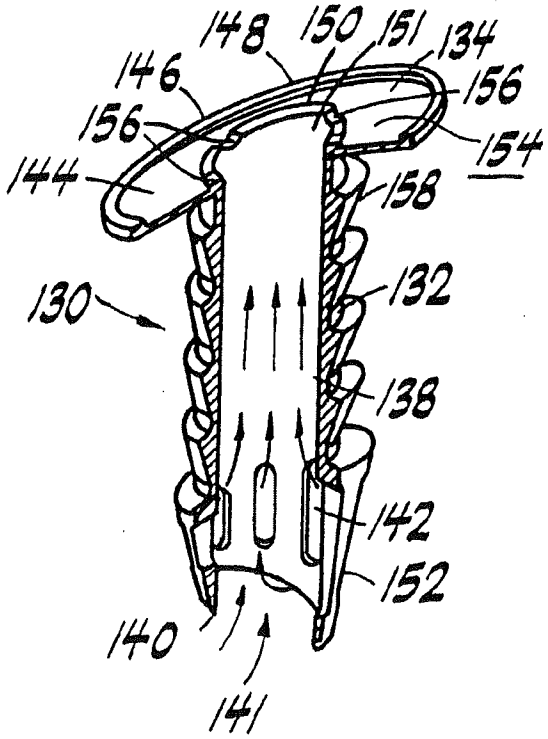

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FIG. 11

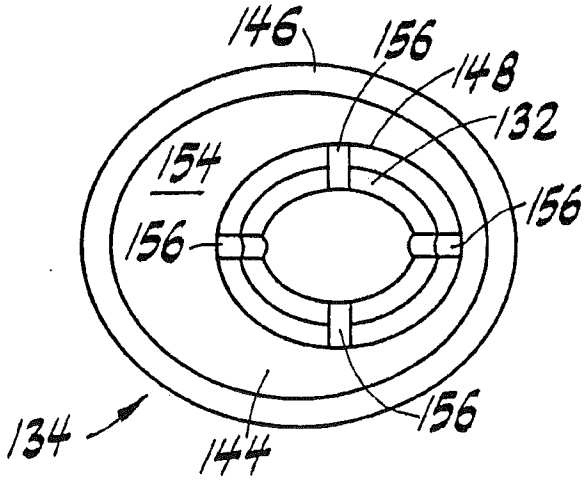
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FIG. 12

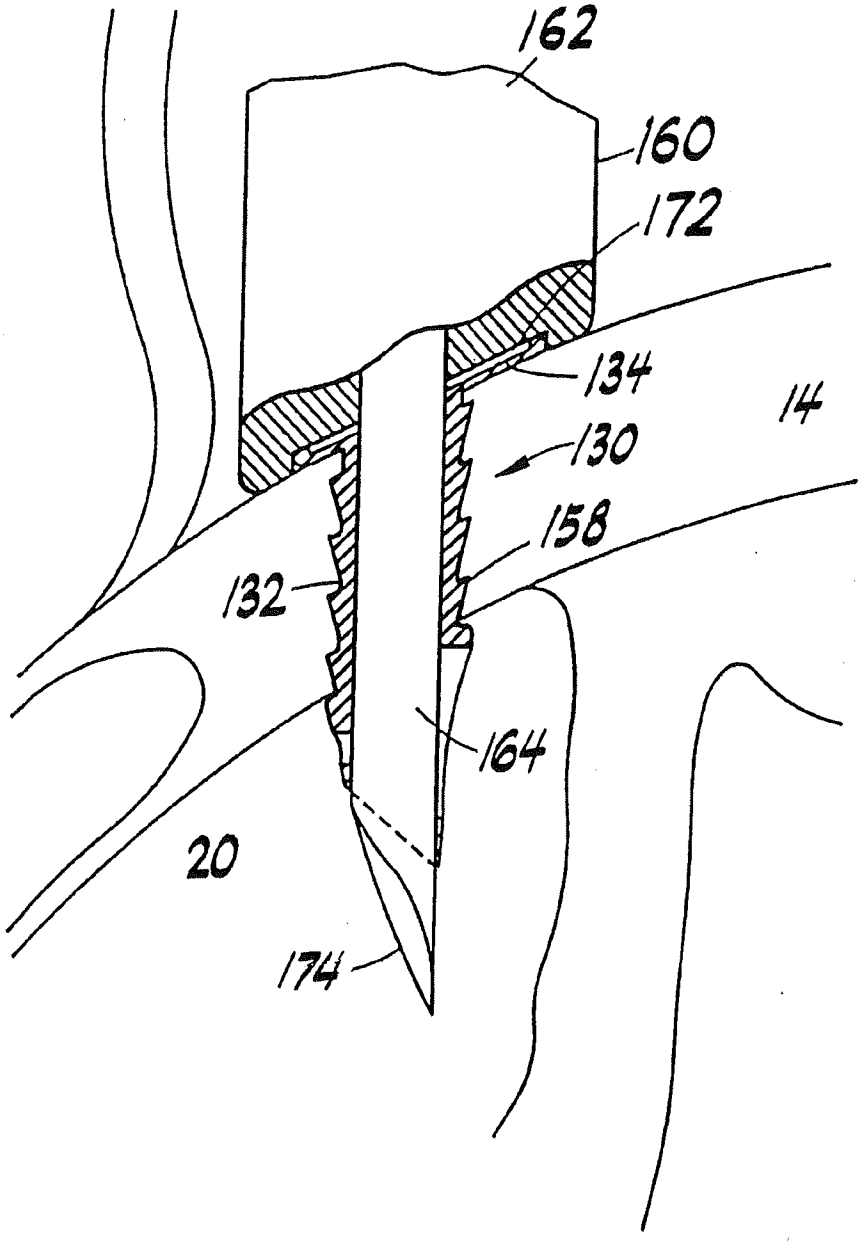

FIG. 13

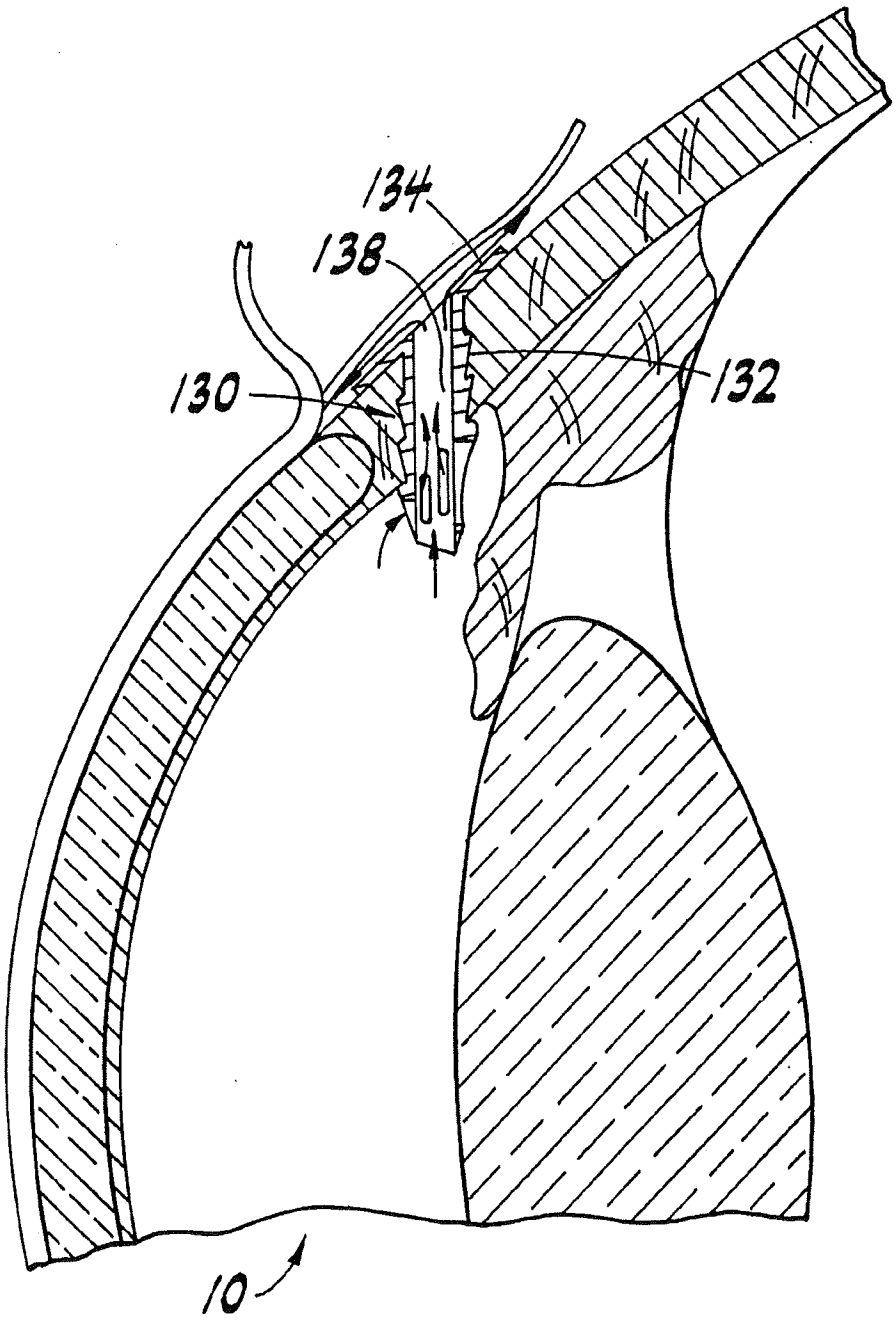

FIG. 14

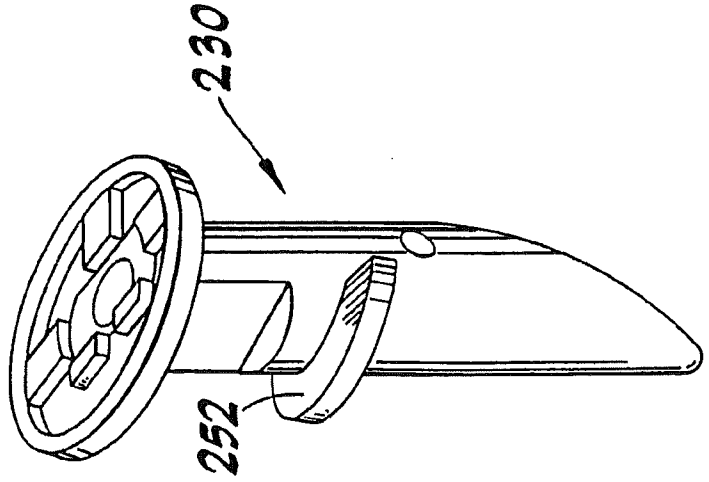

FIG. 16

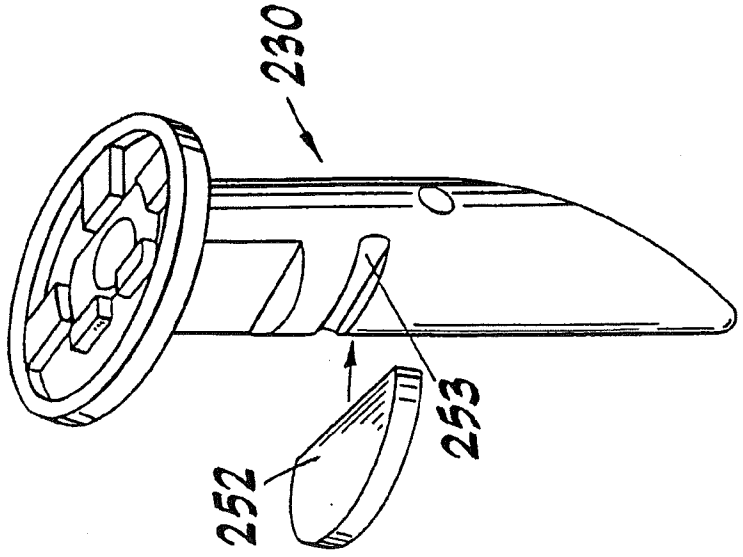

FIG. 15

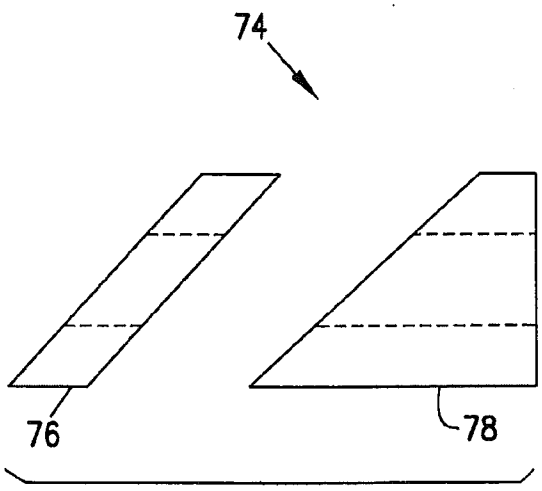

FIG. 17

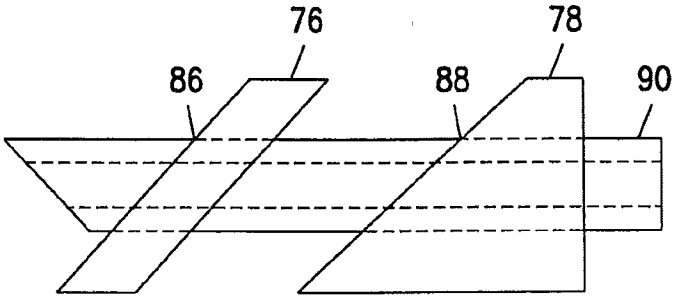

FIG. 18

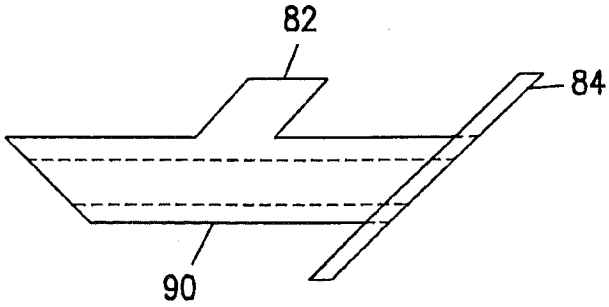

FIG. 19

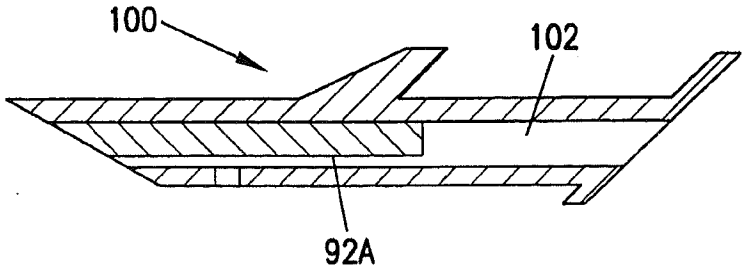

FIG. 20

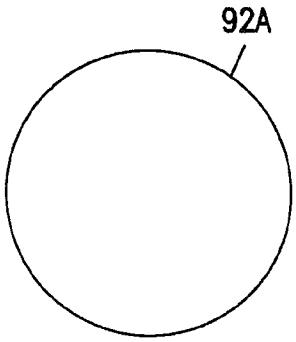

FIG. 21A

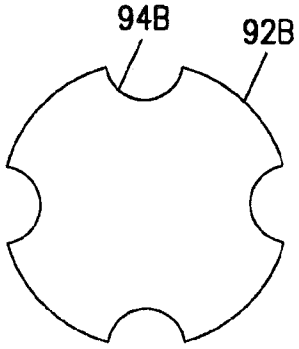

FIG. 21B

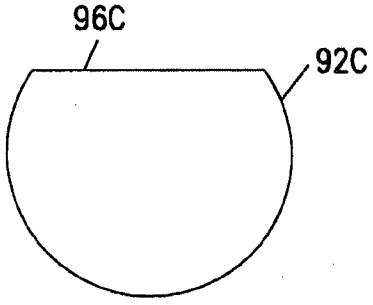

FIG. 21C

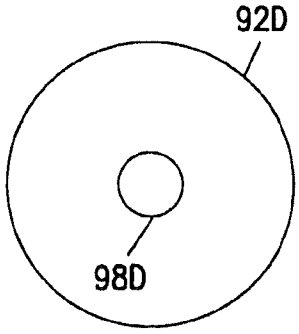

FIG. 21D

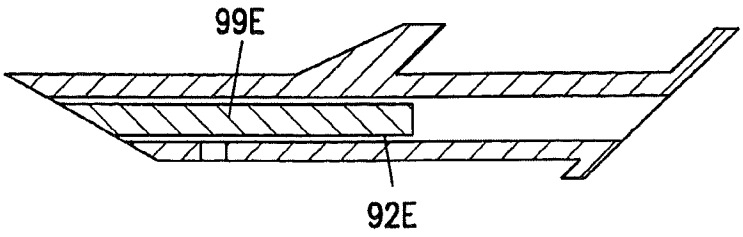

FIG. 22

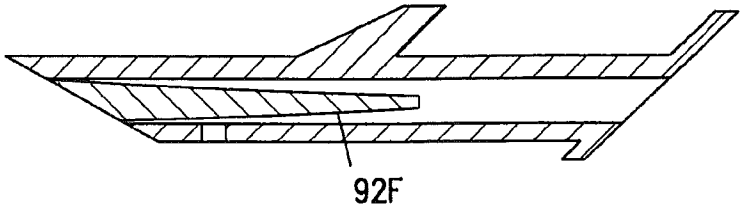

FIG. 23

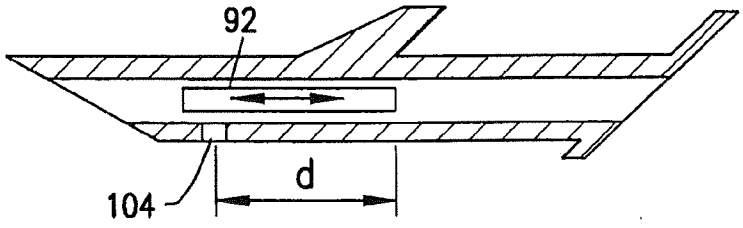

FIG. 24

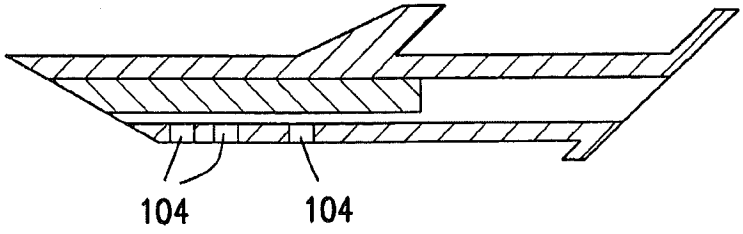

FIG. 25

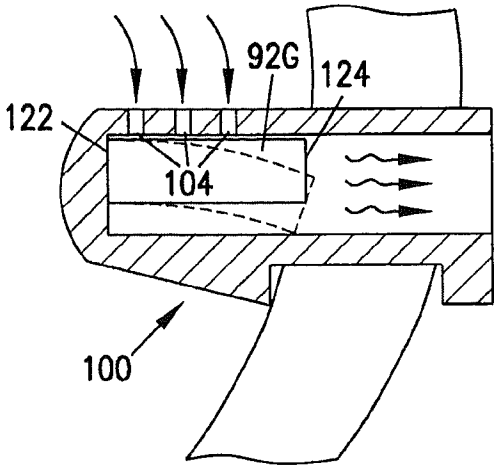

FIG. 26

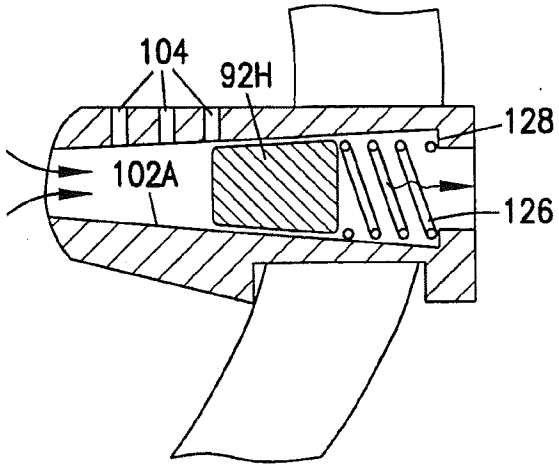

FIG. 27

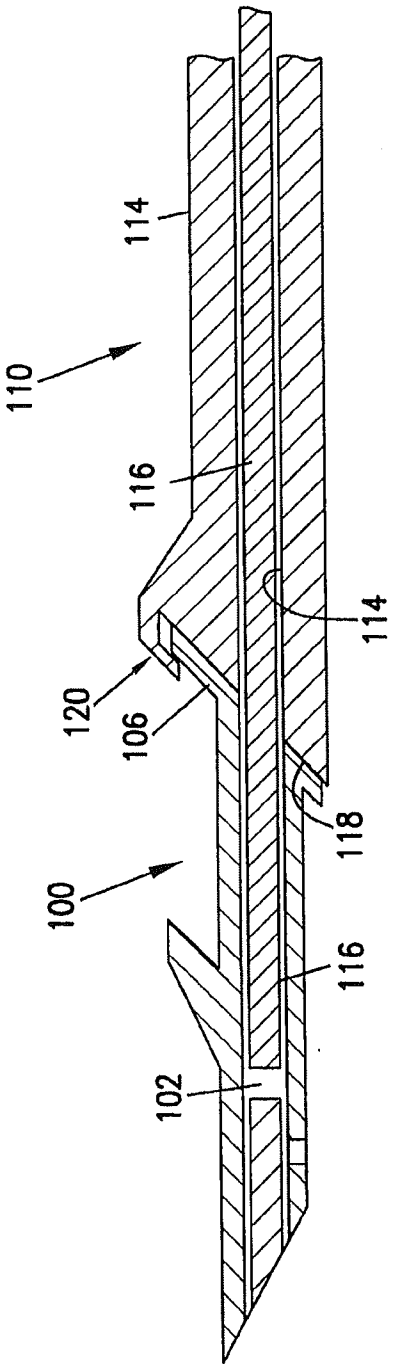
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FIG. 28

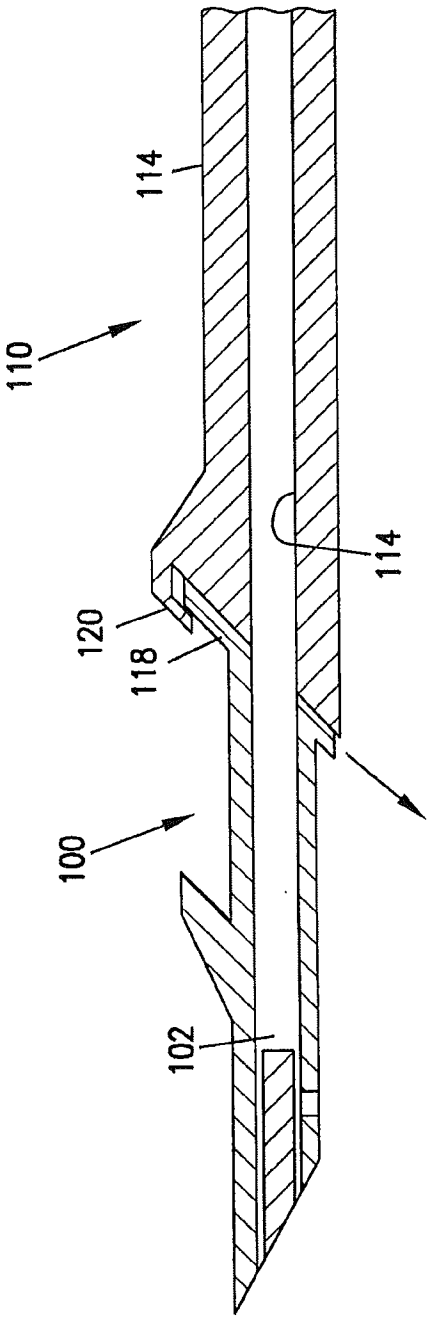
110
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FIG. 29

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/24651

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61F9/007

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 36377 A (OPTONOL LTD ;WERNER MARY C (US)) 21 November 1996 see the whole document ---	1,7,17, 23
A	US 4 402 681 A (HAAS JOSEPH S ET AL) 6 September 1983 see claims; figures ---	1,7,17
A	US 5 626 559 A (SOLOMON ARIE) 6 May 1997 see column 2, line 61 - column 3, line 6; figure 3 --- -/--	1,7,17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document. with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SOVIET PATENTS ABSTRACTS Section PQ, Week 941929 June 1994 Derwent Publications Ltd., London, GB; Class P32, AN 94-157341 XP002093877 & SU 1 797 884 A (TURK EYE DISEASE RES INST), 28 February 1993 see abstract	1,7,17
A	US 5 433 701 A (RUBINSTEIN MARK H) 18 July 1995 see claims 1-12; figures 1-6	1,7,17

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INTERNATIONAL SEARCH REPORT

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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(54) Title: UROGUANYLIN AS AN INTESTINAL CANCER INHIBITING AGENT

(57) Abstract: Disclosed is a method of retarding the development of polyps and prevention, inhibition and treatment of cancer in the intestine of a subject by administration of a composition comprising a peptide with the active domain of uroguanylin, or any agonist peptide or compound binding to the guanylate cyclase receptor GC-C in the intestine.

UROGUANYLIN AS AN INTESTINAL CANCER INHIBITING AGENT

This application claims priority from U.S. provisional application # 60/157,950, filed October 6, 1999, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to the use of certain peptides, more particularly the use of uroguanylin, prouroguanylin, guanylin, and other like peptides to retard the development of polyps and prevent, inhibit or treat cancer in the intestine.

The pathogenesis of colorectal cancer is characterized as a multistep process that begins with increased proliferation and/or decreased apoptosis of colorectal epithelial cells resulting in generation of polyps, followed by adenoma formation and ultimately to adenocarcinoma. Certain individuals develop multiple colorectal adenomas and subsequent carcinomas early in life because of a genetic defect in the APC gene responsible for causing a condition called familial adenomatous polyposis (FAP). Dihlmann et al, *Dominant negative effect of the APC 1309 mutation: a possible explanation for genotype-phenotype correlations in familial adenomatous polyposis*, Cancer Res. 1999 Apr. 15, 59(8): 1857-60. Chemoprevention has evolved during the last decade as a viable strategy for cancer prevention, with the aim of controlling the development of cancer through pharmacological and/or dietary intervention prior to the appearance of a clinically detectable tumor. Reddy, B.S. (1997) *Chemoprevention of colon cancer by dietary administration of naturally-occurring and related synthetic agents*, Adv. Exp. Med. Biol. 400B:931-936.

Uroguanylin and guanylin are structurally related enteric peptide hormones that are secreted intraluminally by different types of cells, include enterochromaffin, goblet and others within the intestinal mucosal lining. A receptor for theses peptides that has been identified at the molecular level is a transmembrane form of guanylate cyclase

(GC) known as GC-C. Krause, W.J. et al, *The guanylin and uroguanylin peptide hormones and their receptors*, Acta. Anat. (Basel) 160:213-231 (1997). GC-C receptors are localized on the luminal surface of enterocytes throughout the GI tract. Swenson, E.S. et al, *The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine*, Biochem. Biophys. Res. Commun. 225:1009-1014 (1996). Binding of uroguanylin or guanylin to the extracellular domain of GC-C receptors stimulates intracellular production of the second messenger cGMP, resulting in activation of cystic fibrosis transmembrane conductance regulator (CFTR), the apical membrane channel for efflux of chloride from enterocytes lining the intestinal tract. Forte, L.R. et al, *Salt and water homeostasis: uroguanylin is a circulating peptide hormone with naturiuretic activity*, Am. J. Kidney Dis. 28:296-304 (1996). Activation of CFTR chloride channel proteins and the subsequent enhancement of transepithelial secretion of chloride leads to stimulation of sodium (Na^+) and water secretion into the intestinal lumen. Forte, L.R. et al, *Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology*, Regul. Pept. 81:25-39 (1999). Therefore, one of the major physiological functions of these hormones is the regulation of fluid and electrolyte transport in the gastrointestinal (GI) tract by serving as paracrine regulators of CFTR activity.

The precursor of uroguanylin is prouroguanylin, which is broken down by endogenous proteases in the intestinal tract to produce the active uroguanylin. Chymotrypsin activates prouroguanylin to cleave it into its active form of uroguanylin. Forte, et el, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone With Natriuretic Activity*, Am. J. Kid. Dis. 1996, 28, No.2, 296-304. Uroguanylin is an acid-stable and proteolysis-resistant peptide, which will remain in tact to act on the intestinal lumen directly rather than being absorbed

systemically. Uroguanylin and guanylin are produced throughout the intestinal mucosa and in the myocardium. Forte et al, *Salt and water homeostasis:uroguanylin is a circulating peptide hormone with natriuretic activity* Am. J. Kidney Dis. 28:296-304 (1996). Human uroguanylin has been isolated from human urine and has been chemically synthesized by solid phase peptide synthesis as described in U.S. Patent Number 5,489,670 for *Human Uroguanylin*. Additionally, human guanylin has been isolated from human intestinal cells and has been chemically synthesized by solid phase peptide synthesis as described in U.S. Patent Number 5,969,097 for *Human Guanylin*.

Binding of uroguanylin or guanylin to the guanylin cyclase receptor stimulates the intracellular production of the cGMP ultimately resulting in the stimulation of salt and water secretion into the intestinal lumen. Uroguanylin and guanylin receptors are found on the luminal surface of epithelial cells lining the intestinal tract and renal proximal tubules as well as in other organs. Forte et al, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone with Natriuretic Activity*, Am. J. Kid. Dis.1996, 28, No. 2, 296-304. Uroguanylin has been found to stimulate increases in cyclic GMP levels in a manner similar to another family of heat stable enterotoxins (STs) secreted by pathogenic strains of E. coli and other enteric bacteria that activate intestinal guanylate cyclase and cause secretory diarrhea, which is a major cause of traveler's diarrhea and many deaths in developing countries. Forte et al, *Lymphoguanylin: Cloning and Characterization of a Unique Member of the Guanylin Peptide Family*, Endocrinology Vol. 140, No. 4, p.1800-1806. These ST peptides act as molecular mimics of the endogenous mammalian peptides of uroguanylin and prouroguanylin. Forte et al, Endocrinology Vol. 140, No. 4, p.1800. Unlike uroguanylin the STs from enteric bacteria do not have a decrease in potency when the pH changes in the colon. STs are more potent than either uroguanylin or guanylin under both acidic and alkaline

conditions. Forte et al, *Guanylin: a peptide regulator of epithelial transport,* The FASEB Journal, vol. 9, 643-650 (1995). Uroguanylin is believed to regulate fluid and electrolyte transport in a manner similar to guanylin and the STs in the GI tract. Therefore, as mentioned in previous publications the human uroguanylin may act as a laxative and be useful in patient suffering from constipation.

SUMMARY OF THE INVENTION

Among the objects and features of the present invention may be noted the provision of a method of modulating polyps in the intestine of a subject, in need thereof; said "modulating" or "modulation" includes retarding the development of polyps, preventing, treating, and inhibiting polyps. Also, the present invention is directed to a method of preventing, inhibiting and treating cancer in the intestine (small intestine and colon) of a subject in need thereof.

Briefly, therefore, the present invention is directed to a process for modulating polyps in the intestine of a subject, in need thereof, which comprises the administration of a peptide including the amino acid sequence:

X_8-Asp- Asp- Cys- X_1- X_2- Cys- X_3- Asn- X_4- X_5- Cys- X_6- X_7- Cys-X_9

wherein each of X_1, X_2, X_3, X_4, X_5, X_6, and X_7 is an amino acid residue, X_8 and X_9 are independently hydrogen or at least one amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X_1 and the cystine residue immediately adjacent the amine group of X_6 and by a disulfide bond between the cystine residue immediately adjacent the amine group of X_3 and the cystine residue immediately adjacent the carboxy group of X_7, together with a pharmaceutically acceptable carrier.

The invention is further directed to a method for modulation of polyps in a subject, and to a process for the prevention, inhibition or treatment of cancer in the intestinal tract by administration of a pharmacuetical composition comprising any one of or combination of the following peptides: uroguanylin, human uroguanylin, pro-uroguanylin, and human pro-uroguanylin, guanylin, lymphoguanylin, prolymphoguanylin and heat stable enterotoxin, together with a pharmaceutically acceptable carrier.

Additionally, the invention is directed to a process for modulating polyps in the intestine of a subject, and a process for the prevention, inhibition or treatment of cancer in the intestine of a subject, in need thereof, by administration of a pharmaceutical composition comprising any one of or a combination of agonist peptides and/or other agonist compounds to the guanylate cyclase receptor GC-C, together with a pharmaceutically acceptable carrier.

Other objects of this invention will be in part apparent and, in part, pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1(a) depicts the effect of human uroguanylin on the stimulation of I_{sc} where fresh mouse duodenum consisting of mucosa and submucosa (~1cm^2) was mounted between two halves of Ussing Chambers and bathed on both sides as described. At the arrows, indicated concentrations of TTX, uroguanylin (uroG) and carbachol were added to the apical reservoir. Electrical measurements were monitored with an automatic voltage clamp.

Figure 1(b) depicts the effect of human uroguanylin on the stimulation of I_{sc} where human intestinal mucosa (~1cm^2) was mounted between two halves of Ussing Chambers and bathed on both sides as described. At the arrows, indicated concentrations of TTX, uroguanylin (uroG) and carbachol were added to the apical reservoir. Electrical measurements were monitored with an automatic voltage clamp.

Figure 2 depicts a graphic demonstration of the effect of human uroguanylin on the inhibition of proliferation of T-84 human carcinoma cells. Cells were inoculated in 96-well plates. After an incubation of 72 hours, indicated concentrations of human uroguanylin were added in the media and cells were allowed to grow until they formed semiconfluent monolayers. Subsequently, 5-bromo-2-deoxyuridine (BrdU) was added (final concentration 100μM) and cells were re-incubated for an additional 24 hours. The incorporation of BrdU was measured at 450 nm as per manufacturer's instructions.

Figure 3 depicts the fragmentation of DNA in T-84, human colon carcinoma cells, after treatment with human uroguanylin as analyzed by electrophoresis using 1.8% agarose gel followed by staining with ethidium bromide. Approximately 2X 10^5 cells were inoculated in 35 mm dishes and cultured for 7 days. Semiconfluent monolayers were washed with serum-free DMEM, and further incubated with the same media containing indicated concentrations of human uroguanylin. Subsequently, the cells were quickly collected by trypsinization and washed twice with PBS. Harvested cells were immediately used for DNA isolation as per the instructions of the DNA fragmentation analysis kit (Boehringer Mannheim Corp., Indianapolis, IN). The fragmentation of DNA was analyzed by electrophoresis using 1.8% agarose gel followed by staining with ethidium bromide. Apoptotic DNA provided with the test kit was used as positive control, M (lane 1) and a functionally inactive variant of human uroguanylin (V) was used as negative control (lane 6). Different concentrations of uroguanylin, as indicated were examined (lanes 2 to 5).

Figure 4 depicts microscopic slides with semi-confluent monolayers of Caco-2 cells demonstrating the effects of human uroguanylin on the induction of apoptosis. Cells were cultured on microscopic slides until they formed semi-confluent monolayers. Subsequently the cells on slide B were treated with human uroguanylin (1 μM) for 48 hours.

Induction of apoptosis was detected by fluorescence microscopy directly after the TUNEL reaction as per the instructions of "In situ cell death detection kit" (Boehringer Mannheim Corp., Indianapolis, Indiana). Slide A depicts vehicle-treated cells. Slide B depicts uroguanylin-treated cells.

Figure 5(a) depicts a Northern blot analysis demonstrating that the expression of uroguanylin and guanylin is suppressed in human colon carcinoma cells.

Figure 5(b) depicts an RT-PCR followed by Southern blotting demonstrating that the expression of uroguanylin and guanylin is suppressed in human colon carcinoma cells.

Figure 6(a) depicts a graphic demonstration of the enhancement of daily food consumption by Min-mice after oral administration of human uroguanylin. Total food consumption per day (24 hours) by five (5) animals in one cage was determined and used for calculation of total food consumption per mouse per day. Results are expressed as an average $\pm$ standard deviation.

Figure 6(b) depicts a graphic demonstration of the enhancement of body weight gain by Min-mice after oral administration of human uroguanylin. Body weights of all animals were measured weekly throughout the study. Results are expressed as average $\pm$ standard deviation of gain in body weight per mouse during the study.

Figure 7 depicts the primary structure of human uroguanylin (*h UroG*) [identified as SEQ. ID. 2], human guanylin (*h Gua*) [identified as SEQ. ID. 3], and bacterial enterotoxins (*E.coli* [identified as SEQ. ID. 4]& *V.cholerae* [identified as SEQ. ID. 5]). Bold and italic letters represent the similar residues in these peptides. These residues are believed to be required for the functional activity of these peptides. *E. coli ST* has three additional residues (Asn-Ser-Ser) and *V.cholerae* has two additional residues (Leu and Ile) at their N-terminii. These N-terminal residues make bacterial ST insensitive towards intestinal pH. Two underlined (Asp-Asp) residues are

believed to be important for regulating the functional activity of uroguanylin only at the acidic environment of the intestinal mucosa.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Uroguanylin is secreted naturally by the goblet cells of the intestinal mucosal lining as prouroguanylin, a functionally inactive form, which is then converted to the functionally active uroguanylin in the intestine by endogenous proteases. Uroguanylin is an acid-stable, proteolysis-resistant peptide. Therefore, orally delivered prouroguanylin and uroguanylin will act on the lumenal intestinal surface and not be absorbed systemically. Oral administration of uroguanylin, prouroguanylin and other like peptides, containing the amino acid sequences similar to the active domain, are expected to induce apoptosis, cell death, in the intestinal mucosal cell lining. The induced apoptosis in the intestinal mucosal cell lining is expected to retard the incidence of polyp formation and subsequent intestinal cancer. Without intending to be bound by any theory, applicants believe that the peptides of the invention exert their effects by increasing the rate of apoptosis, cell death, in the intestinal mucosal cell lining promoting the perfect balance between the cell proliferation and the programmed cell death thereby retarding the growth of polyps and preventing, inhibiting, and treating cancer in the intestine and other epithelial-derived cancer possessing receptors for guanylin, uroguanylin, lymphoguanylin and STa family of peptides.

The rate of cell proliferation and cell death in the intestinal mucosa is very rapid. The cells of the intestinal mucosa are in a steady state of turnover to insure a perfect balance between cell proliferation and cell death. The constant rapid renewal of the GI tract epithelium fulfills the functions of maintaining the integrity of normal mucosa, repairing and replenishing differentiated epithelial cells that have specialized

functions. The prevention of apoptosis in the intestinal mucosal cells creating an imbalance in the renewal process results in an increased incidence of polyp formation and subsequent intestinal cancer. *See* Eastwood et al, *A review of gastrointestinal epithelial renewal and its relevance to the development of adenocarcinomas of the gastrointestinal tract*, J. Clin. Gastroenterol. 21: S1-11 (1995). The process of apoptosis is known to be suppressed in colon cancer tissues. Baretton, et al, *Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomoas and carcinomas. Aspectes of Carcinogenesis and prognostic significance*, Cancer 77:255-264 (1996).

A major cellular characteristic of the apoptotic process is a marked loss of cell volume, which is directly related to the movement of ions, with homeostatsis being achieved by the balance of osmotic pressure across the plasma membrane. Hoffman, E.K. et al, *Membrane mechanisms in intracellular signalling in cell volume regulation*, Int. Rev. Cytol. 161:173-262 (1995). Most mammalian cells achieve and maintain this osmotic pressure through the continuous action of Na^+/K^+ ATPase pump, which creates a gradient of these monovalent cations across the membrane. Several sources of evidence have implicated a potential role of K^+ efflux in the induction of apoptosis. Hughes, F.M. et al, *Intracellular K^+ suppresses the activation of apoptosis in lymphocytes*, J.Biol.Chem. 272:30567-30576 (1997); Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo*, Adv. Enzyme Regul. 39:157-171 (1999). First, a bacterial pore-forming cytolysin, staphylococcal α-toxin, which selectively permeabilizes plasma membranes for monovalent cations, was found to induce apoptosis. Bhakdi, S. et al, *Release of interleukin-1 beta associated with potent cytocidal action of staphylococcal alpha-toxin on human monocytes*, Infect. Immun. 57:3512-3519 (1989). Second, apoptotic and shrunken cells have been shown to contain much lower levels of intracellular K^+ as compared to that in normal cells.

Hughes, F.M et al, *Intracellular K+ suppresses the activation of apoptosis in lymphocytes*, J.Biol.Chem. 272:30567-30576 (1997). Third, an intracellular K+ concentration more than 150mM has been shown to selectively inhibit Caspase-3, a proteolytic enzyme involved in the induction of apoptosis. Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo*, Adv.Enzyme Regul. 39:157-171 (1999). Finally, suppressing K^+ efflux in whole cells prevents the activation of pro-apoptosis nucleases, whereas enhancing the efflux of this ion facilitates enzymatic activities of these nucleases. Hughes, F.M. 39: 157-171 (1999). Thus, intracellular levels of potassium balance appear to be the critical regulator of apoptosis.

Without intending to be bound by any theory, applicants believe that there is a relationship between K^+ channel activity and uroguanylin-induced apoptosis in colon carcinoma cells. Uroguanylin and guanylin have been shown to stimulate Cl^- and K^+ efflux to regulate electrolyte and water transport in the GI tract. Recently, heat-stable enterotoxin (STa) of Escherichia coli, a GC-C agonist peptide that also increases intracellular accumulation of cGMP and stimulates fluid secretion in the lumen of the intestine, has been shown to increase K^+ efflux and Ca^+ influx. Bhattacharya, J. et al, *Rise of intracellular free calcium levels with activation of inositol triphosphate in a human colonic carcinoma cell line (COLO 205) by heat-stable enterotoxin of Escherichia coli*, Biochem. Biophys. Acta. 1403:1-4 (1998). Atrial naturiuretic peptide (ANP), a peptide that stimulates intracellular accumulation of cGMP by binding to a specific GC receptor, has also been shown to activate K^+ conductance in rat mesangial cells, and to induce apoptosis in cardiac myocytes by a cGMP-dependent mechanism. Cermak, R. et al, *Natriuretic peptides increase a K^+ conductance in rat mesangial cells*, Pflugers Arch. 43:571-577 (1996). Furthermore, pretreatment of rat endothelial cells with either ANP (10^{-7}M) or 8-bromo-cGMP(10^{-3}M) caused a

marked accumulation of the nuclear phosphoprotein, p53, a tumor suppresser protein known to induce apoptosis in many cell types. Suenobu, N. et al, *Natriuretic peptides and nitric oxide induce endothelial apoptosis via a cGMP-dependent mechanism*, Arterioscler. Thromb. Vasc. Biol. 19:140-146 (1999). Also, CFTR expression is associated with K^+ and Cl^- efflux and shrinkage of cells, characteristic biochemical changes found in apoptotic cells. Rotoli, B.M. et al, *CFTR expression in C127 cells is associated with enhanced cell shrinkage and ATP extrusion in Cl(-)- free medium*, Biochem. Biophys. Res. Commun. 227:755-61 (1996). Applicants believe that uroguanylin, prouroguanylin, guanylin and other like peptides may induce apoptosis of epithelial cells lining the GI tract mucosa via maintenance of intracellular concentration of K^+ ions as a result of binding to the GC-C receptors. Applicants believe that the binding of the GC-C receptors stimulates the production of cGMP thereby activating the CFTR chlorine channel which causes an increase in K^+ efflux. Thus, the induction of apoptosis is also expected from the administration of agonist peptides which bind to the GC-C receptors, and to other receptors for guanylin, uroguanylin and lymphoguanylin in the intestine.

Additionally, guanylin has been shown to be completely diminished in colon cancer cells and evenly expressed in normal intestinal mucosal cells. This finding suggest that guanylin is involved in the maintenance of colonic differentiation or functions as a tumor modifier gene. Mitchell et al., *Guanylin mRNA Expression in Human Intestine and Colorectal Adenocarcinoma*, Lab. Invest. 1998, Vol. 78, No. 1, 101-108. Recent data demonstrates that the guanylin cyclase receptor known as GC-C receptor is expressed in all primary and metastatic colorectal cancers and it may serve as a specific marker for these tumors. Carrithers, S.L. et al, *Guanylin cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues*, Proc. Natl. Acad. Sci. USA. 93:14827-14832. By contrast, the

expression of guanylin has been shown to be down-regulated in colorectal cancer tissues and cell lines. Cohen, M.B. et al, *Guanylin mRNA expression in human intestine and colorectal adenocarcinoma*, Lab. Invest. 78:101-108.

A study described in the examples to this application shows that uroguanylin is similarly completely diminished in colon cancer cells and evenly distributed in normal intestinal mucosal cells. Additionally, the expression of uroguanylin in human colon cancer and the adjacent normal tissues has been examined. Thus, the expression of both uroguanylin and guanylin is completely diminished in all human colon cancer specimens examined. This study suggests that either the reduced expression of uroguanylin and/or guanylin leads to or is a result of adenocarcinoma formation. The applicants also demonstrate that treatment with uroguanylin results in the induction of apoptosis in T-84, human colon carcinoma cells, and that the oral administration of human uroguanylin leads to inhibition in polyp formation in the intestinal tract of Min-mouse, an animal model for human Familial Adenomatous Polyposis (FAP).

Both guanylin and uroguanylin genes have recently been mapped on the mouse chromosome 4 and to a synthetic position on human chromosome 1p34-35. Sciaky, D. et al, *Mapping of guanylin to murine chromonsome 4 and human chromosome 1p34-35*, Genomics 26:427-429 (1995); Whitaker, T.L. et al, *The uroguanylin gene (Guca 1b) is linked to guanylin (Guca 2) on mouse chromosome 4*, Genomics 45:348-354 (1997). This region is frequently associated with the loss of heterozygosity in human colon carcinoma. Leister, I. et al, *Human colorectal cancer: high frequency of deletions at chromosome 1p35*, Cancer Res. 50:7232-7235 (1990). In the min-mouse tumor model, ademona multiplicity and growth rate are regulated by APC, the tumor suppressor gene, which is also localized to mouse chromosome 4 in a region syntenic with human chromosome 1p34-36. Dietrich, W.F. et al, *Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse*, Cell 75:631-

639 (1992). The APC gene is mutated in the vast majority of humans with colorectal cancer. Miyoshi, Y. et al, *Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene*, Hum. Mol. Genet. 1:229-233 (1992). The principal function of this gene is to regulate cell cycle via the wnt signal transduction cascade. Cadigan, K.M. et al, *Wnt signaling: a common theme in animal development*, Genes Dev. 11:3286-3305 (1997). Thus, the uroguanylin and guanylin peptides may be involved early in the process of colon carcinogenesis.

In accordance with the process of the present invention, therefore, a polypeptide which contains the active domain of human uroguanylin or which binds to the guanylate cyclase receptor GC-C in the intestine of the subject is administered to a subject. While the polypeptide may be administered prophylactically, it will typically be administered to a subject who has been determined to have intestinal cancer, intestinal polyps, or a genetic predisposition for the growth of polyps in the intestine.

In a preferred embodiment of the present invention, the polypeptide is a polypeptide having the sequence as identified in SEQ. ID. 1:

X_8-Asp -Asp -Cys -X_1 -X_2 -Cys -X_3 -Asn -X_4 -X_5 -Cys -X_6 -X_7 -Cys-X_9

wherein each of X_1, X_2, X_3, X_4, X_5, X_6, and X_7 is an amino acid residue, X_8 and X_9 are independently hydrogen or at least one amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X_1 and the cystine residue immediately adjacent the amine group of X_6 and by a disulfide bond between the cystine residue immediately adjacent the amine group of X_3 and the cystine residue immediately adjacent the carboxy group of X_7. Preferably, the polypeptide is guanylan, uroguanylin, pro-uroguanylin, or another polypeptide which contains the active domain of uroguanylin.

As is known in the art, certain amino acids in a peptide or protein can be substituted for other amino acids having a similar hydropathic index or score and produce a resultant peptide or protein having similar biological activity, i.e., which still retains biological functionality. In making such changes, it is preferable that amino acids having hydropathic indices within ±2 are substituted for one another. More preferred substitutions are those wherein the amino acids have hydropathic indices within ±1. Most preferred substitutions are those wherein the amino acids have hydropathic indices within ±0.5.

Like amino acids can also be substituted on the basis of hydrophilicity. U.S. Patent No. 4,554,101 discloses that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. The following hydrophilicity values have been assigned to amino acids: arginine/lysine (+3.0); aspartate/glutamate (+3.0 ±1); serine (+0.3); asparagine/glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ±1); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). Thus, one amino acid in a peptide, polypeptide, or protein can be substituted by another amino acid having a similar hydrophilicity score and still produce a resultant protein having similar biological activity, i.e., still retaining correct biological function. In making such changes, amino acids having hydropathic indices within ±2 are preferably substituted for one another, those within ±1 are more preferred, and those within ±0.5 are most preferred.

As outlined above, amino acid substitutions in the peptides of the present invention can be based on the relative similarity of the amino acid side-chain substituents in the non-active domain of the peptide to create a protein with the same biological activity as the human uroguanylin peptide. Thus, X_1 may be selected from the

group of all amino acid residues, but preferably is selected from the group of amino acid residues consisting of aspartic acid, glutamic acid, glycine, lysine, asparagine, proline, glutamine, arginine, serine and threonine. The more preferred amino acid residues that may be substituted for X_1 are glutamic acid, aspartic acid, arginine, and lysine. The most preferred amino acid residue that may be used for X_1 is glutamic acid. X_2 may be selected from all amino acid residues, however the preferred amino acid residues for substitution are leucine, isoleucine, tyrosine, phenylalanine, tryptophan, valine, methionine, cysteine, alanine, histidine, proline, threonine, glycine, asparagine, and glutamine. The more preferred amino acid residues that may be substituted for X_2 are cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, valine, and tyrosine. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues for substitution for X_2 are leucine, isoleucine, tyrosine, valine, and methionine. The most preferred amino acid residue for substitution for X_2 is leucine.

Additionally, as discussed above, X_3 and X_4 may be selected from all amino acid residues, but the preferred amino acid residues are valine, isoleucine, tyrosine, phenylalanine, tryptophan, methionine, cysteine, alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for X_3 and X_4 are valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues that may be substituted for X_3 and X_4 are valine, isoleucine, leucine, methionine, and cysteine. Even more preferable for substitution for X_3 and X_4 are isoleucine and valine. The most preferred amino acid residue for substitution for X_3 and X_4 is valine. Also, X_5 may be selected from all amino acid residues, but the preferred amino acid residues are alanine, histidine,

cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for X_5 are alanine, histidine, cysteine, methionine, valine, proline, threonine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X_5 are alanine, histidine, cysteine, proline, threonine, glycine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X_5 is alanine.

Moreover, X_6 may be selected from all amino acid residues, but the preferred amino acid residues for substitution are threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues for substitution for X_6 are threonine, proline, alanine, histidine, cysteine, methionine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution threonine, proline, alanine, histidine, and glycine. The most preferred amino acid residue for substitution for X_6 is threonine. Also, X_7 may be selected from all amino acid residues, but the preferred amino acid residues are glycine, threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, glutamine, asparagine, serine, glutamic acid, and aspartic acid. The more preferred amino acid residues for substitution for X_7 are glycine, threonine, proline, alanine, histidine, cysteine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X_7 are glycine, threonine, proline, alanine, histidine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X_7 is glycine.

The polypeptides of the present invention can be combined with various excipient vehicles and/or adjuvants well known in this art which serve as pharmaceutically acceptable carriers to permit drug administration in the

form of, e.g., injections, suspensions, emulsions, tablets, capsules, and ointments. These pharmaceutical compositions may be administered by any acceptable means. For warm-blooded animals, and in particular, for humans, administration can be oral, parenteral, subcutaneous, intravenous, intramuscular and/or intraperitoneal. The specific dose administered will be dependent upon such factors as the general health and physical condition of the subject as well as the subject's age and weight, the stage of the subject's disease condition, the existence of any concurrent treatments, and the frequency of administration; typically, the dose will be in the range of about 0.5 to about 2.0 mg/kg for human subjects. In general, the composition will contain one or more of the polypeptide(s) of the present invention in a concentration of at least about 0.0001% by weight, more typically at least about 0.001% by weight, still more typically at least about 0.01%, still more typically at least about 0.1% and, in some embodiments, in a concentration of at least about 1% by weight of the composition.

Human uroguanylin cDNA has been cloned in bacteria, and chemically synthesized by solid phase peptide synthesis. Uroguanylin peptide can be chemically synthesized by using the procedure as described in U.S. patent number 5,489,670 *Human Uroguanylin* and in U.S. patent number 5,140,102 *Pentadecapeptide, guanylin, which stimulates intestinal guanylate cyclase*. Peptides similar to uroguanylin peptides have been identified in mouse, rat, porcine, and bovine species. The functionally active domain in most of these peptides are highly conserved. Therefore, the physiological functions of these peptides may be similar, and these peptides may be used as intestinal cancer preventative agents as well. Thus, as long as the functionally active domains of these peptides are conserved, substitutions in the non-active domains may be achieved with no change in the activity of the peptides.

All references, patents or applications U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein.

In order to further illustrate the invention, the following exemplary laboratory preparative work was performed. However, it will be appreciated that the invention is not limited to these examples or the details described therein.

EXAMPLES

Materials and Methods

Cell Culture. T-84 cells were obtained from the American Type Culture Collection at passage 52. Cells were grown in 1:1 mixture of Ham's F-12 medium and Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100U penicillin/ml, and 100 μg/ml streptomycin. Cells were fed fresh medium every third day and split at a confluence of approximately 80%.

Human tissue. Samples of normal colon and tumors were obtained following colon resections for adenocarcinoma under a human experimentation protocol that was approved by the Missouri University/Truman VA Hospital Committee. Mucosa samples from normal colon tissues adjacent to the colon adenocarcinomas were isolated from submucosal tissue by scraping the mucosal surface with a microscope slide to separate mucosa from the underlying tissue. Portions of the tumors were collected and processed as an intact tissue. Tissues from eleven subjects between the ages of 48 and 82 years representing female and male patients were used in this study.

EXAMPLE 1

Materials and Methods

Cell proliferation assay. Approximately 10,000 T-84 cells were inoculated in each well of 96-well plates. After an incubation period of 3 days, the indicated concentrations of human uroguanylin were added to the media and cells were allowed to grow until they formed semi-confluent monolayers. Subsequently, BrdU labeling agent (5-bromo-2'-deoxyuridine in PBS) was added (final concentration 100 μM) and cells were re-incubated for an additional 24 hours. Monolayers were washed and incorporation of BrdU was measured following the manufacturer's instructions (Boehringer Mannheim Corp., Indianapolis, IN).

Results:

Uroguanylin treatment caused a dose-dependent inhibition in growth of these cells, reaching an approximately 30% growth inhibition at 1 μM as seen in figure 2. In contrast, a biologically inactive variant of this peptide did not inhibit cell growth suggesting that the growth inhibition was a receptor-mediated event.

EXAMPLE 2

Materials and Methods

Apoptosis assay. T-84 cells were grown in 35 mm dishes for 7 days. The confluent monolayers were washed once with serum-free DMEM, and incubated with the same media containing different concentrations of human uroguanylin for 16 hours. After this incubation, cells were quickly collected by trypsinization, and the cell pellet was washed twice with phosphate buffer saline (PBS). Cells were resuspended in PBS at a concentration of approximately 10^8 cell/ml. For demonstration of nucleosomal ladders, the apoptotic DNA was isolated from these cells by following the instructions of the DNA fragmentation analysis kit (Boehringer Mannheim Corp., Indianapolis, IN). The apoptotic DNA was separated by agarose gel electrophoresis followed by staining with ethidium bromide. Induction of

apoptosis by uroguanylin was further demonstrated by using the TUNEL assay as per the instructions of the '*In situ* cell death detection kit' (Boehringer Mannheim Corp., Indianapolis, IN).

Results:

As shown in figure 3, the DNA isolated from the control (lane 2) as well as from the biologically inactive variant of uroguanylin treated cells (lane 6) exhibited very low levels of DNA fragmentation, consistent with a low basal rate of apoptosis under serum-free conditions. On the other hand, DNA from the uroguanylin treated cells exhibited extensive DNA fragmentation in a dose-dependent manner. The induction of apoptosis by uroguanylin treatment was further supported by the terminal dexoynucleotidayl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay using CaCo-2 cells. Uroguanylin treatment significantly augmented the generation of apoptotic cells compared to the vehicle treated cells as seen in figure 4. These results confirmed that uroguanylin induces apoptosis in human colon cancer cells (T-84 and CaCo-2).

EXAMPLE 3

Uroguanylin functional assay. Human uroguanylin (NDDCELCVNVACTGCL) peptide was custom synthesized by Multiple Peptide System, San Diego, CA. The biological activity of the synthetic peptide was assayed by a modified cell-based assay. Briefly, the confluent monolayers of T-84 cells in 24-well plates were washed twice with 250 μl of DMEM containing 50 mM HEPES (pH 7.4), preincubated at 37° C for 10 min with 250 μl of DMEM containing 50 mM HEPES (pH 7.4) and 1 mM isobutylmethylxanthine (IBMX), followed by incubation with different concentration of human uroguanylin (10^{-6} to 10^{-10} M) for 30 min. The medium was aspirated, and the reaction was terminated by the addition of 3% perchloric acid. The plate was centrifuged at 1000xg for 5 min and the supernatant was collected. After neutralization with 0.1N NaOH, the supernatant was used directly for measurements of

cGMP by using the ELISA kit from Caymen Chemical, Ann Arbor, MI. Results are expressed as an average of three determinations.

Results:

Biological activity was observed in several isoforms of this peptide as indicated by the cGMP levels observed.

EXAMPLE 4

Ussing chamber assay. The seromuscular layer of human intestinal mucosa was removed by blunt dissection and one to four mucosal sheets from each specimen (~1cm^2) were used. To collect intestinal mucosa from mice, animals were sacrificed by 100% CO_2 inhalation. A mid-line abdominal incision was used to excise the intestinal mucosal layer. The dissected intestinal tissue was opened along the mesenteric border in ice-cold, oxygenated Krebs-Ringer-bicarbonate (KRB) solutions and pinned luminal-side down on a pliable silicone surface. The outer muscle layers were striped by shallow dissection with a scalpel and fine forceps. Mouse intestine and human colon tissue, consisting of mucosa and submucosa, were mounted between two ussing half-chambers and bathed on both sides. Electrical measurements were monitored with an automatic voltage clamp, and direct-connecting voltage and current passing difference and I_{sc}. Tissues were equilibrated under short-circuit conditions until I_{sc} had stabilized and the potential difference across the epithelium was measured intermittently.

Human uroguanylin peptide was chemically synthesized and the relative potencies of various synthetic forms were evaluated by their abilities to stimulate cGMP accumulation in intact T-84 cells. The biological activity of several isoforms of this peptide that exhibited similar physico-chemical properties was observed. The major isoform, exhibiting a potent biological activity, was further purified to about 99% purity and used for this study. To ensure that the synthetic form of human uroguanylin was equally effective in mouse and human GI mucosa, its activity

was examined in the Ussing chamber using the mouse duodenum and the human colonic mucosa.

Results:

The pattern of I_{sc} responses to sequential treatment with specific agents on the mouse duodenum (Fig. 1a) and human colon (Fig. 1b) were recorded. In both tissues, the addition of TTX (0.1 μM in serosal bath) resulted in a decrease in the baseline I_{sc} to a stable value within 20 minutes. Subsequent addition of uroguanylin (0.1 μM in the luminal bath) resulted in a rapid increase in I_{sc}, which was sustained for a 60 minute period. Carbachol, a known stimulator of ion transport across the membrane, further increased the I_{sc}. These results confirmed that the synthetic human uroguanylin peptide was effective in stimulating electrolytes transport in human as well as in mouse intestinal mucosa.

EXAMPLE 5

Methods and Materials

Min-mouse model. Male Min mice (C57BL/6J-APC^{Min}/+), a strain containing a fully penetrant dominant mutation in the APC gene, were obtained at 4-5 weeks of age from The Jackson Laboratory, Bar Harbor, ME. All mice were fed a high-fat AIN-93G diet, tap water to drink and housed in a humidity and temperature controlled room with a 12 hour light-dark cycle. Animals consumed approximately 5 grams of the diet per day. After one week of quarantine period, animals were randomly divided in three groups of 10 animals each. These groups of animals were fed the same diet containing different concentrations of (0, 10, and 20μg/5grams of the diet) of human uroguanylin. Animals were also given additional amounts of human uroguanylin (vehicle, 10 and 20 μg) in 0.2 ml of PBS containing 20% polyethylene glycol by oral gavage twice a week. Food consumption and body weight of these animals were monitored weekly. At the end of the 17th week, animals were sacrificed by CO_2 asphyxiation and the GI tracts were removed. After flushing with PBS to

remove food materials, the GI tract was divided as sections of duodenum (two (2) inches from the stomach), jejunum (middle portion, approx. 4-5 inches from the stomach), ileum (two (2) inches from the cecum) and colon. These sections were opened longitudinally, washed with tissue fixative (Streck Laboratories, Inc., Omaha, NE) and placed between two layers of blotting paper in a tray containing the tissue fixative. Polyps and tumors were counted independently by four different observers. Results are expressed as the average of the total number of polyps for each individual animal by four different observers. Analysis of the data obtained from all observers revealed insignificant inter-observer variance. Sections of these tissues were viewed under a constant magnification (10 X)to gauge the differences between polyp diameter between animals.

Results:

The Min-mouse, the most widely used animal model to assess the chemopreventive properties of dietary nutrients and therapeutic agents, carries a dominant mutation in one of the alleles of the APC gene. Thus, when these mice are raised on a high fat diet, they begin to develop polyps throughout the intestine at around 55 days of age. Development of polyps causes blockage in the movement of intestinal contents, which leads to decreased food consumption and reduced gain in body weight as the disease progresses. The test results for oral administration of uroguranylin to the min-mouse showed a dose-dependent increase in the food consumption as shown in figure 6a, and in the body weight gain as shown in figure 6b. The average body weight for the control group was 25.1± 0.9 g, and that for the uroguanylin-treated (20μg) group was 29.4 ± 1.07 g at 17. In addition, animals treated with uroguanylin were visibly more healthy and active.

At the end of the study, all animals were sacrificed and the GI tract was removed to determine the number and distribution of polyps in the small intestine and colon tissues as shown in table I. The GI tract in the untreated

control group contained 48.3 ± 7.7 polyps per mouse. A majority of the polyps were located throughout the small intestine and only a few polyps were found in the colon. The sizes of the polyps in the control group of mice were in the range of approximately 3 to 5 mm. Three animals in the control group had also developed globular tumors in the duodenum. Administration of uroguanylin reduced the total number of polyps (23.3 ± 3.1) by approximately 50%. In addition, polyps in uroguanylin-treated group of mice were significantly smaller in size (<2.0 mm). There were no polyps observed in the colons of any animals in this group, nor were there any globular tumors in these animals. Since the appearance of polyps in colon of Min-mice occurs only during the severe cases of diseases, the absence of polyps in colon of the uroguanylin-treated group of mice suggest that this peptide might also inhibit the progression of colon cancer. These results suggest that the oral administration of uroguanylin suppresses both the formation as well as the progression of polyp formation in this animal model for colon cancer.

TABLE 1: Inhibition of Polyps formation in Min-mouse by oral administration of human uroguanylin.

Treatments	Average Numbers of Polyps/mouse				Total	Remarks
	Duodenum	Jejunum	Ileum	Colon		
Vehicle Control Group A*	6.4 ± 0.8	26.9 ± 5.4	14.3 ± 2.6	0.8 ± 0.2	48.3 ± 7.7	Three animals showe globular tumor in the duodenum. The average size of polyps was in the range of 3.0 to 5.0 mm.
Uroguanylin Group B	2.0 ± 0.5	21.2 ± 4.2	8.7 ± 1.6	0.2 ± 0.1	32.1 ± 5.2	The average size of polyps was 2.0-3.0 mm.
Uroguanylin Group C	2.2 ± 0.6	12.5 ± 1.6	8.7 ± 1.8	0.0	23.3 ± 3.1**	Polyps were <2.0 mm size.

All results are shown as mean ± SEM, n=10.
*Uroguanylin was administered by *p.o.* twice a week: Group A, vehicle control; group B, 10 μg uroguanylin and Group C, 20 μg.
**P<0.05 compared with the control group.

EXAMPLE 4

Expression of Uroguanylin in human colon cancer tissues.

Methods and Materials

Isolation of RNA. RNA was extracted from tissue using a combination of the TRI reagent method (Molecular Research Center, Inc., Cincinnati, OH) and the RNAeasy Kit (Qiagen, Valencia, CA). The tissue was homogenized in TRI reagent following the manufacturer's protocol. After phase separation with chloroform, the aqueous supernatant phase containing total RNA was removed and mixed with an equal volume of 70% ethanol and lysis buffer without beta-mercaptoethanol. The resulting mixture was loaded onto the RNAeasy columns and then processed following the protocol provided by the manufacturer.

Northern blotting. Total RNA (20 μg) was subjected to electrophoresis in formaldehyde-agarose gels and then transferred to nylon membranes (Zeta-Probe, Bio-Rad Laboratories, Inc., Hercules, CA). The membranes were prehybridized for two hours at 65°C in ExpressHyb solution (Clontech, Palo Alto, CA) and then hybridized with human guanylin, uroguanylin and GC-C cDNAs overnight at 65°C. All cDNA probes were labeled with ^{32}P by random priming (Boehringer Mannheim, Indianapolis, IN). RNA blots were then washed twice with 2X SSC-0.1% SDS for 5 min at room temperature followed by a 15 min wash at 60°C with 0.2X SSC-0.1% SDS. Exposure to X-ray film was performed at -80°C with intensifying screens.

RT-PCR. Oligo(deoxythymidine)$_{18}$-primed cDNAs were synthesized from 3 mμg total RNA using reverse transcriptase (Superscript II, Life Technologies, Gaithersburg, MD). Two PCR primers, 5'-primer (5'-GAACCCAGGGAGCGCGAT-3') [identified as SEQ. ID. 6] and 3'-primer (5'-CTGGTGGGCTCAGGGTACC-3') [identified as SEQ. ID 7], were designed from regions flanking the open reading frame of human pre-prouroguanylin cDNA. A PCR product of the expected size of 384 bp was

amplified from colon cDNAs after 25 cycles at 93°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min using Taq DNA polymerase (U.S. Biochemical Corp., Cleveland, OH). The pair of primers for RT-PCR of guanylin were 5'-primer (5'-AACTCAGGAACTTTGCAC-3') [identified as SEQ. ID. 8] and 3'-primer (5'-CGTAGGCACAGATTTCAC-3') [identified as SEQ. ID. 9]. These primers produced a 174 bp cDNA for human guanylin using the PCR conditions of 25 cycles at 93°C for 1 min, 59°C for 1 min and 72°C for 1.5 min. The PCR-generated cDNA products were subjected to electrophoresis on 1% agarose gels in TAE buffer containing ethidium bromide and then transferred to nylon membranes. Southern hybridization was carried out using the urogyanylin and guanylin cDNA probes. Prehybridization was for 1 hour at 65°C with ExpressHyb solution and then hybridization was for 3 hours at 65°C. Blots were washed as described above and exposed to X-ray films at -80°C with intensifying screens.

Results:

The expression of uroguanylin, guanylin and GC-C receptor in eleven samples of human colon carcinoma and the surrounding normal tissues. Northern blot analysis showed that the expression of uroguanylin and guanylin was completely suppressed in all specimens examined, whereas the adjacent non-cancerous tissue from the same patient exhibited a robust expression of these transcripts as shown in figure 5a. A similar expression pattern was observed when these tissue specimens were analyzed by a more sensitive RT-PCR followed by Southern blotting based analysis as shown in figure 5b. Despite the fact that these specimens were from different stages of colon cancer and were from a different age group of patients, the expression of guanylin and uroguanylin was severely suppressed in all eleven tissue specimens examined. These results raise the possibility that the loss of these intestinal hormones either leads to or is a result of adenocarcinoma formation.

In light of the detailed description of the invention and the examples presented above, it can be appreciated that the several objects of the invention are achieved.

The explanations and illustrations presented herein are intended to acquaint others skilled in the art with the invention, its principles, and its practical application. Those skilled in the art may adapt and apply the invention in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of the present invention as set forth are not intended as being exhaustive or limiting of the invention.

WHAT IS CLAIMED IS:

1. A method of modulating polyps in the intestine of a subject, the process comprising administering to the subject, in need thereof, a pharmaceutical composition comprising a polypeptide having the sequence:

X_8-Asp -Asp -Cys -X_1 -X_2 -Cys -X_3 -Asn -X_4 -X_5 -Cys -X_6 -X_7 -Cys-X_9

wherein each of X_1, X_2, X_3, X_4, X_5, X_6, and X_7 is an amino acid residue, X_8 and X_9 are independently hydrogen or at least one amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X_1 and the cystine residue immediately adjacent the amine group of X_6 and by a disulfide bond between the cystine residue immediately adjacent the amine group of X_3 and the cystine residue immediately adjacent the carboxy group of X_7, together with a pharmaceutically acceptable carrier.

2. A method of modulating polyps in the intestine of a subject, the process comprising administering to the subject, in need thereof, a pharmaceutical composition comprising an agonist peptide or compound which binds to the guanylate cyclase receptor GC-C in the intestine of the subject, together with a pharmaceutically acceptable carrier.

3. A method of claim 1 wherein the concentration of the peptide in the composition is at least 0.0001 percent by weight of the composition.

4. A method of claim 1 wherein the concentration of the peptide in the composition is at least 0.001 percent by weight of the composition.

5. A method of claim 1 wherein the concentration of the peptide in the composition is at least 0.01 percent by weight of the composition.

6. A method of claim 1 wherein the concentration of the peptide in the composition is at least 0.1 percent by weight of the composition.

7. A method of claim 1 wherein the concentration of the peptide in the composition is at least 1 percent by weight of the composition.

8. The method of claim 1 wherein said subject has been determined to have a genetic predisposition for the growth of polyps in the intestine.

9. The method of claim 1 wherein polyps have been identified in the intestine of said subject.

10. The method of claim 1 wherein said subject has been identified as having intestine cancer.

11. A method of claim 2 wherein the concentration of the peptide in the composition is at least 0.0001 percent by weight of the composition.

12. A method of claim 2 wherein the concentration of the peptide in the composition is at least 0.001 percent by weight of the composition.

13. A method of claim 2 wherein the concentration of the peptide in the composition is at least 0.01 percent by weight of the composition.

14. A method of claim 2 wherein the concentration of the peptide in the composition is at least 0.1 percent by weight of the composition.

15. A method of claim 2 wherein the concentration of the peptide in the composition is at least 1 percent by weight of the composition.

16. The method of claim 2 wherein said subject has been determined to have a genetic predisposition for the growth of polyps in the intestine.

17. The method of claim 2 wherein polyps have been identified in the intestine of said subject.

18. The method of claim 2 wherein said subject has been identified as having intestine cancer.

19. The method of claim 1 wherein X_1 is selected from a group of amino acid residues consisting of aspartic acid, glutamic acid, glycine, lysine, asparagine, proline, glutamine, arginine, serine, and threonine.

20. The method of claim 1 wherein X_1 is selected from a group of amino acid residues consisting of glutamic acid, arginine, lysine, serine, aspartic acid, asparagine, glutamine, and glycine.

21. The method of claim 1 wherein X_1 is selected from a group of amino acid residues consisting of glutamic acid, aspartic acid, arginine, and lysine.

22. The method of claim 1 wherein X_1 is glutamic acid.

23. The method of claim 1 wherein X_2 is selected from a group of amino acid residues consisting of leucine, isoleucine, tyrosine, phenylalanine, tryptophan, valine, methionine, cysteine, alanine, histidine, proline, threonine, glycine, asparagine, and glutamine.

24. The method of claim 1 wherein X_2 is selected from a group of amino acid residues consisting of cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, valine, and tyrosine.

25. The method of claim 1 wherein X_2 is selected from a group of amino acid residues consisting of leucine, isoleucine, tyrosine, valine, methionine.

26. The method of claim 1 wherein X_2 is selected from a group of amino acid residues consisting of leucine, and isoleucine.

27. The method of claim 1 wherein X_2 is leucine.

28. The method of claim 1 wherein X_3 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, methionine, cysteine, alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine.

29. The method of claim 1 wherein X_3 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline.

30. The method of claim 1 wherein X_3 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, methionine, and cysteine.

31. The method of claim 1 wherein X_3 is valine.

32. The method of claim 1 wherein X_3 is isoleucine.

33. The method of claim 1 wherein X_4 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan,

methionine, cysteine, alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine.

34. The method of claim 1 wherein X_4 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline.

35. The method of claim 1 wherein X_4 is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, methionine, and cysteine.

36. The method of claim 1 wherein X_4 is valine.

37. The method of claim 1 wherein X_5 is alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, proline, threonine, glycine, glutamine, asparagine, and serine.

38. The method of claim 1 wherein X_5 is selected from the group of amino acid residues consisting of alanine, histidine, cysteine, methionine, valine, proline, threonine, glycine, glutamine, asparagine, and serine.

39. The method of claim 1 wherein X_5 is selected from the group of amino acid residues consisting of alanine, histidine, cysteine, proline, threonine, glycine, glutamine, asparagine, and serine.

40. The method of claim 1 wherein X_5 is alanine.

41. The method of claim 1 wherein X_6 is selected from a group of amino acid residues consisting of threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, glycine, glutamine, asparagine, and serine.

42. The method of claim 1 wherein X_6 is selected from a group of amino acid residues consisting of threonine, proline, alanine, histidine, cysteine, methionine, glycine, glutamine, asparagine, and serine.

43. The method of claim 1 wherein X_6 is selected from a group of amino acid residues consisting of threonine, proline, alanine, histidine, and glycine.

44. The method of claim 1 wherein X_6 is threonine.

45. The method of claim 1 wherein X_7 is selected from a group of amino acid residues consisting of glycine, threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, glutamine, asparagine, serine, glutamic acid, and aspartic acid.

46. The method of claim 1 wherein X_7 is selected from a group of amino acid residues consisting of glycine, threonine, proline, alanine, histidine, cysteine, glutamine, asparagine, and serine.

47. The method of claim 1 wherein X_7 is selected from a group of amino acid residues consisting of glycine, threonine, proline, alanine, histidine, glutamine, asparagine, and serine.

48. The method of claim 1 wherein X_7 is glycine.

49. The method of claim 1 wherein the polypeptide is uroguanylin.

50. The method of claim 1 wherein the polypeptide is human uroguanylin.

51. The method of claim 1 wherein the composition comprises pro-uroguanylin.

52. The method of claim 1 wherein the composition comprises human pro-uroguanylin.

53. The method of claim 2 wherein the composition comprises guanylin.

54. The method of claim 2 wherein the composition comprises lymphoguanylin.

55. The method of claim 2 wherein the composition comprises prolymphoguanylin.

56. The method of claim 2 wherein the composition comprises heat stable enterotoxin.

57. The method of claim 1 wherein the composition comprises a polypeptide, which is degraded with endogenous proteases of the subject, into uroguanylin.

58. The method of claim 1 wherein about 0.5 mg to about 2 mg of the polypeptide is administered per kilogram of the subject's weight.

59. The method of claim 1 wherein the subject is human.

60. The method of claim 2 wherein the composition comprises a polypeptide, which is degraded with endogenous proteases of the subject, into guanylin.

61. The method of claim 1 wherein X_1 is glutamic acid, X_2 is leucine, X_3 is isoleucine, X_4 is valine, X_5 is alanine, X_6 is threonine, and X_7 is glycine.

62. A method for the prevention, inhibition and treatment of cancer in the intestine of a subject, the

process comprising administering to the subject the composition of claim 1.

63. A method for the prevention, inhibition and treatment of cancer in the intestine of a subject, the process comprising administering to the subject the composition of claim 2.

64. The method of claim 62 wherein the composition comprises uroguanylin.

65. The method of claim 63 wherein the composition comprises uroguanylin.

66. The method of claim 62 wherein the composition comprises pro-uroguanylin.

67. The method of claim 63 wherein the composition comprises pro-uroguanylin.

68. The method of claim 62 wherein the composition comprises human uroguanylin.

69. The method of claim 63 wherein the composition comprises human uroguanylin.

70. The method of claim 62 wherein the composition comprises human pro-uroguanylin.

71. The method of claim 63 wherein the composition comprises human pro-uroguanylin.

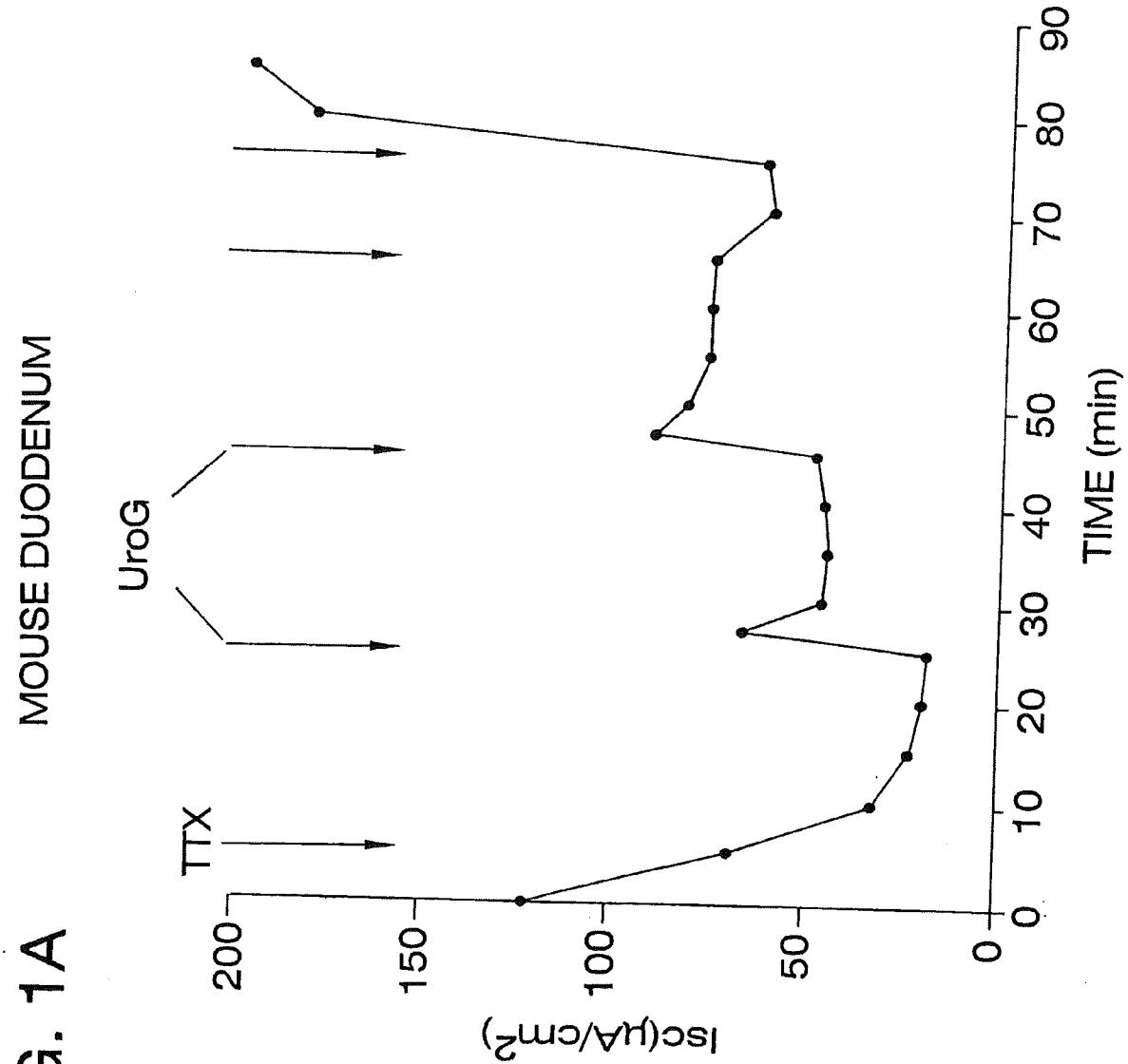
72. The method of claim 63 wherein the composition comprises guanylin.

73. The method of claim 63 wherein the composition comprises lymphoguanylin.

74. The method of claim 63 wherein the composition comprises heat stable enterotoxin.

75. The method of claim 63 wherein the composition comprises pro-lymphoguanylin.

FIG. 1A


MOUSE DUODENUM
TTX
UroG
Isc(μA/cm²)
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
TIME (min)

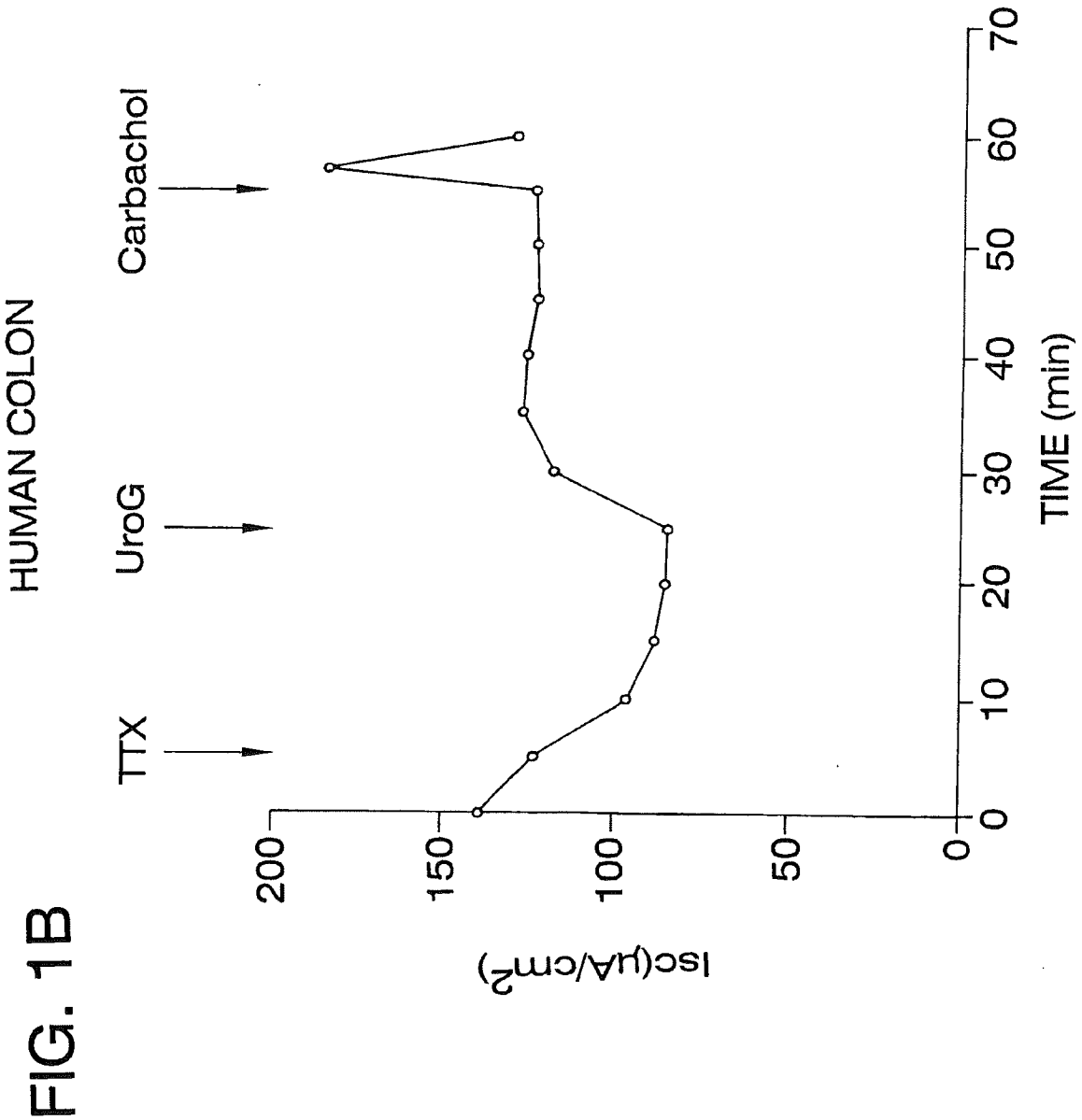

HUMAN COLON
TTX
UroG
Carbachol
Isc(μA/cm²)
200
150
100
50
0
0
10
20
30
40
50
60
70
TIME (min)

FIG. 1B

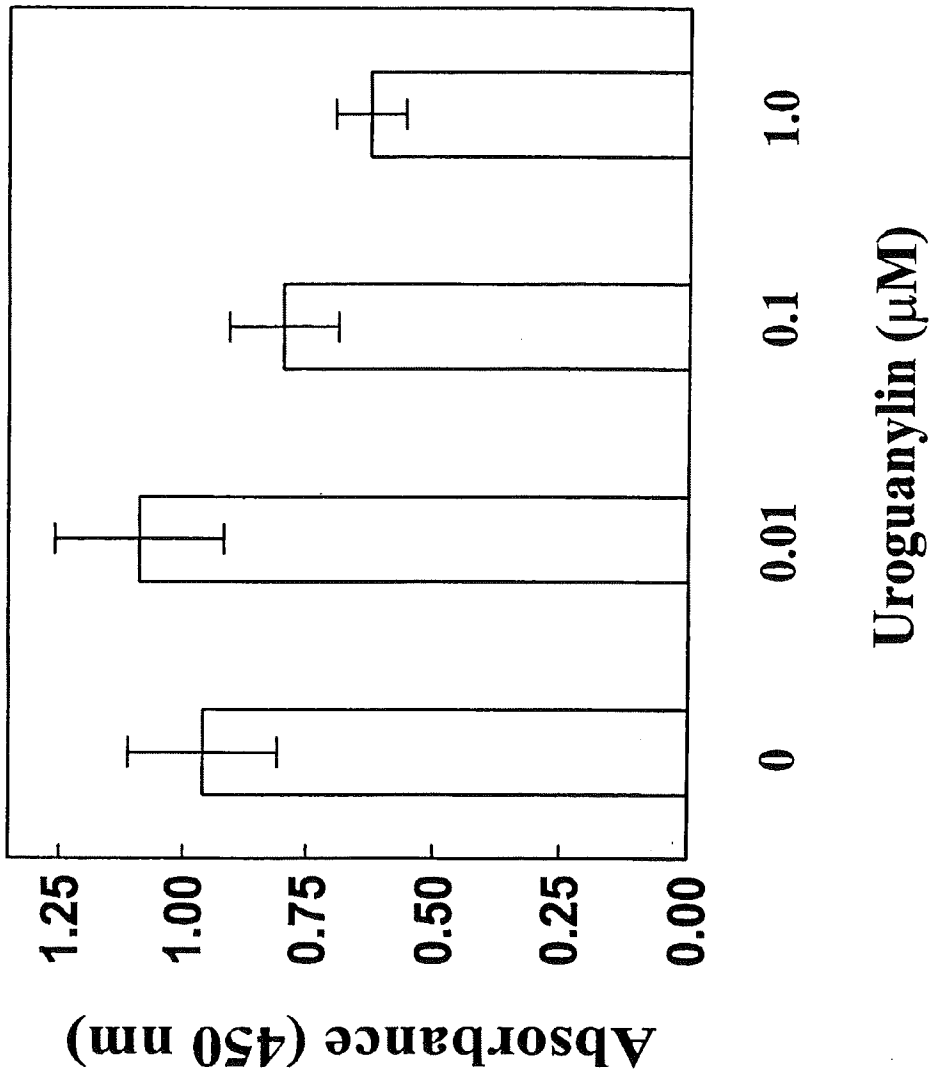

Absorbance (450 nm)
1.25
1.00
0.75
0.50
0.25
0.00
0
0.01
0.1
1.0
Uroguanylin (μM)

FIG. 2

FIG. 3

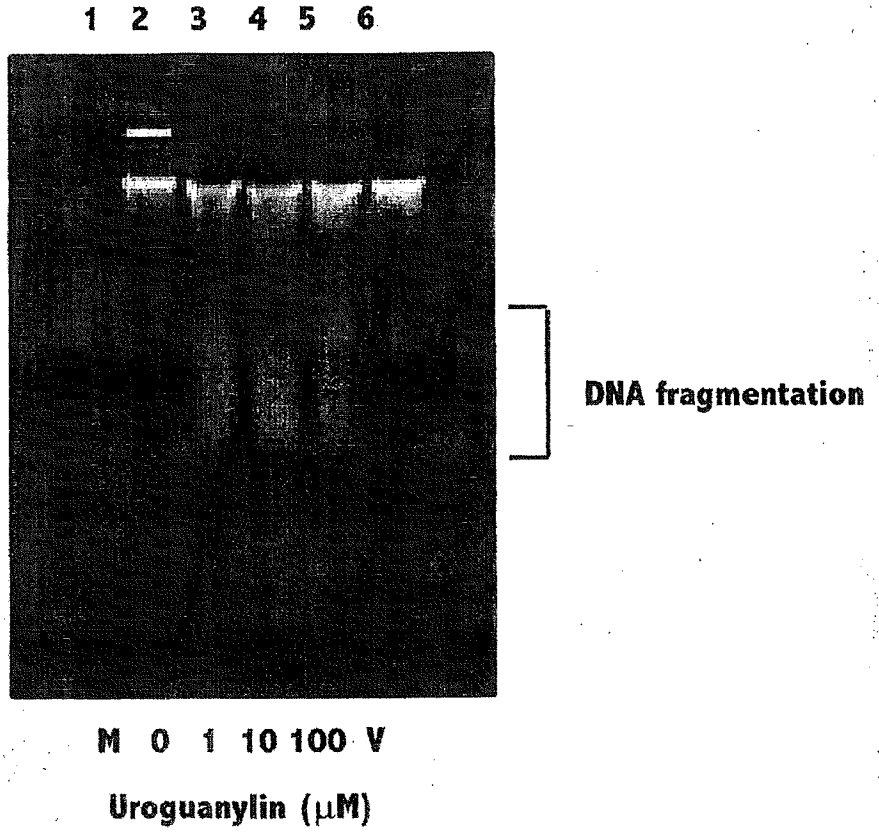


FIG. 4A

FIG. 4B

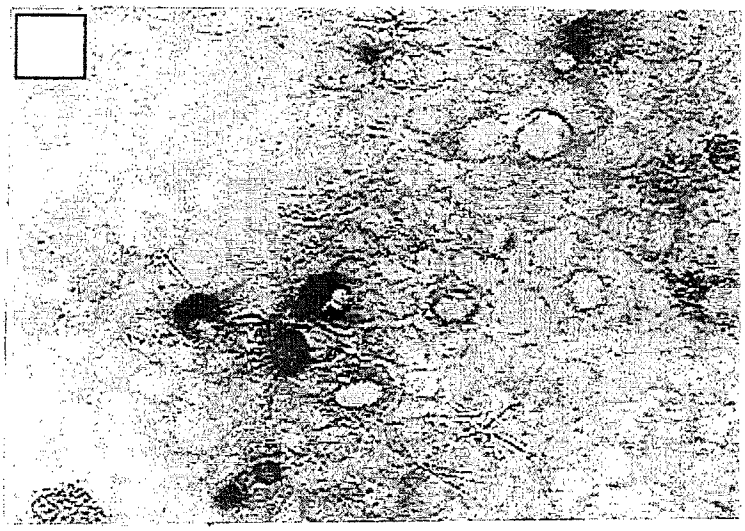


FIG. 5A

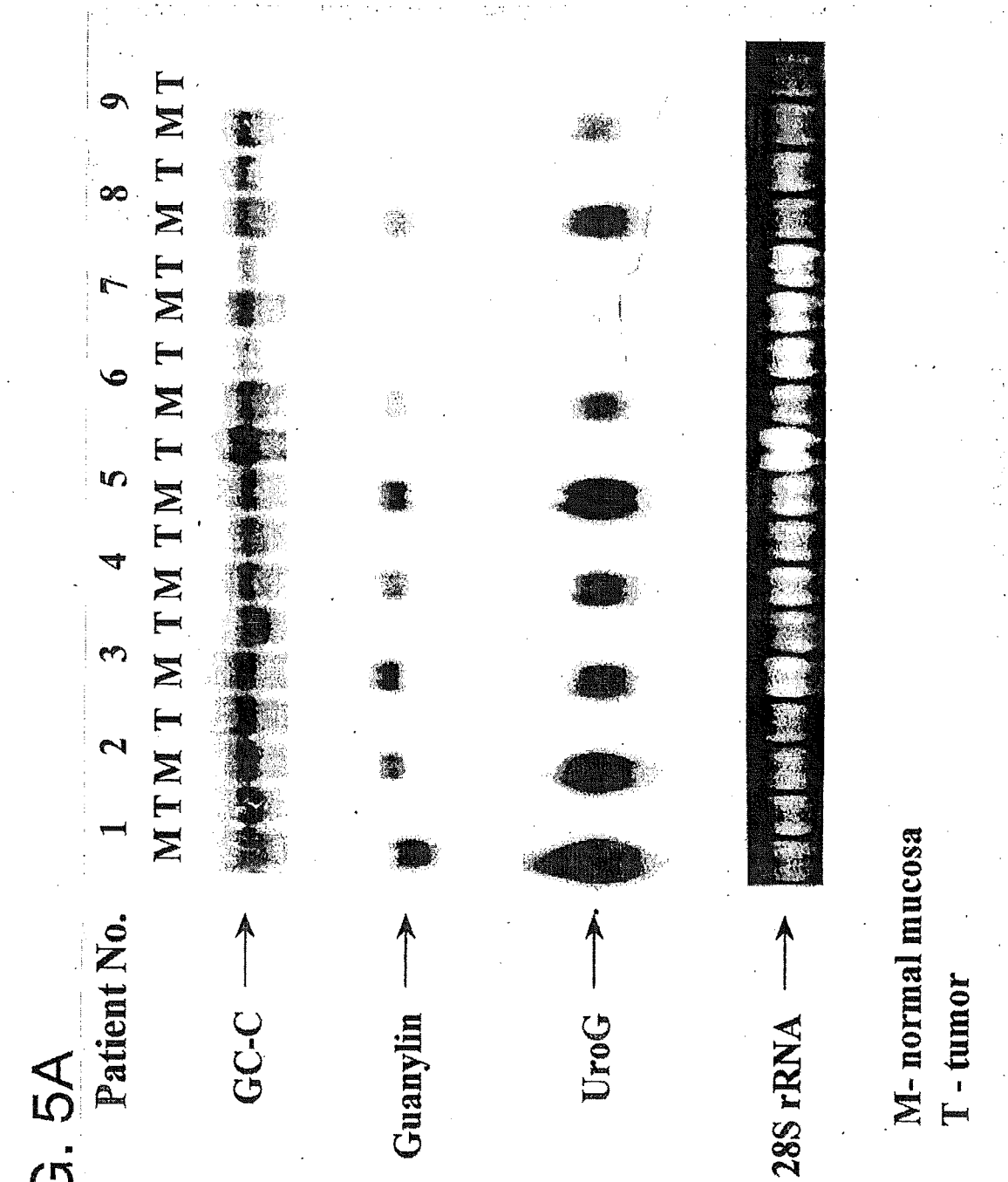
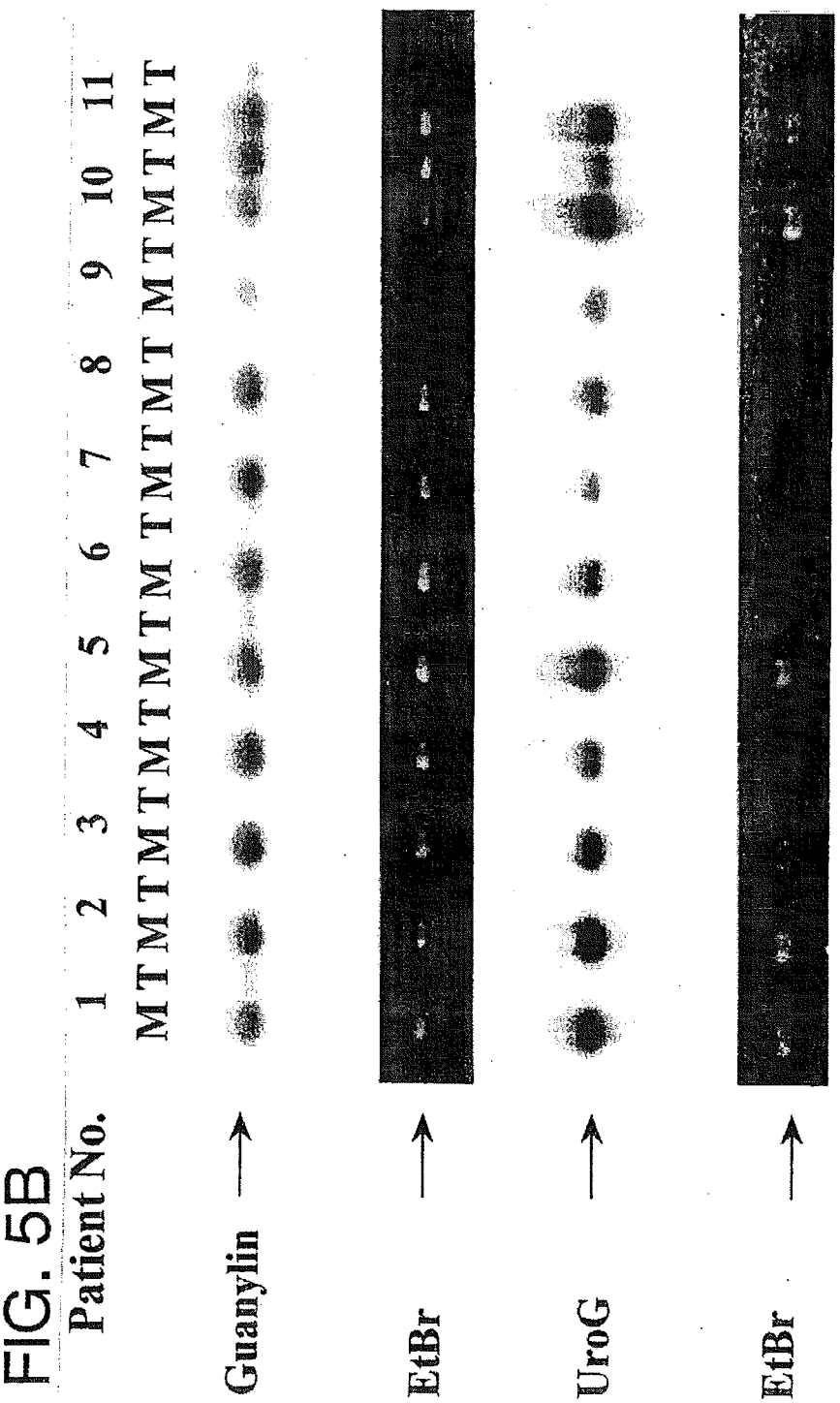

Patient No.
1 2 3 4 5 6 7 8 9
M T M T M T M T M T M T M T M T M T
GC-C
Guanylin
UroG
28S rRNA
M- normal mucosa
T - tumor

FIG. 5B


Patient No.
1 2 3 4 5 6 7 8 9 10 11
M T M T M T M T M T M T M T M T M T M T M
Guanylin
EtBr
UroG
EtBr
M- normal mucosa
T - tumor

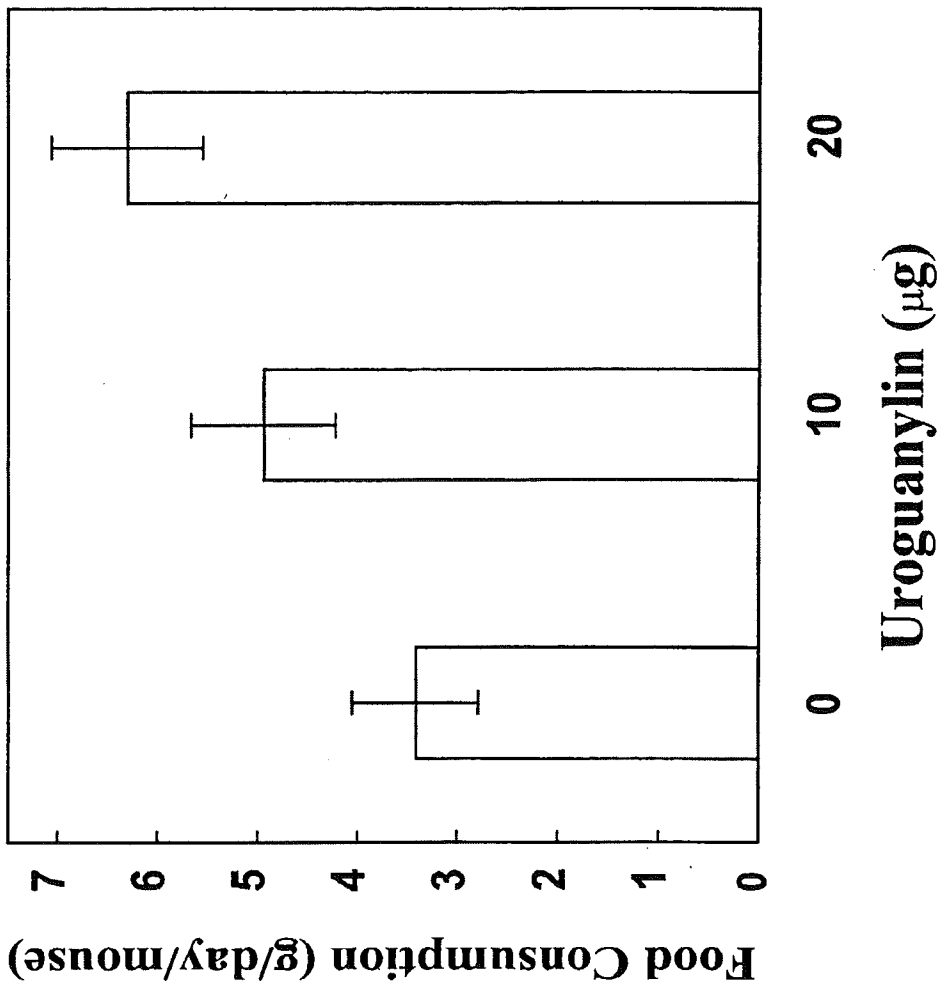

Food Consumption (g/day/mouse)
0
1
2
3
4
5
6
7
0
10
20
Uroguanylin (μg)

FIG. 6A

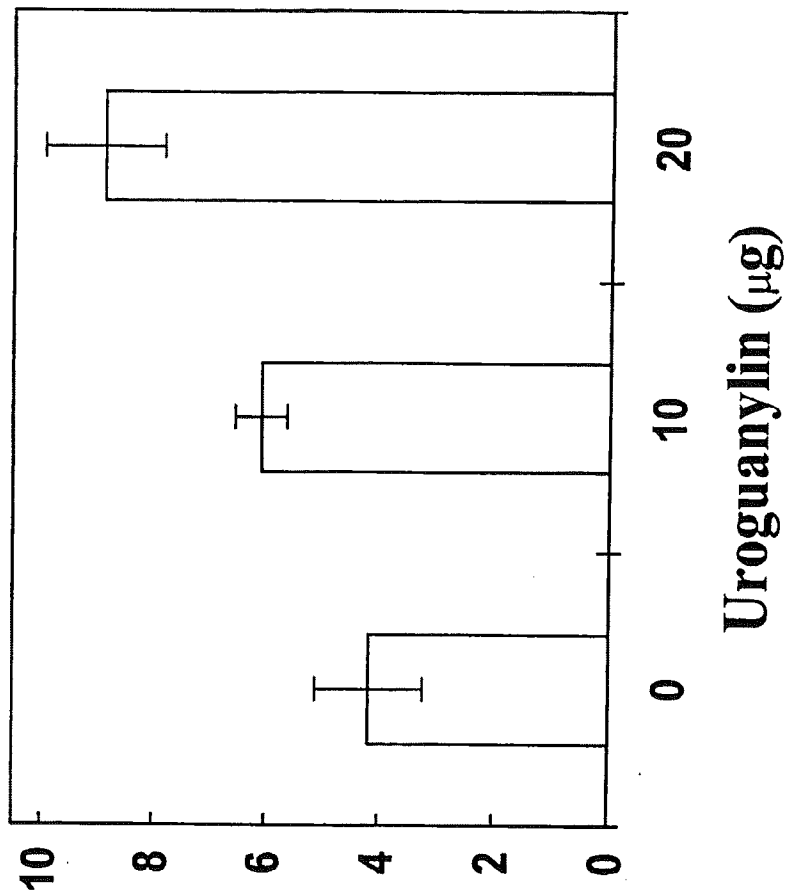


FIG. 6B

FIG. 7

Asn Asp Asp ***Cys Glu*** Leu ***Cys*** Val *Asn* Val Ala ***Cys*** Thr ***Gly Cys*** Leu h UroG.

Pro Gly Thr ***Cys Glu*** Ile ***Cys*** Ala Tyr Ala ***Cys*** Thr ***Gly Cys*** h Gua

Asp Ser Ser Asn Tyr Cys ***Cys Glu*** Leu ***Cys*** *Cys Asn* Pro Ala ***Cys*** Thr ***Gly Cys*** Tyr E. *coli*

Leu Ile Ile Asp Cys ***Cys Glu*** Ile ***Cys*** *Cys Asn* Pro Ala ***Cys*** Phe ***Gly Cys*** Leu Asn V. *cholerae*

SEQUENCE LISTING

<110> Monsanto Company

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<130> MTC6591.1

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<141>

<150> US 60/157,950
<151> 1999-10-06

<160> 9

<170> PatentIn Ver. 2.1

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<212> PRT
<213> Artificial Sequence

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pro-uroguanylin, or another polypeptide which
contains the active domain of uroguanylin

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<222> (5)
<223> X= any amino acid residue

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1 5 10 15

<210> 3
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1 5 10 15

<210> 4
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<213> Escherichia coli

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Gly Cys Tyr

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<212> PRT
<213> Vibrio cholerae

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1 5 10 15

Cys Leu Asn

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designed from regions flanking the open reading
frame of human pre-prouroguanylin cDNA

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 00/21998

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/08 A61K38/10 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SHAILUBHAI, KUNWAR ET AL.: "UROGUANYLIN TREATMENT SUPPRESSES POLYP FORMATION IN THE APCMIN/+ MOUSE AND INDUCES APOPTOSIS IN HUMAN COLON ADENOCARCINOMA CELLS VIA CYCLIC GMP" CANCER RES (2000) 60(18) 5151-5157, XP002159386 the whole document ---	1-75
X	US 5 879 656 A (WALDMAN SCOTT A) 9 March 1999 (1999-03-09) abstract; claims 1-3,50-55 column 8, line 40 - line 50 column 9, line 50 - line 60 --- -/--	1-75

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2001	21/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

1

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 00/21998

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	FORTE LEONARD R: "Guanylin regulatory peptides: Structures, biological activities mediated by cyclic GMP and pathobiology." REGULATORY PEPTIDES, vol. 81, no. 1-3, 31 May 1999 (1999-05-31), pages 25-39, XP000979549 ISSN: 0167-0115 page 36, paragraph 9.3 ---	1-75
X	WO 97 42220 A (UNIV JEFFERSON ;WALDMAN SCOTT A (US)) 13 November 1997 (1997-11-13) abstract page 12, last paragraph -page 13, paragraph 1 page 7, line 17 ---	2,53,63, 72,74
X	US 5 962 220 A (WALDMAN SCOTT A) 5 October 1999 (1999-10-05) abstract column 7 -column 8 ---	2,53,63, 72,74
X	WO 99 39748 A (MATTHEWS DEREK PETER ;NYCOMED IMAGING AS (NO)) 12 August 1999 (1999-08-12) abstract page 38 -page 39 ---	2,53,63, 72,74
A	FORSSMANN W -G ET AL: "REVIEW: GUANYLIN IS A NEW GASTROINTESTINAL HORMONE REGULATING WATER-ELECTROLYTE TRANSPORT IN THE GUT" FALK SYMPOSIUM,US,UNIVERSITY PARK PRESS, BALTIMORE, vol. 77, 12 June 1994 (1994-06-12), pages 279-292, XP000618033 ISSN: 0161-5580 -----	

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. tional Application No
PCT/US 00/21998

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5879656 A	09-03-1999	US 5518888 A	21-05-1996
		AU 681920 B	11-09-1997
		AU 8124994 A	22-05-1995
		CA 2174928 A	04-05-1995
		EP 0734264 A	02-10-1996
		JP 9506340 T	24-06-1997
		NO 961706 A	20-06-1996
		WO 9511694 A	04-05-1995
		US 6087109 A	11-07-2000
		US 5962220 A	05-10-1999
		US 6060037 A	09-05-2000
WO 9742220 A	13-11-1997	CA 2254082 A	13-11-1997
		EP 0951475 A	27-10-1999
US 5962220 A	05-10-1999	US 5518888 A	21-05-1996
		US 6087109 A	11-07-2000
		AU 681920 B	11-09-1997
		AU 8124994 A	22-05-1995
		CA 2174928 A	04-05-1995
		EP 0734264 A	02-10-1996
		JP 9506340 T	24-06-1997
		NO 961706 A	20-06-1996
		WO 9511694 A	04-05-1995
		US 5879656 A	09-03-1999
		US 6060037 A	09-05-2000
WO 9939748 A	12-08-1999	AU 2530199 A	23-08-1999

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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WO 02/062369 A2

(51) International Patent Classification[7]: A61K 38/00

(21) International Application Number: PCT/US02/03201

(22) International Filing Date: 4 February 2002 (04.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/265,955 2 February 2001 (02.02.2001) US

(71) Applicant *(for all designated States except US)*: **PHARMACIA CORPORATION** [US/US]; 800 North Lindbergh Blvd., 04E, St. Louis, MO 63167 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **MASFERRER, Jaime, L.** [CL/US]; 1213 Blairshire, Ballwin, MO 63011 (US).

(74) Agents: **WARNER, J., Michael** et al.; Corporate Patent Department, Pharmacia Corporation, 800 North Lindbergh Blvd., 04E, St. Louis, MO 63167 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/062369 A2

(54) Title: UROGUANYLIN AND CYCLOOXYGENASE-2 INHIBITOR COMBINATIONS FOR INHIBITION OF INTESTINAL CANCER

(57) Abstract: Disclosed is a method of retarding the development of polyps and prevention, inhibition and treatment of cancer in the intestine of a subject by administration of a composition comprising a peptide with the active domain of uroguanylin or any agonist peptide or compound binding to the guanylate cyclase receptor GC-C in the intestine in combination with a naturally occurring, derived from a naturally occurring, or a chemically synthesized cycloogenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor.

UROGUANYLIN AND CYCLOOXYGENASE-2 INHIBITOR COMBINATIONS FOR INHIBITION OF INTESTINAL CANCER

BACKGROUND OF THE INVENTION

The present invention relates to the use of certain peptides, more particularly the use of uroguanylin and prouroguanylin in combination with any one of or combination of naturally occurring, extract of a naturally occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors, to retard the development of polyps and prevent, inhibit or treat cancer in the intestine.

The pathogenesis of colorectal cancer is characterized as a multistep process that begins with increased proliferation and/or decreased apoptosis of colorectal epithelial cells resulting in generation of polyps, followed by adenoma formation and ultimately to adenocarcinoma. Certain individuals develop multiple colorectal adenomas and subsequent carcinomas early in life because of a genetic defect in the APC gene responsible for causing a condition called familial adenomatous polyposis (FAP). Dihlmann et al, *Dominant negative effect of the APC 1309 mutation: a possible explanation for genotype-phenotype correlations in familial adenomatous polyposis*, Cancer Res. 1999 Apr. 15, 59(8): 1857-60. Chemoprevention has evolved during the last decade as a viable strategy for cancer prevention, with the aim of controlling the development of cancer through pharmacological and/or dietary intervention prior to the appearance of a clinically detectable tumor. Reddy, B.S. (1997) *Chemoprevention of colon cancer by dietary administration of naturally-occurring and related synthetic agents*, Adv. Exp. Med. Biol. 400B:931-936.

Uroguanylin and guanylin are structurally related enteric peptide hormones that are secreted intraluminally by different types of cells, include enterochromaffin, goblet and others within the intestinal mucosal lining. A receptor for theses peptides that has been identified at the molecular level is a transmembrane form of guanylate cyclase (GC) known as GC-C. Krause, W.J. et al, *The guanylin and uroguanylin peptide hormones and their receptors*, Acta. Anat. (Basel) 160:213-231 (1997). GC-C receptors are localized on the luminal surface of enterocytes throughout the GI tract. Swenson, E.S. et al, *The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine*, Biochem. Biophys. Res. Commun. 225:1009-1014 (1996). Binding of uroguanylin or guanylin to the extracellular domain of GC-C receptors stimulates intracellular production of the second messenger cGMP, resulting in activation of cystic fibrosis transmembrane conductance regulator (CFTR), the apical membrane channel for efflux of chloride from enterocytes lining the intestinal tract. Forte, L.R. et al, *Salt and water homeostasis: uroguanylin is a circulating peptide hormone with naturiuretic activity*, Am. J. Kidney Dis. 28:296-304 (1996). Activation of CFTR chloride channel proteins and the subsequent enhancement of transepithelial secretion of chloride leads to stimulation of sodium (Na^+) and water secretion into the intestinal lumen. Forte, L.R. et al, *Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology*, Regul. Pept. 81:25-39 (1999). Therefore, one of the major physiological functions of these hormones is the regulation of fluid and electrolyte transport in the gastrointestinal (GI) tract by serving as paracrine regulators of CFTR activity.

The precursor of uroguanylin is prouroguanylin, which is broken down by endogenous proteases in the intestinal tract to produce the active uroguanylin. Chymotrypsin activates prouroguanylin to cleave it into its active form of uroguanylin. Forte, et el, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone With Natriuretic Activity*, Am. J. Kid. Dis. 1996, 28, No.2, 296-304. Uroguanylin is an acid-stable and proteolysis-resistant peptide, which will remain intact to act on the intestinal lumen directly rather than being absorbed systemically. Uroguanylin and guanylin are produced throughout the intestinal mucosa and in the myocardium. Forte et al, *Salt and water homeostasis:uroguanylin is a circulating peptide hormone with natriuretic activity* Am. J. Kidney Dis. 28:296-304 (1996). Human uroguanylin has been isolated from human urine and has been chemically synthesized by solid phase peptide synthesis as described in U.S. Patent Number 5,489,670 for *Human Uroguanylin*.

Binding of uroguanylin or guanylin to the guanylin cyclase receptor stimulates the intracellular production of the cGMP ultimately resulting in the stimulation of salt and water secretion into the intestinal lumen. Uroguanylin and guanylin receptors are found on the luminal surface of epithelial cells lining the intestinal tract and renal proximal tubules as well as in other organs. Forte et al, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone with Natriuretic Activity*, Am. J. Kid. Dis.1996, 28, No. 2, 296-304. Uroguanylin has been found to stimulate increases in cyclic GMP levels in a manner similar to another family of heat stable enterotoxins (STs) secreted by pathogenic strains of E. coli and other enteric bacteria that activate intestinal guanylate cyclase and cause secretory diarrhea, which is

a major cause of traveler's diarrhea and many deaths in developing countries. Forte et al, *Lymphoguanylin: Cloning and Characterization of a Unique Member of the Guanylin Peptide Family*, Endocrinology Vol. 140, No. 4, p.1800-1806. These ST peptides act as molecular mimics of the endogenous mammalian peptides of uroguanylin and prouroguanylin. Forte et al, Endocrinology Vol. 140, No. 4, p.1800. Unlike uroguanylin the STs from enteric bacteria do not have a decrease in potency when the pH changes in the colon. STs are more potent than either uroguanylin or guanylin under both acidic and alkaline conditions. Forte et al, *Guanylin: a peptide regulator of epithelial transport*, The FASEB Journal, vol. 9, 643-650 (1995). Uroguanylin is believed to regulate fluid and electrolyte transport in a manner similar to guanylin and the STs in the GI tract. Therefore, as mentioned in previous publications the human uroguanylin may act as a laxative and be useful in patient suffering from constipation.

Prostaglandins play a major role in the inflammation process and the inhibition of prostaglandin production, especially production of PGG_2, PGH_2 and PGE_2, has been a common target of anti-inflammatory drug discovery. However, common non-steroidal anti-inflammatory drugs (NSAID's) that are active in reducing the prostaglandin-induced pain and swelling associated with the inflammation process are also active in affecting other prostaglandin-regulated processes not associated with the inflammation process. Thus, use of high doses of most common NSAID's can produce severe side effects, including life threatening ulcers, that limit their therapeutic potential. An alternative to NSAID's is the use of corticosteroids, which also produce severe adverse effects, especially when long term therapy is involved.

NSAIDs have been found to prevent the production of prostaglandins by inhibiting enzymes in the human arachidonic acid/prostaglandin pathway, including the enzyme cyclooxygenase (COX). The recent discovery of an inducible enzyme associated with inflammation (named "cyclooxygenase-2 (COX-2)" or "prostaglandin G/H synthase II") provides a viable target of inhibition which more effectively reduces inflammation and produces fewer and less drastic side effects.

Compounds that selectively inhibit cyclooxygenase-2 have also been described in the following individual publications.

U.S. Patent No. 5,380,738.

U.S. Patent No. 5,344,991.

U.S. Patent No. 5,393,790.

U.S. Patent No. 5,434,178.

U.S. Patent No. 5,474,995.

U.S. Patent No. 5,510,368.

WO 96/06840.

WO 96/03388.

WO 96/03387.

WO 96/19469.

WO 96/25405.

WO 95/15316.

WO 94/15932.

WO 94/27980.

WO 95/00501.

WO 94/13635.

WO 94/20480.

WO 94/26731.

Further, natural Cyclooxygenase-2 inhibitors have been disclosed in "Selective Cyclooxygenase-2 Inhibition

from Edible Plant Extracts", US Non-provisional Application number 09/737892, filed Jan. 03, 2001; "Selective Cyclooxygenase-2 Inhibition from Non-edible Plant Extracts", US Non-provisional Application number 09/737701, filed Jan. 03, 2001; and "Selective Cyclooxygenase-2 Inhibition from Plant Extracts", US Non-provisional Application number 09/738041, filed Jan. 03, 2001. [Pyrazol-1-yl]benzenesulfonamides have been described as inhibitors of cyclooxygenase-2 and have shown promise in the treatment of inflammation, arthritis, and pain, with minimal side effects in pre-clinical and clinical trials. Their use for preventing colon cancer has been described in U.S. Patent No. 5,466,823. However, their use for treating or preventing intestinal cancer, in combination with uraguanylin, has not been described.

SUMMARY OF THE INVENTION

The combination of certain peptides, particularly uroguanylin or prouroguanylin, with a single or multiple natural occurring, extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors, may be useful in treating, preventing, inhibiting or retarding the development of polyps and cancer in the intestine.

Among the objects and features of the present invention may be noted the provision of a process for retarding the development of polyps and preventing, and a process for inhibiting and treating cancer or neoplasia in a subject. Preferably the method is useful for treating the development of polyps and preventing, and a process for inhibiting and treating cancer in the

intestine of a subject, more preferably the small intestine or the colon.

Briefly, therefore, the present invention is directed to a process for retarding the development of polyps in a subject which comprises the administration of a peptide including the amino acid sequence:

Asp- Asp- Cys- X_1- X_2- Cys- X_3- Asn- X_4- X_5- Cys- X_6- X_7- Cys

wherein each of X_1, X_2, X_3, X_4, X_5, X_6, and X_7 is an amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X_1 and the cystine residue immediately adjacent the amine group of X_6 and by a disulfide bond between the cystine residue immediately adjacent the amine group of X_3 and the cystine residue immediately adjacent the carboxy group of X_7, in combination with any one of or combination of naturally occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

US Patent application number PCT/US00/21998 (herein incorporated by reference) describes the use of uroguanylin as an intestinal cancer inhibiting agent.

The invention is further directed to a process for retarding the development of polyps and to a process for the prevention, inhibition or treatment of cancer in a subject by administration of a composition comprising any one of or combination of the following peptides: uroguanylin, human uroguanylin, pro-uroguanylin, and human pro-uroguanylin, guanylin, lymphoguanylin, prolymphoguanylin and heat stable enterotoxin in combination with any one of or combination of naturally occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

Additionally, the invention is directed to a process for retarding the development of polyps and a process for the prevention, inhibition or treatment of cancer by administration of a composition comprising any one of or a combination of agonist peptides and/or other agonist compounds to the guanylate cyclase receptor GC-C in combination with any one of or combination of naturally occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

The cancer or neoplasia which can be treated with the present inventive method can be located anywhere in the body, for example, the head, neck, chest, lungs, skin, liver, blood, kidneys, heart, intestines, bladder, gall bladder, brain, throat, musculoskeletal system, lymphatic system, central nervous system, and others. Preferably, the methods of the present invention are used to treat cancer or neoplasia located in the intestine, for example, the small intestine or colon.

Other objects of this invention will be in part apparent and, in part, pointed out hereinafter.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "treatment" includes partial or total inhibition of the tumor growth, either benign or malignant, spreading or metastasis, as well as partial or total destruction of the neoplastic cells.

The term "prevention" includes either preventing the onset of clinically evident neoplasia altogether or preventing the onset of a preclinically evident stage of neoplasia in individuals at risk. Also intended to be encompassed by this definition is the prevention of initiation for malignant cells or to arrest or reverse the progression of premalignant cells to malignant cells. This includes prophylactic treatment of those at risk of developing the neoplasia.

The phrase "therapeutically-effective" is intended to qualify the amount of each agent which will achieve the goal of improvement in disease severity and the frequency of incidence over treatment of each agent by itself, while avoiding adverse side effects typically associated with alternative therapies.

The term "subject" for purposes of treatment includes any human or animal subject who has any one of the known neoplasia or tumor disorders, and preferably is a human subject. For methods of prevention, the subject is any human or animal subject, and preferably is a human subject who is at risk for obtaining an intestinal cancer or neoplasia-related disorder, either

benign or malignant, including metastasis. The subject may be at risk due to exposure to carcinogenic agents, being genetically predisposed to have the neoplasia, and the like.

The term neoplasia includes both benign and cancerous tumors and growths.

In the method above, the epithelial cell-derived neoplasia includes epithelial carcinomas such as basal cell carcinoma, adenocarcinoma, colon cancer, prostate cancer, renal cell carcinoma, and other known neoplasias that effect epithelial cells throughout the body. Preferably, the epithelial cell-derived neoplasia is selected from gastrointestinal cancer, liver cancer, prostate cancer, kidney cancer, brain cancer, bladder cancer, cervical cancer, lung cancer, breast cancer and skin cancer.

Inhibitors of the cyclooxygenase pathway in the metabolism of arachidonic acid used in the prevention and treatment of cancer or neoplasia may inhibit enzyme activity through a variety of mechanisms. The use of cyclooxygenasse-2 selective inhibitors is highly advantageous in that it minimize the gastric side effects that can occur with non-selective NSAID's, especially where prolonged prophylactic treatment is expected.

The term "cyclooxygenase-2 inhibitor" denotes a compound able to inhibit cyclooxygenase-2 without significant inhibition of cyclooxygenase-1. Preferably, it includes compounds which have a cyclooxygenase-2 IC_{50}

of less than about 0.2 μM, and also have a selectivity ratio of cyclooxygenase-2 inhibition over cyclooxygenase-1 inhibition of at least 50, and more preferably of at least 100. Even more preferably, the compounds have a cyclooxygenase-1 IC_{50} of greater than about 1 μM, and more preferably of greater than 10 μM.

The term "purified" means partially purified and/or completely purified. Thus a "purified composition" may be either partially purified or completely purified. An extract of a naturally occurring cyclooxygenase-2 inhibitor may be paritially purified or purified.

Uroguanylin is secreted naturally by the goblet cells of the intestinal mucosal lining as prouroguanylin, a functionally inactive form, which is then converted to the functionally active uroguanylin in the intestine by endogenous proteases. Uroguanylin is an acid-stable, proteolysis-resistant peptide. Therefore, orally delivered prouroguanylin and uroguanylin will act on the lumenal intestinal surface and not be absorbed systemically. Oral administration of uroguanylin, prouroguanylin and other like peptides, containing the amino acid sequences similar to the active domain, are expected to induce apoptosis, cell death, in the intestinal mucosal cell lining. The induced apoptosis in the intestinal mucosal cell lining is expected to retard the incidence of polyp formation and subsequent intestinal cancer. Without intending to be bound by any theory, applicants believe that the peptides of the invention exert their effects by increasing the rate of apoptosis, cell death, in the intestinal mucosal cell lining promoting the perfect balance between the cell proliferation and the programmed cell death thereby retarding the growth of polyps and preventing, inhibiting, and treating cancer

in the intestine and other epithelial-derived cancer possessing receptors for guanylin, uroguanylin, lymphoguanylin and STa family of peptides.

The rate of cell proliferation and cell death in the intestinal mucosa is very rapid. The cells of the intestinal mucosa are in a steady state of turnover to insure a perfect balance between cell proliferation and cell death. The constant rapid renewal of the GI tract epithelium fulfills the functions of maintaining the integrity of normal mucosa, repairing and replenishing differentiated epithelial cells that have specialized functions. The prevention of apoptosis in the intestinal mucosal cells creating an imbalance in the renewal process results in an increased incidence of polyp formation and subsequent intestinal cancer. *See* Eastwood et al, *A review of gastrointestinal epithelial renewal and its relevance to the development of adenocarcinomas of the gastrointestinal tract,* J. Clin. Gastroenterol. 21: S1-11 (1995). The process of apoptosis is known to be suppressed in colon cancer tissues. Baretton, et al, *Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomoas and carcinomas. Aspectes of Carcinogenesis and prognostic significance,* Cancer 77:255-264 (1996).

A major cellular characteristic of the apoptotic process is a marked loss of cell volume, which is directly related to the movement of ions, with homeostatsis being achieved by the balance of osmotic pressure across the plasma membrane. Hoffman, E.K. et al, *Membrane mechanisms in intracellular signalling in cell volume regulation,* Int. Rev. Cytol. 161:173-262 (1995). Most mammalian cells achieve and maintain this osmotic pressure through the continuous action of Na^+/K^+ ATPase pump, which creates a gradient of these

monovalent cations across the membrane. Several sources of evidence have implicated a potential role of K^+ efflux in the induction of apoptosis. Hughes, F.M. et al, *Intracellular K^+ suppresses the activation of apoptosis in lymphocytes,* J.Biol.Chem. 272:30567-30576 (1997); Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo,* Adv. Enzyme Regul. 39:157-171 (1999). First, a bacterial pore-forming cytolysin, staphylococcal α-toxin, which selectively permeabilizes plasma membranes for monovalent cations, was found to induce apoptosis. Bhakdi, S. et al, *Release of interleukin-1 beta associated with potent cytocidal action of staphylococcal alpha-toxin on human monocytes,* Infect. Immun. 57:3512-3519 (1989). Second, apoptotic and shrunken cells have been shown to contain much lower levels of intracellular K^+ as compared to that in normal cells. Hughes, F.M et al, *Intracellular K+ suppresses the activation of apoptosis in lymphocytes,* J.Biol.Chem. 272:30567-30576 (1997). Third, an intracellular K+ concentration more than 150mM has been shown to selectively inhibit Caspase-3, a proteolytic enzyme involved in the induction of apoptosis. Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo,* Adv.Enzyme Regul. 39:157-171 (1999). Finally, suppressing K^+ efflux in whole cells prevents the activation of pro-apoptosis nucleases, whereas enhancing the efflux of this ion facilitates enzymatic activities of these nucleases. Hughes, F.M. 39: 157-171 (1999). Thus, intracellular levels of potassium balance appear to be the critical regulator of apoptosis.

Additionally, guanylin has been shown to be completely diminished in colon cancer cells and evenly

expressed in normal intestinal mucosal cells. This finding suggest that guanylin is involved in the maintenance of colonic differentiation or functions as a tumor modifier gene. Mitchell et al., *Guanylin mRNA Expression in Human Intestine and Colorectal Adenocarcinoma*, Lab. Invest. 1998, Vol. 78, No. 1, 101-108. Recent data demonstrates that the guanylin cyclase receptor known as GC-C receptor is expressed in all primary and metastatic colorectal cancers and it may serve as a specific marker for these tumors. Carrithers, S.L. et al, *Guanylin cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues*, Proc. Natl. Acad. Sci. USA. 93:14827-14832. By contrast, the expression of guanylin has been shown to be down-regulated in colorectal cancer tissues and cell lines. Cohen, M.B. et al, *Guanylin mRNA expression in human intestine and colorectal adenocarcinoma*, Lab. Invest. 78:101-108.

In PCT/US00/21998 (herein incorporated by reference) uroguanylin was shown to be completely diminished in colon cancer cells and evenly distributed in normal intestinal mucosal cells. Additionally, the expression of uroguanylin and guanylin in human colon cancer and the adjacent normal tissues was reportedly completely diminished in all human colon cancer specimens examined. That study suggested that either the reduced expression of uroguanylin and/or guanylin leads to or is a result of adenocarcinoma formation. In the same application, it was demonstrated that treatment with uroguanylin resulted in the induction of apoptosis in T-84, human colon carcinoma cells, and that the oral administration of human uroguanylin leads to inhibition in polyp formation in the intestinal tract of Min-mouse,

an animal model for human Familial Adenomatous Polyposis (FAP).

Both guanylin and uroguanylin genes have recently been mapped on the mouse chromosome 4 and to a synthetic position on human chromosome 1p34-35. Sciaky, D. et al, *Mapping of guanylin to murine chromonsome 4 and human chromosome 1p34-35*, Genomics 26:427-429 (1995); Whitaker, T.L. et al, *The uroguanylin gene (Guca 1b) is linked to guanylin (Guca 2) on mouse chromosome 4*, Genomics 45:348-354 (1997). This region is frequently associated with the loss of heterozygosity in human colon carcinoma. Leister, I. et al, *Human colorectal cancer: high frequency of deletions at chromosome 1p35*, Cancer Res. 50:7232-7235 (1990). In the min-mouse tumor model, ademona multiplicity and growth rate are regulated by APC, the tumor suppressor gene, which is also localized to mouse chromosome 4 in a region syntenic with human chromosome 1p34-36. Dietrich, W.F. et al, *Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse*, Cell 75:631-639 (1992). The APC gene is mutated in the vast majority of humans with colorectal cancer. Miyoshi, Y. et al, *Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene*, Hum. Mol. Genet. 1:229-233 (1992). The principal function of this gene is to regulate cell cycle via the wnt signal transduction cascade. Cadigan, K.M. et al, *Wnt signaling: a common theme in animal development*, Genes Dev. 11:3286-3305 (1997). Thus, the uroguanylin and guanylin peptides may be involved early in the process of colon carcinogenesis.

In accordance with the process of the present invention, therefore, a polypeptide which contains the active domain of human uroguanylin or which binds to the

guanylate cyclase receptor GC-C in the intestine of the subject is administered to a subject. While the polypeptide may be administered prophylactically, it will typically be administered to a subject who has been determined to have intestinal cancer, intestinal polyps, or a genetic predisposition for the growth of polyps in the intestine.

In a preferred embodiment of the present invention, the polypeptide is a polypeptide having the sequence as identified in SEQ. ID. 1:

X_8-Asp -Asp -Cys -X_1 -X_2 -Cys -X_3 -Asn -X_4 -X_5 -Cys -X_6 -X_7 -Cys-X_9

wherein each of X_1, X_2, X_3, X_4, X_5, X_6, and X_7 is an amino acid residue, X_8 and X_9 are independently hydrogen or at least one amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X_1 and the cystine residue immediately adjacent the amine group of X_6 and by a disulfide bond between the cystine residue immediately adjacent the amine group of X_3 and the cystine residue immediately adjacent the carboxy group of X_7. Preferably, the polypeptide is guanylan, uroguanylin, pro-uroguanylin, or another polypeptide which contains the active domain of uroguanylin.

As is known in the art, certain amino acids in a peptide or protein can be substituted for other amino acids having a similar hydropathic index or score and produce a resultant peptide or protein having similar biological activity, i.e., which still retains biological functionality. In making such changes, it is preferable that amino acids having hydropathic indices within .2 are substituted for one another. More

preferred substitutions are those wherein the amino acids have hydropathic indices within .1. Most preferred substitutions are those wherein the amino acids have hydropathic indices within .0.5.

Like amino acids can also be substituted on the basis of hydrophilicity. U.S. Patent No. 4,554,101 discloses that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. The following hydrophilicity values have been assigned to amino acids, (according to the Hopp-Woods values): arginine/lysine (+3.0); aspartate/glutamate (+3.0 ±1); serine (+0.3); asparagine/glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ±1); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). Thus, one amino acid in a peptide, polypeptide, or protein can be substituted by another amino acid having a similar hydrophilicity score and still produce a resultant protein having similar biological activity, i.e., still retaining correct biological function. In making such changes, amino acids having hydropathic indices within ±2 are preferably substituted for one another, those within ±1 are more preferred, and those within ±0.5 are most preferred.

As outlined above, amino acid substitutions in the peptides of the present invention can be based on the relative similarity of the amino acid side-chain substituents in the non-active domain of the peptide to create a protein with the same biological activity as the human uroguanylin peptide. Thus, X_1 may be selected from the group of all amino acid residues, but

preferably is selected from the group of amino acid residues consisting of aspartic acid, glutamic acid, glycine, lysine, asparagine, proline, glutamine, arginine, serine and threonine. The more preferred amino acid residues that may be substituted for X_1 are glutamic acid, aspartic acid, arginine, and lysine. The most preferred amino acid residue that may be used for X_1 is glutamic acid. X_2 may be selected from all amino acid residues, however the preferred amino acid residues for substitution are leucine, isoleucine, tyrosine, phenylalanine, tryptophan, valine, methionine, cysteine, alanine, histidine, proline, threonine, glycine, asparagine, and glutamine. The more preferred amino acid residues that may be substituted for X_2 are cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, valine, and tyrosine. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues for substitution for X_2 are leucine, isoleucine, tyrosine, valine, and methionine. The most preferred amino acid residue for substitution for X_2 is leucine.

Additionally, as discussed above, X_3 and X_4 may be selected from all amino acid residues, but the preferred amino acid residues are valine, isoleucine, tyrosine, phenylalanine, tryptophan, methionine, cysteine, alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for X_3 and X_4 are valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues that may be substituted for X_3 and X_4 are valine, isoleucine, leucine, methionine, and cysteine.

Even more preferable for substitution for X_3 and X_4 are isoleucine and valine. The most preferred amino acid residue for substitution for X_3 and X_4 is valine. Also, X_5 may be selected from all amino acid residues, but the preferred amino acid residues are alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for X_5 are alanine, histidine, cysteine, methionine, valine, proline, threonine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X_5 are alanine, histidine, cysteine, proline, threonine, glycine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X_5 is alanine.

Moreover, X_6 may be selected from all amino acid residues, but the preferred amino acid residues for substitution are threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues for substitution for X_6 are threonine, proline, alanine, histidine, cysteine, methionine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution threonine, proline, alanine, histidine, and glycine. The most preferred amino acid residue for substitution for X_6 is threonine. Also, X_7 may be selected from all amino acid residues, but the preferred amino acid residues are glycine, threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, glutamine, asparagine, serine, glutamic acid, and aspartic acid. The more preferred amino acid residues for substitution for X_7 are glycine,

threonine, proline, alanine, histidine, cysteine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X_7 are glycine, threonine, proline, alanine, histidine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X_7 is glycine.

The polypeptides and compounds of the present invention can be combined with various excipient vehicles and/or adjuvants well known in this art which serve as pharmaceutically acceptable carriers to permit drug administration in the form of, e.g., injections, suspensions, emulsions, tablets, capsules, and ointments. These pharmaceutical compositions may be administered by any acceptable means. For warm-blooded animals, and in particular, for humans, administration can be oral, parenteral, subcutaneous, intravenous, intramuscular and/or intraperitoneal. The specific dose administered will be dependent upon such factors as the general health and physical condition of the subject as well as the subject's age and weight, the stage of the subject's disease condition, the existence of any concurrent treatments, and the frequency of administration; typically, the dose will be in the range of about 0.5 to about 2.0 mg/kg for human subjects. In general, the composition will contain one or more of the polypeptide(s) of the present invention in a concentration of at least about 0.0001% by weight, more typically at least about 0.001% by weight, still more typically at least about 0.01%, still more typically at least about 0.1% and, in some embodiments, in a concentration of at least about 1% by weight of the composition.

For oral administration, the pharmaceutical composition may be in the form of, for example, a