			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			
L9	107	L6 with L7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
L10	2	L9 and L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
L11	364	L6 same L7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
L12	7	L11 and L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
L13	7	L2 near5 L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
L14	8	L2 with L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:17
L15	183540	Freeze$1drying lyophilisation lyophilization cryodesiccation lyophilized lyophilize	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:22
L16	516	L15 and L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:23

L17	22	L15 same L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:23
L18	93	Mundipharma.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:29
L19	0	Mundipharma.as. and L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:30
L20	34	L2 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:36
L21	1160	bendamustine "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" Treakisym Ribomustin Treanda "SDX-105" "IMET 3393"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:53
L22	273	34/284.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
L23	0	34/284.ccls. and L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
L24	273	34/284.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
L25	2	"5977129".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	ON	2012/08/20 18:39

			DERWENT; IBM_TDB			
L26	904	548/304.4.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:00
L27	11	L26 and (nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:01
L28	593	548/304.7.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:06
L29	14	L28 and (nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:06
L30	9	(brittain.in. franklin.in. cephalon.as.) and bendamustine.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:08
S1	2	treanda	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:41
S2	0	bendamustine same (lyophilize lyphilized)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:41
S3	10	bendamustine and (lyophilize lyphilized)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:42
S4	46	bendamustine and (lyophilize lyphilized freeze$1dried)	US-PGPUB; USPAT; USOCR;	OR	ON	2010/08/14 19:42

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S5	3	bendamustine same (lyophilize lyphilized freeze$1dried)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:42
S6	88851	lyophilize lyophilization freeze$dry freeze$dried free$1drying	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:56
S7	22	S6 same (alkylating adj agent)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:57
S8	2	bendamustine same (aqueous adj solution) same unstable	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:03
S9	0	"cephalon.in"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:04
S10	563	cephalon.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:05
S11	11	S10 and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:05
S12	4	bendamustine same (aqueous adj solution)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:06
S13	458	bendamustine	US-PGPUB;	OR	ON	2010/08/14

			USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			20:06
S14	30	bendamustine adj hydrochloride	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:06
S15	58	bendamustine same injection	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:07
S16	18	bendamustine same solid	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:12
S17	2	bendamustine same unstable	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:13
S18	2	"0656211"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:29
S19	0	"0656211"	EPO	OR	ON	2010/08/14 20:29
S20	610	ku.in.	EPO	OR	ON	2010/08/14 20:29
S21	1	S20 and thiotepa	EPO	OR	ON	2010/08/14 20:30
S22	0	"5330835".pn.	EPO	OR	ON	2010/08/17 12:07
S23	2	"5330835".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/17 12:08
S24	3	"4145400".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	2010/08/17 12:10

			JPO; DERWENT; IBM_TDB			
S25	3	"4145440".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/17 12:10
S26	1	10/417631.app.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/24 13:13
S27	0	benadmustine with mannitol with alcohol	EPO	OR	ON	2011/04/22 20:07
S28	0	benadmustine	EPO	OR	ON	2011/04/22 20:07
S29	11	bendamustine ribomustin treanda "SDX-105" bendamustin Cytostasan "IMET 3393" "Zimet 3393" "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" "16506-27-7"	EPO	OR	ON	2011/04/22 20:20
S30	775	bendamustine ribomustin treanda "SDX-105" bendamustin Cytostasan "IMET 3393" "Zimet 3393" "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" "16506-27-7"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:20
S31	10	S30 with mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S32	13	S30 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S33	13	S30 with alcohol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S34	22	S30 same alcohol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	ON	2011/04/22 20:22

			DERWENT; IBM_TDB			
S35	23	S30 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:24
S36	345	S30 and mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:36
S37	52	S36 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:38
S38	108	(mannitol "(2R,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol" Osmitrol Osmofundin) with (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:44
S39	31	S38 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:44
S40	2	"5362718".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:52
S41	1	S30 same (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:55
S42	15	S30 and (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:55

S43	18	S30 with rapamycin	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:56
S44	23	S30 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:01
S45	6	S30 same (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:01
S46	132	S30 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:01
S47	299	(mannitol "(2R,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol" Osmitrol Osmofundin) same (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:02
S48	7	S47 and S30	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:02
S49	65	cyclophosphamide with mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:07
S50	17	S49 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:07

S51	0	S50 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:12
S52	17166	(nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S53	113050	S52 sme (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S54	6	S52 same (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S55	2335	S52 and (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S56	4	S35 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:15
S57	3	S30 same tablet	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:18
S58	60242	(t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:22
S59	81388	lyophilization lyophilize freeze$1dry freeze$1drying	US-PGPUB; USPAT;	OR	ON	2011/04/22 21:22

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			
S60	477	S58 same S59	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:22
S61	52	S60 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:23
S62	7	chlorambucil same lyophilization	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:41
S63	49972	freeze$1dry freez$1drying lyophilisation lyophilization cryodesiccation	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:45
S64	82	S63 and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:45
S65	6	S38 and S64	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:46
S66	13	S30 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:48
S67	10	fishman.in. and K4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:50

S68	0	fishman.in. and S30	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:50
S69	2	"20020102215"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:53
S70	986	brittain.in. franklin.in. and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 22:53
S71	2	(brittain.in. franklin.in.) and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 22:54
S72	0	"4670262".pn.	EPO	OR	ON	2011/04/25 11:15
S73	2	"4670262".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:15
S74	626	jenapharm.as. ribosepharm.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:43
S75	0	S74 and (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:44
S76	28	S74 and (powder)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:44
S77	396	GlOlA.in.	US-PGPUB; USPAT; USOCR;	OR	ON	2011/04/25 15:35

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S78	0	S77 and dinitroalanine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 15:35
S79	4	S77 and dinitroaniline	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 15:35

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PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	11/330,868	Filing Date	2006-01-12	Docket Number (if applicable)	CP391/CEPH-4391	Art Unit	1617
First Named Inventor	Jason Edward Brittain			Examiner Name	Ali Soroush		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☐ Amendment/Reply
- ☒ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☒ Other WO2006065392

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 233050

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

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Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Signature of Registered U.S. Patent Practitioner			
Signature	/Stephanie A. Barbosa/	Date (YYYY-MM-DD)	2012-11-15
Name	Stephanie A. Barbosa	Registration Number	51430

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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Examiner Initials	Cite No.	Document Number Number – Kind Code (if known)	Publication or Grant Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document
	1	5,192,743	03-09-1993	Hsu et al.
	2	5,183,746	02-02-1993	Shaked et al.

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(54) Title: CANCER TREATMENTS

(57) Abstract: Methods and compositions for treating cancers characterized by death-resistant cancer cells are described. In general, such methods involve administration of a therapeutically effective amount of a compound that induces mitotic catastrophe in the some, and preferably most or all, of the cancerous cells. Methods for assessing the efficacy of such treatments are also provided.

CANCER TREATMENTS

FIELD OF THE INVENTION

[0001] This invention relates generally to cancer treatment, particularly cancers resistant to drug-induced apoptosis.

BACKGROUND OF THE INVENTION

1. Introduction.

[0002] This application claims the benefit of, and priority to, each of the following U.S. provisional patent applications: serial numbers 60/625,193, entitled "Cancer Treatments" and filed November 5, 2004; and 60/660,266, entitled "Cancer Treatments" and filed March 10, 2005. Each of these applications is incorporated herein by reference in its entirety, including figures, tables, and claims.

[0003] The following description includes information that may be useful in understanding the present invention. It is not an admission that any such information is prior art, or relevant, to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

2. Background.

[0004] Cancer is now the second leading cause of death in the United States and over 8,000,000 persons in the United States have been diagnosed with cancer. In 1995, cancer accounted for 23.3% of all deaths in the United States. See U.S. Dept. of Health and Human Services, National Center for Health Statistics, Health United States 1996-97 and Injury Chartbook 117 (1997).

[0005] Cancer is not fully understood on the molecular level. It is known that exposure of a cell to a carcinogen such as certain viruses, certain chemicals, or radiation, leads to DNA alteration that inactivates a "suppressive" gene or activates an "oncogene".

Suppressive genes are growth regulatory genes, which upon mutation, can no longer control cell growth. Oncogenes are initially normal genes (called proto-oncogenes) that by mutation or altered context of expression become transforming genes. The products of transforming genes cause inappropriate cell growth. More than twenty different normal cellular genes can become oncogenes by genetic alteration. Transformed cells differ from normal cells in many ways, including cell morphology, cell-to-cell interactions, membrane content, cytoskeletal structure, protein secretion, gene expression and mortality (transformed cells can grow indefinitely).

[0006] A neoplasm, or tumor, is an abnormal, unregulated, and disorganized proliferation of cell growth, and is generally referred to as cancer. A neoplasm is malignant, or cancerous, if it has properties of destructive growth, invasiveness, and metastasis. Invasiveness refers to the local spread of a neoplasm by infiltration or destruction of surrounding tissue, typically breaking through the basal laminas that define the boundaries of the tissues, thereby often entering the body's circulatory system. Metastasis typically refers to the dissemination of tumor cells by lymphatics or blood vessels. Metastasis also refers to the migration of tumor cells by direct extension through serous cavities, or subarachnoid or other spaces. Through the process of metastasis, tumor cell migration to other areas of the body establishes neoplasms in areas away from the site of initial appearance.

[0007] Cancer is now primarily treated with one or a combination of three types of therapies: surgery; radiation; and chemotherapy. Surgery involves the bulk removal of diseased tissue. While surgery is sometimes effective in removing tumors located at certain sites, for example, in the breast, colon, and skin, it cannot be used in the treatment of tumors located in other areas, such as the backbone, nor in the treatment of disseminated neoplastic conditions such as leukemia. Radiation therapy involves the exposure of living tissue to ionizing radiation causing death or damage to the exposed cells. Side effects from radiation therapy may be acute and temporary, while others may be irreversible. Chemotherapy involves the disruption of cell replication or cell metabolism. It is used most often in the treatment of breast, lung, and testicular cancer.

[0008] The adverse effects of systemic chemotherapy used in the treatment of neoplastic disease are most feared by patients undergoing treatment for cancer. Of these adverse effects, nausea and vomiting are the most common. Other adverse side effects include cytopenia, infection, cachexia, mucositis in patients receiving high doses of chemotherapy with bone marrow rescue or radiation therapy; alopecia (hair loss); cutaneous complications such as pruritis, urticaria, and angioedema; neurological complications; pulmonary and cardiac complications; and reproductive and endocrine complications. Chemotherapy-induced side effects significantly impact the quality of life of the patient and may dramatically influence patient compliance with treatment. As such, improved methods of treatment are needed.

3. Definitions.

[0009] An "alkylating agent" refers to a chemotherapeutic compound that chemically modifies DNA and disrupts its function. Some alkylating agents cause formation of cross links between nucleotides on the same strand, or the complementary strand, of a double-stranded DNA molecule, while still others cause base-pair mismatching between DNA strands.. Exemplary alkylating agents include bendamustine, busulfan, carboplatin, carmustine, cisplatin, chlorambucil, cyclophosphamide, dacarbazine, hexamethylmelamine, ifosphamide, lomustine, mechlorethamine, melphalan, mitotane, mytomycin, pipobroman, procarbazine, streptozocin, thiotepa, and triethylenemelamine.

[00010] An "anti-metabolite" refers to a chemotherapeutic agent that interferes with the synthesis of biomolecules, including those required for DNA synthesis (*e.g.*, nucleosides and nucleotides) needed to synthesize DNA. Examples of anti-metabolites include capecitabine, chlorodeoxyadenosine, cytarabine (and its activated form, ara-CMP), cytosine arabinoside, dacabazine, floxuridine, fludarabine, 5-fluorouracil, gemcitabine, hydroxyurea, 6-mercaptopurine, methotrexate, pentostatin, trimetrexate, and 6-thioguanine.

[00011] An "anti-mitotic" refers to a chemotherapeutic agent that interferes with mitosis, typically through disruption of microtubule formation. Examples of anti-mitotic compounds include navelbine, paclitaxel, taxotere, vinblastine, vincristine, vindesine, and vinorelbine.

[00012] In the context of this invention, a "chemotherapeutic agent" refers to a chemical intended to destroy malignant cells and tissues. Chemotherapeutic agents include small molecules, nucleic acids (*e.g.*, anti-sense molecules, ribozymes, small interfering RNA molecules, *etc.*), and proteins (e.g., antibodies, antibody fragments, cytokines, enzymes, and peptide hormones) that have anti-tumor effects when administered to a patient in order to prevent or treat a cancer or other malignancy. Chemotherapeutic agents are often divided classes based on mechanism of action, *e.g.*, alkylating agents, anti-metabolites, and anti-mitotic agents.

[00013] The term "combination therapy" refers to a therapeutic regimen that involves the provision of at least two distinct therapies to achieve an indicated therapeutic effect. For example, a combination therapy may involve the administration of two or more chemically distinct active ingredients, for example, a fast-acting chemotherapeutic agent and a myeloprotective agent. Alternatively, a combination therapy may involve the administration of one or more chemotherapeutic agents as well as the delivery of radiation therapy and/or surgery or other techniques to either improve the quality of life of the patient or to treat the cancer. In the context of the administration of two or more chemically distinct active ingredients, it is understood that the active ingredients may be administered as part of the same composition or as different compositions. When administered as separate compositions, the compositions comprising the different active ingredients may be administered at the same or different times, by the same or different routes, using the same of different dosing regimens, all as the particular context requires and as determined by the attending physician. Similarly, when one or more chemotherapeutic agents are combined with, for example, radiation and/or surgery, the drug(s) may be delivered before or after surgery or radiation treatment.

[00014] An "intercalating agent" refers to a chemotherapeutic agent that inserts itself between adjacent base pairs in a double-stranded DNA molecule, disrupting DNA structure and interfering with DNA replication, gene transcription, and/or the binding of DNA binding proteins to DNA.

[00015] "Monotherapy" refers to a treatment regimen based on the delivery of one therapeutically effective compound, whether administered as a single dose or several doses over time.

[00016] In the context of the commercialization of pharmaceuticals, the terms "promotion", "promote", "promoting", and the like refer to any and all informational, persuasive, and scientific activities conducted by or on behalf of a manufacturer, distributor, or other entity involved in the discovery, research, development, and/or commercialization of the particular pharmaceutical compound, composition, or treatment regimen intended, directly or indirectly, to induce the prescription, supply, purchase, and/or use of the compound, composition, or treatment regimen. Such activities may be directed toward anyone in the in the supply and distribution chain, including, without limitation, medical professionals (e.g., physicians and nurses), pharmacists, health care administrators, insurance company or government representatives, and patients (including potential patients). In other words, the primary aim of promotion is to stimulate the sale or use of, and/or interest in, a particular pharmaceutical compound, composition, or treatment regimen, and thus any activity intended to serve this aim constitutes "promotion" of the particular pharmaceutical compound, composition, or treatment regimen.

[00017] A "patentable" composition, process, machine, or article of manufacture according to the invention means that the subject matter satisfies all statutory requirements for patentability at the time the analysis is performed. For example, with regard to novelty, non-obviousness, or the like, if later investigation reveals that one or more claims encompass one or more embodiments that would negate novelty, non-obviousness, *etc.*, the claim(s), being limited by definition to "patentable" embodiments, specifically exclude the unpatentable embodiment(s). Also, the claims appended hereto are to be interpreted both to provide the broadest reasonable scope, as well as to preserve their validity. Furthermore, if one or more of the statutory requirements for patentability are amended or if the standards change for assessing whether a particular statutory requirement for patentability is satisfied from the time this application is filed or issues as a patent to a time the validity of one or more of the appended claims is questioned, the

claims are to be interpreted in a way that (1) preserves their validity and (2) provides the broadest reasonable interpretation under the circumstances.

[00018] The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto. Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids, while pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. For a review of pharmaceutically acceptable salts see Berge, *et al.* ((1977) *J. Pharm. Sci.*, vol. 66, 1). The expression "non-toxic pharmaceutically acceptable salts" refers to non-toxic salts formed with nontoxic, pharmaceutically acceptable inorganic or organic acids or inorganic or organic bases. For example, the salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, fumaric, methanesulfonic, and toluenesulfonic acid and the like. Salts also include those from inorganic bases, such as ammonia, hydroxyethylamine and hydrazine. Suitable organic bases include methylamine, ethylamine, propylamine, dimethylamine, diethylamine, trimethylamine, triethylamine, ethylenediamine, hydroxyethylamine, morpholine, piperazine, and guanidine.

[00019] A "plurality" means more than one.

[00020] The term "rituximab refractory" means prior treatment with rituximab, but inappropriate for further treatment due to disease refractory to rituximab therapy, given either as a single agent or in combination (defined as no response, or progression within 6 months of completing rituximab treatment), and/or untoward reaction to prior rituximab therapy, making further treatment unwarranted, as determined by the physician or treating specialist.

[00021] The term"anti-CD20 refractory" means prior treatment with an agent that interacts with the CD20 antigen, but inappropriate for further treatment due to disease refractory to the anti-CD20 agent given either as a single agent or in combination (defined as not response, or progression within 6 months of completing the anti-CD20 treatment), and/or untoward reaction to prior anti-CD20 therapy, making further treatment unwarranted, as determined by the physician or treating specialist.

[00022] The "S phase" of the cell cycle refers to the phase in which the chromosomes are replicated.

[00023] The term "species" is used herein in various contexts, *e.g.*, a particular species of chemotherapeutic agent. In each context, the term refers to a population of chemically indistinct molecules of the sort referred in the particular context.

[00024] A "subject" or "patient" refers to an animal in need of treatment that can be effected by molecules of the invention. Animals that can be treated in accordance with the invention include vertebrates, with mammals such as bovine, canine, equine, feline, ovine, porcine, and primate (including humans and non-humans primates) animals being particularly preferred examples.

[00025] A "therapeutically effective amount" refers to an amount of an active ingredient sufficient to effect treatment when administered to a subject in need of such treatment. In the context of cancer therapy, a "therapeutically effective amount" is one that produces an objectively measured change in one or more parameters associated with cancer cell survival or metabolism, including an increase or decrease in the expression of one or more genes correlated with the particular cancer, reduction in tumor burden, cancer cell lysis, the detection of one or more cancer cell death markers in a biological sample (*e.g.*, a biopsy and an aliquot of a bodily fluid such as whole blood, plasma, serum, urine, *etc.*), induction of induction apoptosis or other cell death pathways, *etc.* Of course, the therapeutically effective amount will vary depending upon the particular subject and condition being treated, the weight and age of the subject, the severity of the disease condition, the particular compound chosen, the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which can readily be determined by one of ordinary skill in the art. It will be appreciated that in the

context of combination therapy, what constitutes a therapeutically effective amount of a particular active ingredient may differ from what constitutes a therapeutically effective amount of the active ingredient when administered as a monotherapy (*i.e.*, a therapeutic regimen that employs only one chemical entity as the active ingredient).

[00026] The term "treatment" or "treating" means any treatment of a disease or disorder, including preventing or protecting against the disease or disorder (that is, causing the clinical symptoms not to develop); inhibiting the disease or disorder (*i.e.*, arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder (*i.e.*, causing the regression of clinical symptoms). As will be appreciated, it is not always possible to distinguish between "preventing" and "suppressing" a disease or disorder since the ultimate inductive event or events may be unknown or latent. Accordingly, the term "prophylaxis" will be understood to constitute a type of "treatment" that encompasses both "preventing" and "suppressing". The term "protection" thus includes "prophylaxis".

SUMMARY OF THE INVENTION

[00027] One object of this invention is to provide patentable methods of treating cancers characterized by death-resistant cancer cells by administration of a compound (*e.g.*, bendamustine) that induces mitotic catastrophe in the cancer cells, alone or in conjunction with other compounds and/or treatments. In preferred embodiments, these methods involve determining whether a patient has a cancer characterized by death-resistant cancer cells, and, if so, then administering to the patient a therapeutically effective amount of bendamustine. Still another object of the invention concerns methods of assessing the efficacy of cancer treatments based on the detection of a cancer cell death marker in a biological sample taken from a patient at one or more periods during or after the administration of a cancer therapy.

[00028] Thus, one aspect of the invention relates to patentable methods of treating cancer patients whose cancers are characterized by death-resistant cancer cells, *i.e.*, cancer cells that resist apoptosis or other programmed cell death pathways, as well as cells that exhibit multi-drug resistance (MDR), as may be induced, for example, by administration of one or more alkylating agents, alone or in conjunction with an anti-

CD20 agent, *e.g.*, rituximab. These methods comprise administering to a patient a therapeutically effective amount of a compound that induces mitotic catastrophe in the death-resistant cancer cells. Such cells include those that are resistant to drug-induced apoptosis. Examples of such cells include those that have a p53 deficiency, typically as a result of a mutation of, including deletions in or of, a gene encoding p53. Representative examples of such cancers include non-Hodgkin's lymphoma ("NHL") and chronic lymphocytic leukemia ("CLL"). A particularly preferred compound for inducing mitotic catastrophe is the alkylating agent bendamustine. Thus, a related aspect concerns methods of treatment that involve characterization of the cells of a particular cancer as death-resistant cancer cells, followed by treatment with a compound (*e.g.*, bendamustine) that induces mitotic catastrophe in such cells, alone or in conjunction with other chemotherapeutic agents, adjuvants, surgery, and/or radiation. In addition, the efficacy of such treatment regimens can be monitored to assess whether the particular monotherapy or combination therapy treatment is achieving the desired effect.

[00029] Another aspect of the invention concerns certain related patentable methods for treating a cancer, particularly cancers characterized by death-resistant cancer cells. These methods comprise the administration to a patient of a therapeutically effective amount of a compound at a time when at least a portion of the cells comprising the cancer are in the S phase of the cell cycle. In some embodiments, at least a portion of the patient's cancerous cells are driven into the S phase as a result of administering to the patient a compound that drives cells into the S phase. Bendamustine is a particularly preferred compound for driving cancer cells into the S phase. Because bendamustine is useful in driving cancer cells into the S phase, additional preferred embodiments involve the subsequent administration of one or more other chemotherapeutic agent species that are more active (i.e., exert a greater therapeutic effect, for example, cytotoxicity, when cells are in the S-phase of the cell cycle. In such methods, the subsequent administration of one or more other chemotherapeutic agents preferably occurs at least about 10 minutes, and preferably at least about 30 to about 60 minutes or more after bendamustine administration, although it is preferred that the administration of such other agent(s) occurs within about 72 hours, preferably about 48 hours or less, after bendamustine is administered. In some of these preferred embodiments, the other chemotherapeutic

agent(s) is(are) given within about 30 minutes to about 36 hours after the administration of bendamustine, preferably within about 30 minutes to 24 hours after administration of bendamustine, and in some cases, within about 30 minutes to six to about twelve hours after administration of bendamustine. Related methods involve reducing toxicity associated with a cancer therapy. Such methods comprise administering a plurality of doses of therapeutically effective amounts bendamustine to a cancer patient. The first dose may well result in an undesired toxicity. In such event, the administration of the second (or other subsequent doses) may be delayed until after the undesired toxicity begins to subside. In some cases, the doses of bendamustine administered at different times may also vary.

[00030] Yet another aspect of the invention thus relates to patentable methods for assessing the efficacy of a cancer treatment based on the administration of an alkylating agent (*e.g.*, bendamustine), either during the course of or after completion of the treatment, be it a monotherapy or a combination therapy. When the assessment is performed after administration of a therapeutic regimen that involves administration of an alkylating agent (*e.g.*, bendamustine), preferably a sufficient period is allowed to elapse so that the alkylating agent can exert its intended, or desired, therapeutic effect. In such methods, a marker of cancer cell death (*i.e.*, a molecule (*e.g.*, a protein, carbohydrate, lipid, nucleic acid, or other molecule) produced by or released from a dying or dead cancer cell, as well as a phenotype such as a lack of cell viability, inability to proliferate, senescence, etc.) that correlates with treatment efficacy is detected in a biological sample obtained from the patient to determine if the treatment with was efficacious. Preferred markers of cell death include adenylate kinase activity levels, the level of PARP cleavage products, and reduced cell viability. Depending on the marker, such detection may be qualitative, semi-quantitative, or quantitative. The presence, or level, of the marker detected indicates whether the treatment is, or has been, efficacious.

[00031] In still another aspect of the invention, the invention concerns treatments for cancer based on administering bendamustine to patients who have a cancer resistant, or refractory, to one or more alkylating agents and an anti-CD20 agent (for example, rituximab). Preferably, these methods are deployed against cancers characterized by death-resistant cancer cells. A related aspect of the invention concerns methods of doing

business in the treatment of such cancers, which involve promoting bendamustine use to treat a refractory cancer or a cancer characterized by death-resistant cancer cells, particularly a cancer refractory to treatment with a combination of one or more alkylating agents and an anti-CD20 agent, *e.g.*, rituximab. Still another aspect concerns whether a patient's cancer is amenable to bendamustine treatment. As will be appreciated, any suitable assessment of bendamustine susceptibility can be employed. In some preferred embodiments of these methods, some or all of a cell sample from cancerous tissue taken from a patient is exposed to bendamustine under growth conditions which, in the absence of a compound that is toxic to cancer cells, allows the cancer cells to proliferate. The assessment of susceptibility is then made based on the results of the assay. For example, reduced proliferation, as compared to controls, would indicate that the cells, and hence the patient's cancer, are susceptible to a bendamustine-based therapy. In contrast, no effect on (or enhanced proliferation) would indicate a lack of susceptibility.

[00032] Yet another aspect of the invention relates to the use of bendamustine in the manufacture of a medicament for treatment of a cancer characterized by death-resistant cancer cells or for treatment of a refractory cancer, particularly a cancer refractory to treatment with a combination of one or more alkylating agents and an anti-CD20 agent *e.g.*, rituximab. Preferably, such medicaments include a therapeutically effective amount of bendamustine.

BRIEF DESCRIPTION OF THE DRAWINGS

[00033] This patent application contains at least one figure executed in color. Copies of this patent application with color drawing(s) will be provided upon request and payment of the necessary fee.

[00034] Figure 1 has two panels, A and B, each which show gene expression profiles. The panels show changes in gene expression measured in the Non-Hodgkin's Lymphoma cell line, SU-DHL-1, using an Affymetrix gene chip (U133A) containing more than 12,000 known genes. Bendamustine was tested at IC_{50} (25 μM; lane 1) and IC_{90} (35 μM; lane 2). Chlorambucil (5 μM; lane 3) and phosphoramide mustard, a cyclophosphamide

metabolite (50 μM; lane 4), were tested at IC_{90}. Isolation of mRNA was performed 8h after exposure. *A.* The clustergram shown represents the top 100 most modulated genes as compared to a control (diluent, DMSO). The red color represents the genes that were up-modulated; blue represents the genes that were down-regulated. *B.* The clustergram represents genes that are concomitantly induced by all three tested drugs.

[00035] Figure 2 has three bar graphs, 2A, 2B, and 2C. Q-PCR analysis was performed as described in the Methods section, below, in SU-DHL-1 cells exposed to equitoxic concentrations of bendamustine, phosphoramide mustard, and chlorambucil. The levels of input cDNA were normalized using an assay for 18s RNA, and the level of transcripts in the untreated sample was set to 1. Figure 2 A shows the relative RNA levels of two representative p53-dependent genes, p21 and NOXA. Figure 2 B shows the RNA levels of four genes involved in the M-phase cell cycle checkpoint, polo-like-kinase 1 (PLK-1), the aurora kinases A and B, and cyclin B1. Figure 2 C shows the relative RNA levels of genes involved in DNA-repair mechanisms, EXO1 and Fen1. The columns represents the mean +/- SE of the fold changes from DMSO-treated controls. The results were obtained from three independent experiments.

[00036] Figure 3 shows several immunoblots that demonstrate that enhanced apoptotic effect of bendamustine (50 μM) as compared to cyclophosphamide (50 μM) and chlorambucil (4 μM) in NHL cells (SU-DHL-1). To generate these immunoblots, cell lysates were prepared after 20 hours exposure as described in the Methods section, below. Probing the membrane with ß-actin served as a loading control and is shown below the regulated proteins. The top-left panel represents the expression of Ser15-phosphorylated p53, detected using a phospho-specific antibody. The middle-left panel shows total p53 and p21 expression. The lower-left panel represents the expression of Bax. The right panels shows the expression of the full-length PARP (top) and the caspase-cleaved fragment of PARP using an antibody that recognizes the specific caspase-cleavage site.

[00037] Figure 4 consists of two graphs, A and B that represent functional analyses of selected DNA repair mechanisms. Figure 4 A shows that bendamustine, but not cyclophosphamide, leads to DNA damage repair via base excision repair (BER). The role of the repair enzyme Ape-1, an apurininc endonuclease that plays a critical role in the

BER pathway in the cytotoxic activity of bendamustine and a cyclophosphamide metabolite, phosphoramide mustard (PM), was assessed using the Ape-1 inhibitor methoxyamine (MX). The left shift of the curve observed with bendamustine and MX shows that DNA damage produced by bendamustine is repaired by BER. Figure 4 B shows that inhibition of MGMT repair activity does not affect bendamustine cytotoxicity. The role of the repair enzyme MGMT (O^6-methylguanine-DNA methyltransferase) in the cytotoxic activity of bendamustine was assessed using the MGMT inhibitor O^6-benzylguanine (O^6-BG). The addition of O^6-benzylguanine did not significantly change the IC_{50} of bendamustine, so it is unlikely that bendamustine induces O^6-alkylguanine DNA adducts. In contrast, O^6-benzylguanine significantly sensitizes cells to other nitrogen mustards such as carmustine and phosphoramide mustard (PM).

[00038] Figure 5 illustrates that bendamustine efficiently enters tumor cells and induces prolonged and extensive DNA damage, which results in the initiation of at least three signaling pathways: 1) activation of "canonical" p53-dependent stress pathway resulting in a strong activation of intrinsic apoptosis, probably mediated by pro-apoptotic BCL-2 family members such as NOXA and Bax; 2) activation of a DNA repair mechanism, such as the base-excision repair machinery, that are not activated by other alkylating agents frequently used in NHL or CLL patients; and 3) inhibition of several mitotic checkpoints, such as the kinases PLK-1 and Aurora A and B. While not wishing to be bound to a particular theory, the concomitant induction of DNA damage and inhibition of mitotic checkpoints presumably prevents tumor cells exposed to bendamustine from efficiently repairing DNA damage before undergoing mitosis. Cells thus enter mitosis with damaged DNA, or cells that can not proceed to "conventional" p53-dependent apoptosis, will undergo death by mitotic catastrophe. This alternative programmed cell death pathway, together with the strong activation of traditional apoptosis, is believed to be why bendamustine is very effective in killing drug-resistant cancer cells *in vitro*, as well as in patients having chemo-refractory tumors.

[00039] Figure 6 is a histogram that shows the results of adenylate kinase assays performed in the course of several of the "wash-out" experiments described in Example 3, below. In these experiments, SU-DHL-1 cells were treated with either 50 μM bendamustine, 20 μM phosphoramide mustard, or 2 μM chlorambucil for either 30, 60, or

90 minutes. After the timed drug incubation, the cells were washed in 1X PBS to "wash out" the particular chemotherapeutic agent and then fresh medium was added. Cells were then cultured for 48 hours, after which time adenylate kinase assays were performed on the cell supernatants. The pink bars represent zero minutes of drug (or no drug) incubation. The green bars represent 30 minute incubations, the orange bars represent 60 minute incubations, and the purple bars represent 120 minute incubations. The results plot the level of adenylate kinase activity in the supernatants versus the three drugs and a "no drug" control. Standard deviation are represented at the top of each bar on the graph.

[00040] Figure 7, like Figure 6, is a histogram that shows the results of adenylate kinase assays performed in the course of several of the "wash-out" experiments described in Example 3, below. The difference between the results depicted in Figures 6 and 7 is that the data represented in Figure 6 concerns 48 hours of cell culture after each of the drugs was "washed out" of the culture, whereas the data in Figure 7 concerns 72 hours of cell culture post "washing out" the particular drug.

[00041] As those in the art will appreciate, the following description describes certain preferred embodiments of the invention in detail, and is thus only representative and does not depict the actual scope of the invention. Before describing the present invention in detail, it is understood that the invention is not limited to the particular molecules, systems, and methodologies described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the invention defined by the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[00042] The present invention is based on the surprising discovery that the alkylating agent bendamustine exerts very rapid cytotoxic effects on a number of cancer cell types, including those refractory to conventional chemotherapeutic regimens. It has also been discovered that bendamustine exerts its toxic effects through distinct modes of action, as compared to other anti-cancer drugs, as described in detail below.

[00043] Bendamustine, 4-{5-[bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl}, is a chemotherapeutic agent of the nitrogen mustard class. Bendamustine primarily

exhibits alklyating activity, *i.e.*, it is a DNA-damaging agent. When administered to humans (typically by bolus intravenous infusion), bendamustine has a short serum half-life, on the order of 2 hours. Thus, it is rapidly cleared from a patient's system. Surprisingly, it has been discovered that, after cell uptake, bendamustine rapidly exerts its durable cytotoxic effects. Indeed, as reported in Example 3, below, the vast majority of the compound's cytotoxic effects are exerted upon exposing cancer cells to the agent for as little as about 30 minutes.

[00044] Current protocols for bendamustine treatment typically involve the delivery of three separate bolus intravenous infusions each containing an equivalent amount of bendamustine. The second infusion is generally given one day after the first infusion, followed by the third infusion three weeks after the first infusion. This regimen has been used due toxicities related to bendamustine treatment, including myelosuppression. Given the short serum half-life of bendamustine and its fast-acting nature, drug-related toxicity can be reduced by delaying the second and subsequent administrations. Indeed, because extensive and perhaps lethal tumor lysis has been occasionally been reported in connection with bendamustine treatment of non-Hodgkin's lymphoma, greater spacing of the multiple administrations of the drug may serve to reduce the incidence of tumor lysis. In addition to reducing unwanted toxicity, greater spacing of bendamustine administrations in a particular treatment regimen will also serve to increase the therapeutic window, *i.e.*, the time period over which the drug is exerting its intended therapeutic benefit.

[00045] The composition(s) used in the practice of the invention may be processed in accordance with conventional methods of pharmaceutical compounding techniques to produce medicinal agents (*i.e.*, medicaments or therapeutic compositions) for administration to subjects, including humans and other mammals, *i.e.*, "pharmaceutical" and "veterinary" administration, respectively. *See*, for example, the latest edition of Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA). Typically, a compound such as bendamustine is combined as a composition with a pharmaceutically acceptable carrier. The composition(s) may also include one or more of the following: preserving agents; solubilizing agents; stabilizing agents; wetting agents; emulsifiers; sweeteners; colorants; odorants; salts; buffers; coating agents; and antioxidants.

[00046] The drugs used in the practice of the invention may be prepared as free acids or bases, which are then preferably combined with a suitable compound to yield a pharmaceutically acceptable salt. The expression "pharmaceutically acceptable salts" refers to non-toxic salts formed with nontoxic, pharmaceutically acceptable inorganic or organic acids or inorganic or organic bases. For example, the salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, fumaric, methanesulfonic, and toluenesulfonic acid and the like. Salts also include those from inorganic bases, such as ammonia, hydroxyethylamine and hydrazine. Suitable organic bases include methylamine, ethylamine, propylamine, dimethylamine, diethylamine, trimethylamine, triethylamine, ethylenediamine, hydroxyethylamine, morpholine, piperazine, and guanidine.

[00047] In any event, the therapeutic compositions are preferably made in the form of a dosage unit containing a given amount of a desired therapeutic agent (*e.g.*, bendamustine) and a carrier (*i.e.*, a physiologically acceptable excipient). What constitutes a therapeutically effective amount of any such molecule for a human or other mammal (or other animal) will depend on a variety of factors, including, among others, the type of disease or disorder, the age, weight, gender, medical condition of the subject, the severity of the condition, the route of administration, and the particular compound employed. Thus, dosage regimens may vary widely, but can be determined routinely using standard methods. In any event, an "effective amount" of chemotherapeutic agent is an amount that elicits the desired cytotoxic. The quantity of such a therapeutic molecule required to achieve the desired effect will depend on numerous considerations, including the particular molecule itself, the disease or disorder to be treated, the capacity of the subject's cancer to respond to the molecule, route of administration, *etc.* Precise amounts of the molecule required to achieve the desired effect will depend on the judgment of the practitioner and are peculiar to each individual subject. However, suitable dosages may range from about several nanograms (ng) to about several milligrams (mg) of active ingredient per kilogram body weight per day.

[00048] The preparation of therapeutic compositions is well understood in the art. Typically, such compositions are prepared as injectable, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are physiologically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water for injection, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, anti-pyretics, stabilizing agents, thickening agents, suspending agents, anesthetics, preservatives, antioxidants, bacteriostatic agents, analgesics, pH buffering agents, *etc.* that enhance the effectiveness of the active ingredient. Such components can provide additional therapeutic benefit, or act towards preventing any potential side effects that may be posed as a result of administration of the pharmaceutical composition.

[00049] The compositions of the invention may be administered orally, parentally, by inhalation spray, rectally, intranodally, intrathecally, or topically in dosage unit formulations containing conventional carriers, adjuvants, and vehicles. In the context of therapeutic compositions intended for human administration, pharmaceutically acceptable carriers are used. The terms "pharmaceutically acceptable carrier" and "physiologically acceptable carrier" refer to molecular entities and compositions that are physiologically tolerable and do not typically produce an unintended allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a subject.

[00050] For oral administration, the composition may be of any suitable form, including, for example, a capsule, tablet, lozenge, pastille, powder, suspension, or liquid, among others. Liquids may be administered by injection as a composition with suitable carriers including saline, dextrose, or water. The term "parenteral" includes infusion (including continuous or intermittent infusion) and injection via a subcutaneous, intravenous, intramuscular, intrasternal, or intraperitoneal route. Suppositories for rectal administration can be prepared by mixing the active ingredient(s) with a suitable non-irritating excipient such as cocoa butter and/or polyethylene glycols that are solid at ordinary temperatures but liquid at physiological temperatures.

[00051] The compositions may also be prepared in a solid form (including granules, powders or suppositories). The compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers *etc.* Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert excipient such as sucrose, lactose, or starch. Such dosage forms may also comprise additional substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting sweetening, flavoring, and perfuming agents.

[00052] Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions, may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Suitable vehicles and solvents that may be employed are water for injection, Ringer's solution, and isotonic sodium chloride solution, among others. In addition, sterile, fixed oils can be employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[00053] For topical administration, a suitable topical dose of a composition may be administered one to four, and preferably two or three, times daily. The dose may also be administered with intervening days during which no dose is applied. Suitable compositions for topical delivery often comprise from 0.001% to 10% w/w of active ingredient, for example, from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w, but preferably not more than 5% w/w, and more preferably from 0.1% to 1% of the formulation. Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through

the skin (*e.g.*, liniments, lotions, ointments, creams, or pastes), and drops suitable for administration to the eye, ear, or nose.

[00054] Exemplary methods for administering the compositions of the invention (*e.g.*, so as to achieve sterile or aseptic conditions) will be apparent to the skilled artisan. Certain methods suitable for such purposes are set forth in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th Ed. (1985). The administration to the patient can be intermittent; or at a gradual, continuous, constant, or controlled rate.

[00055] Typical therapeutically effective doses for bendamustine for the treatment of non-Hodgkin's lymphoma can be from about 60-120 mg/m^2 given as a single dose on two consecutive days, or with several days between doses. The cycle can be repeated about every three to four weeks. For the treatment of chronic lymphocytic leukemia (CLL) bendamustine can be given at about 80-100 mg/ m^2 on days 1 and 2. The cycle can be repeated after about 4 weeks. For the treatment of Hodgkin's disease (stages II-IV), bendamustine can be given in the "DBVBe regimen" with daunorubicin 25 mg/ m^2 on days 1 and 15, bleomycin 10 mg/ m^2 on days 1 and 15, vincristine 1.4 mg/ m^2 on days 1 and 15, and bendamustine 50 mg/ m^2 on days 1-5 with repetition of the cycle about every 4 weeks. For breast cancer, bendamustine (120 mg/ m^2) on days 1 and 8 can be given in combination with methotrexate 40 mg/ m^2 on days 1 and 8, and 5-fluorouracil 600 mg/ m^2 on days 1 and 8 with repetition of the cycle about every 4 weeks. As a second-line of therapy for breast cancer, bendamustine can be given at about 100-150 mg/ m^2 on days 1 and 2 with repetition of the cycle about every 4 weeks.

[00056] The methods of the invention involve both monotherapy and combination therapy. In the context of combination therapy, the invention envisions the administration of two or more chemotherapeutic agents. A wide variety of chemotherapeutic agents are known in the art. Some of these compounds have already been approved for use in treating one or more cancer indications. Others are in various stages of pre-clinical and clinical development. Examples of chemotherapeutic agents useful in the practice of combination therapies according to the invention include the alkylating agents busulfan, carboplatin, carmustine, cisplatin, chlorambucil, cyclophosphamide, dacarbazine, hexamethylmelamine, ifosphamide, lomustine, mechlorethamine, melphalan, mitotane,

mytomycin, pipobroman, procarbazine, streptozocin, thiotepa, and triethylenemelamine. Preferred anti-metabolites for use in conjunction with bendamustine include capecitabine, chlorodeoxyadenosine, cytarabine (and its activated form, ara-CMP), cytosine arabinoside, dacabazine, floxuridine, fludarabine, 5-fluorouracil, gemcitabine, hydroxyurea, 6-mercaptopurine, methotrexate, pentostatin, trimetrexate, and 6-thioguanine. Preferred anti-mitotic compounds that can be used in combination therapies with bendamustine include navelbine, paclitaxel, taxotere, vinblastine, vincristine, vindesine, and vinorelbine.

[00057] Other classes of chemotherapeutic agents include topoisomerase I inhibitors (*e.g.*, camptothecin, irinotecan. topotecan, *etc.*); topoisomerase II inhibitors such as daunorubicin, doxorubicin, etoposide, idarubicin, mitoxantrone, and teniposide; angiogenesis inhibitors (*e.g.*, dalteparin, suramin, *etc.*); antibodies, including alemtuzumab, bevacizumab, bexarotene, epratuzumab, gemtuzumab ozogamicin, ibritumomab tiuxetan, imatinib mesylate, raltitrexed, revlimid, rituximab, trastuzumab; tyrosine kinase inhibitors; intercalating agents; and hormones, such as anastrozole, estrogen, anti-estrogen (*e.g.*, fulvestrant and tamoxifen), exemestane, flutamide, goserelin, leuprolide, nilutamide, levimasole, letrozole, prednisone, and toremifene. Other chemotherapeutic agents include proteins such as angiostatin, asparaginase, deniluekin diftitox, endostatin, imiquimod, interferon, interleukin-11, and pegaspargase. Still other chemotherapeutic agents include molecules such as alitretinoin, altretamine, amifostine, amsacrine, arsenic trioxide, bleomycin, capecitabine, carboxyamidotriazole, celecoxib, dactinomycin, epirubicin, geldanmycin, 17-Allylamino-17-demethoxygeldanamycin (17 AAG), irinotecan, 2-methoxyestradiol, mithramycin, mytomycin C, oxaliplatin, squalamine, temozolamide, thalidomide, tretinoin triapine, and valrubicin. As those in the art will appreciate, these and other chemotherapeutic agents now known or later developed may be used in combination with bendamustine to treat various neoplasias, including cancers.

EXAMPLES

[00058] The following examples are provided to illustrate certain aspects of the present invention and to aid those of skill in the art in practicing the invention. These examples are in no way to be considered to limit the scope of the invention in any manner.

Example 1

Molecular Analysis of the Mechanism of Action of Bendamustine

A. Introduction.

[00059] Bendamustine (Treanda™, Salmedix, Inc. CA; Ribomustin™ (Ribosepharm GmbH, Munich Germany)) is an anti-tumor agent with demonstrated preclinical and clinical activity against various human cancers, such as Non-Hodgkin's Lymphomas (NHL), chronic lymphocytic leukemias, solid tumors, breast and small cell lung cancers, and multiple myelomas, including those refractory to conventional DNA-damaging agents. Bendamustine, 4-{5-[bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl} butyric acid hydrochloride, was originally synthesized with the intention of producing an agent with low toxicity and both alkylating and anti-metabolite properties. It has three sub-structural elements: a 2-chloroethylamine alkylating group; a benzimidazole ring; and a butyric acid side-chain. The 2-chloroethylamine alkylating group is shared with other nitrogen mustards, such as cyclophosphamide, chlorambucil, and melphalan. The benzimidazole central ring system is a unique feature of bendamustine, although the butyric acid side chain is present in chlorambucil. This multi-faceted structure may contribute to its unique anti-neoplastic activity profile and distinguishes it from conventional alkylating agents.

[00060] DNA alkylating agents are extremely useful in the chemotherapy armamentarium. Such drugs may possess unexpected mechanisms of action, such as a capacity of some of these compounds to induce programmed necrosis and the capacity of others (*e.g.*, platins) to induce apoptosis even in cells deprived of nuclei. In the case of the "nitrogen mustards", major differences exist in their profile of activity as reflected by their differentiated use in various indications: cyclophosphamide, which is used primarily in treating NHL; chlorambucil, which is used in treating chronic lymphocytic leukemia; and melphalan, which is used in treating multiple myeloma.

[00061] The main anti-tumor action of bendamustine, in common with other alkylating agents, results from the formation of cross-links between the paired strands of DNA, although other modes of action may also be involved. Thus, the anti-tumor action of bendamustine may derive from mechanisms which are more complex than simply classic

alkylation activity, as DNA double-strand breaks caused by bendamustine are significantly more durable than those caused by cyclophosphamide or BNCU, bendamustine shows activity against cell lines which are resistant *in vitro* and *ex vivo* to other alkylating agents, and unique pro-apoptotic activity has been demonstrated by bendamustine as a single agent and in combination with other anti-cancer agents in several *in vitro* tumor models. Detailed molecular studies on the exact mechanism of action of bendamustine remain sparse. For this reason, state-of-the art molecular tools were used to fully dissect the mechanism of action of bendamustine. This example presents results derived from pharmacogenomic assays to analyze the gene expression profile changes induced by bendamustine in NHL cell lines. These pharmacogenomic analyses were validated by functional assays dealing with the initiation of apoptotic signaling, the mechanism of DNA repair, and the modulation of mitotic checkpoints. Finally, bendamustine has been profiled in the National Cancer Institute's human tumor 60 cell line in vitro screen, and its comparative activity against a library of other alkylating agents (*i.e.*, chlorambucil and phosphoramide mustard (the metabolite of cyclophosphamide)) was studied. Results were also generated using pharmacogenomic assays to analyze the gene expression profile changes induced by bendamustine in NHL cell lines. These pharmacogenomic analyses were validated by Q-PCR and functional assays dealing with the initiation of apoptotic signaling, mechanisms of DNA repair, and the modulation of mitotic checkpoints. Together, these results demonstrate that bendamustine possesses multiple mechanisms of action that are distinct from other DNA alkylating drugs, explaining bendamustine's activity in patients having tumors refractory to conventional therapy.

B. Materials and Methods.

a. Cells.

[00062] SU-DHL-1 cells were obtained from the University California San Diego. Cells were grown in RPMI 1640 (Hyclone) supplemented with 10% FBS (Invitrogen) and 100 units/ml penicillin/streptomycin.

b. Reagents.

[00063] Bendamustine hydrochloride was obtained from Fujisawa Deutschland (Munich, Germany). Phosphoramide mustard cyclohexylamine salt (PM, NSC69945), an active metabolite of cyclophosphamide, was obtained from the synthetic repository of the Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI). All other reagents were obtained from commercial sources such as Sigma-Aldrich.

c. Drug Treatments.

[00064] For most of the assays presented in this example, the concentrations used for bendamustine, phosphoramide mustard (the active metabolite of cyclophosphamide), and chlorambucil were selected based on their cytotoxic activity measured with the MTT assay over a period of three days. Drugs were prepared in DMSO and then diluted in culture medium.

d. Preparation of RNA Samples and Analysis of Expression Data.

[00065] Cells were harvested (5×10^6 cells) in 1mL TRIZOL solution (Invitrogen, San Diego, CA) and total RNA was isolated as per manufacturer's instructions. Biotin-labeled cDNA (15 μg) was hybridized to each GeneChip array (Affymetrix, Santa Clara). Briefly, the procedure to prepare material for hybridization to the chips involved multiple steps. Total RNA was isolated and quantified by optical density. cDNA was generated using a specific primer that recognizes the poly A tail coupled with a T7 promoter (dT7-(T)24) with dNTP, DTT, and Superscript II to generate the first strand cDNA. This approach alleviated the need to isolate poly-A(+) mRNA. The second strand was synthesized by adding dNTPs with DNA ligase, DNA pol I, and RNAse H, and incubating for 2 h at 16°C before adding T4 DNA polymerase for an additional 5 min. cDNA was column purified and quantified. In vitro transcription (IVT) was performed prior to hybridization to the high-density oligonucleotide arrays. The starting material for this reaction was 1 μg of cDNA to which NTPs were added with 25 % less CTP and UTP to be compensated by adding 10 mM biotinylated-11-CTP and 10 mM biotinylated-16-UTP. The final addition of T7 enzyme in the appropriate buffer for 6 h at 37°C yielded the biotinylated IVT RNA which was then column purified (RNeasy, Qiagen).

Chemically fragmented IVT RNA (15 μg) was mixed with control oligonucleotides, standards (including a housekeeping gene), and salmon sperm DNA in the appropriate buffer, heated to 95°C for 5 minutes, and hybridized to the chip for 16 h at 42°C. Non-hybridized material was washed off with 2XSSPE and phycoerythrin-labeled avidin was then added to the reaction. The excess fluorochrome was washed off and the chip was then scanned for intensity of fluorescence in each synthesis feature (synthesis features are 7.5 square microns).

e. Bioinformatics Analysis.

[00066] A strategy and a process for the analysis of gene expression data was developed, which involved the use of the CORGON method to analyze scanned images of Affymetrix GeneChips. CORGON is freely available software, whose core statistical method is known (Sasik, *et al.* (2002), Bioinformatics, vol. 18, no. 12:1633-40). Only genes that were present at $p < 0.05$ (95% confidence level) in at least one of the conditions were considered for further analysis. A comparison of CORGON with the Affymetrix Microarray Suite (AMS) 5.0 software revealed a 4.4% false positive error rate for CORGON as compared to 29% for AMS 5.0. The genes selected were sorted according to the average or peak magnitude of modulation. The top 100 most modulated genes were chosen for clustering based on the similarity of their expression pattern. Hierarchical clustering methods were used. This initial classification was extremely useful in determining what were the primary genes and pathways modulated by the process under investigation. Clusters of genes that appeared to be co-regulated were subjected to promoter analysis. The next step was GO3 analysis, an unbiased and unsupervised tool for finding statistically significant terms in the Gene Ontology database (website: www.geneontology.org) related to the process. GO3 facilitates the process of identifying the critical components of the system that were modulated significantly. There were three ontologies in the database: molecular function; biological process; and cellular component. The analysis was performed at the UCSD Center for AIDS Research Genomics Core Facility.

f. Quantitative PCR Analysis.

[00067] The expression levels of specific transcripts were determined using quantitative PCR (Q-PCR). Total RNA from each treated SU-DHL-1 cell pellet was isolated using an RNeasy mini-prep kit (Qiagen, Valencia, CA). cDNAs were made using a ThermoScript reverse-transcriptase kit (Invitrogen) and oligo-dT primers according to the manufacturer's protocol. Q-PCR amplification and quantitation was carried out using an iCycler machine (Bio-RAD, Hercules, CA). Sample amplification was performed in a volume of 25 μL containing 12.5 μL of 2 x IQ SybrGreen™ Mix (Bio-Rad), 1μM of each primer, and a volume of cDNA corresponding to 80 ng of total RNA. Cycling conditions were: 95°C for 5 seconds; 30 seconds at the appropriate annealing temperature for each primer; and 72°C for 30 seconds. Target specificity of the assays was validated by melt curve analysis. The expression of each gene was normalized relative to 18s expression levels for each sample. The expression of each gene relative to untreated control was then calculated per the method of Livak and Schmittgen ((2001), Methods, vol. 25:402-408). Primers were designed using Beacon Designer™ (Premier Biosoft, Palo Alto, CA) or designed based on the literature. Primer sequences and annealing temperatures are as follows (each primer is written 5' to 3', followed by its SEQ ID NO):

Gene ID	Forward Primer	Reverse Primer	Anneal Temp
18s	CGCCGCTAGAGGTGAAATTC (1)	TTGGCAAATGCTTTCGCT (2)	55°C
p21	CCTCATCCCGTGTTCTCCTTT (3)	GTACCACCCAGCGGACAAGT (4)	57°C
Noxa	ATTTCTTCGGTCACTACACAA (5)	AACGCCCAACAGGAACAC (6)	55°C
PLK-1	CTCAACACGCCTCATCCT (7)	GTGCTCGCTCATGTAATTGC (8)	57°C
Aurora A	TCCTTGTCAGAATCCATTACCTGT (9)	GAATGCGCTGGGAAGAATTTG (10)	55°C
Aurora B	AGAGTGCATCACACAACGAGA (11)	CTGAGCAGTTTGGAGATGAGGTC (12)	56°C
Cyclin B1	AGTGTGACCCAGACTGCCTC (13)	CAAGCCAGGTCCACCTCCTC (14)	57°C
Exo1	TTGGTCTGGAGGTCTTGGAGA (15)	GAATCGCTCTTTCTTCGGAACTG (16)	57°C

g. COMPARE Analysis.

[00068] Bendamustine was tested in the NCI's *in vitro* anti-tumor screen consisting of 60 human tumor cell lines. Testing involved a minimum of five concentrations at 10-fold dilutions, and each screen was repeated twice. A 48 hour continuous drug exposure

protocol was used. A Sulforhodamine B protein assay estimated cell viability or growth. The COMPARE method and associated data are freely available on the Developmental Therapeutics Program (DTP) website (website: dtp.nci.nih.gov). The NCI assigned bendamustine the number: NSC138783.

h. Western Blot Analysis.

[00069] SU-DHL-1 cells were incubated with 50 μM bendamustine, 2 μM chlorambucil, or 20 μM phosphoramide mustard for 20 hours. Cells were washed twice with 1 x PBS and lysed for 1 hour with ice cold lysis buffer (1 M Tris-HCl (pH 7.4), 1 M KCl, 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholine, with 1mM sodium orthovanidate, 1 mM sodium fluoride, protease inhibitor cocktail (Roche, Nutley, NJ), and phosphatase inhibitor cocktail (Sigma, St. Louis, MO)) added directly before lysis. Non-soluble membranes, DNA, and other precipitants were pelleted and the protein supernatant obtained. Protein concentrations were determined using the Bradford assay (Pierce, Rockford, IL). 20 μg of lysate were separated by gel electrophoresis on a 4-12% polyacrylamide gel, transferred to nitrocellulose membranes (Invitrogen), and detected by immunoblotting using the following primary monoclonal antibodies: anti-p53, anti-phosphorylated p53 (Ser15-specific), anti-p21, and anti-cleaved PARP (caspase-specific cleavage site), which were all purchased from Cell Signaling (Beverly, MA); anti-Bax and anti-PARP, which were purchased from BD Pharmingen (San Diego, CA), and anti-beta-actin, used for a loading control, which was purchased from Sigma (St. Louis, MO). Primary antibodies were incubated overnight at 4°C with gentle shaking. Membranes were washed three times with 1 x PBS and incubated with Alexa Flour 680 goat anti-mouse secondary antibody (1:4000) (Molecular Probes, Eugene, OR) for 2 hours at room temperature with gentle shaking. Blots were washed three times with 1 x PBS and scanned on a LiCor Odyssey scanner.

i. In vitro cell based Ape-1 and AGT assays.

[00070] Cells were pre-incubated for 30 minutes with either 6 mM methoxyamine (Sigma) or 50 μM O^6-benzylguanine (Sigma), inhibitors of Ape-1 base excision repair enzyme and alkylguanyl transferase (AGT) enzyme, respectively. The cells were then exposed to various concentrations of the indicated agents for 72 hrs. Cytotoxicity was

evaluated by the MTT assay (13) and an IC_{50} was measured as the drug concentration that inhibited by 50% the value of the untreated control. Analyses were performed using GraphPad Prism version 3.00 GraphPad Software (San Diego, CA).

j. Cell cycle analyses.

[00071] SU-DHL-1 cells were incubated with equitoxic (IC_{50}) concentrations of bendamustine (50 μM), chlorambucil (4 μM), or phosphoramide mustard (50 μM) for 8 hours. Cells were washed with PBS and fixed in 70% ethanol 20° C for at least one hour. Fixed cells were re-hydrated by washing with PBS. Cells were resuspended in a propidium iodide staining solution consisting of 10 μg/ml propidium iodide (Calbiochem, La Jolla, CA), 10 μg/ml RNAse A (DNase free, Novagen, Madison, WI), and 10 μl/ml Triton-X (Sigma) in PBS. Samples were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA). Analyses of cell cycle distribution were performed using DNA ModFit LT (Verity House Software, Inc. Sunnyvale, CA) modeling software.

k. H2AX foci formation.

[00072] Cell were grown on Lab-Tek chamber slides (Nalge Nunc Intl., Naperville, IL) in RPMI 1640 media supplemented with 10% FBS. After allowing the cells to attach for at least one day, cells were treated in media with either DMSO or 50 μM bendamustine. The cells were incubated for 30 minutes at 37°C and then washed two times with PBS. They were incubated for an additional 4 hours at 37°C. The cells were then washed twice with 1 x PBS and incubated 10 minutes in -20°C 100% methanol to fix the cells. They were then washed three times for five minutes each with 1 x PBS. They were incubated at room temperature for 1 hour in blocking buffer (10% FBS in 1 x PBS, 1% BSA). The slides were incubated at 4°C with rocking overnight with the primary polyclonal anti-H2AX antibody (R & D Systems, Minneapolis, MN). The antibody was diluted in blocking buffer at a ratio of 1:10,000. Slides were washed three times with 1 x PBS and incubated with Alexa Flour 488 goat anti-rabbit secondary antibody (1:4000) (Molecular Probes, Eugene, OR) for 45 minutes at room temperature with gentle shaking. Slides were washed three times with 1 x PBS and then the chambers removed and SlowFade Light Antifade with DAPI (Molecular Probes) was added to the cells and coverslips sealed on the slides. Analysis was performed using a motorized Zeiss

AxioPlan 2e imaging microscope with DIC optics and fluorescence, a Zeiss AxioCam HRm camera and Zeiss Axiovision software Version 4.2.

1. Phosphorylation of H2AX at residue Ser139 immunoblot.

[00073] Cell lines were grown to confluency in RPMI 1640 media supplemented with 10% FBS. The cells were then washed twice with 1 x PBS and lysed for 1 hour with ice cold lysis buffer (1 M Tris-HCl (pH 7.4), 1 M KCl, 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholine, with 1mM sodium orthovanidate, 1 mM NaF, protease inhibitor cocktail (Roche, Nutley, NJ), and phosphatase inhibitor cocktail (Sigma, St. Louis, MO)) added directly before lysis. Non-soluble membranes, DNA, and other precipitants were pelleted and the protein supernatant obtained. Protein concentrations were determined using the Bradford assay (Pierce, Rockford, IL). Twenty micrograms of lysate were separated by gel electrophoresis on a 4-12% polyacrylamide gel, transferred to nitrocellulose membranes (Invitrogen, Carlsbad, CA), and detected by immunoblotting using a polyclonal anti-H2AX antibody (R & D Systems, Minneapolis, MN). The antibody was diluted in blocking buffer at a ratio of 1:2000, and the membranes were incubated for 2 hours at room temperature with gentle shaking. Membranes were washed three times with 1 x PBS and incubated with Alexa Flour 680 goat anti-rabbit secondary antibody (1:5000) (Molecular Probes, Eugene, OR) for 2 hours at room temperature with gentle shaking. Blots were washed three times with 1 x PBS and scanned on a LiCor Odyssey scanner.

C. Results.

a. Gene expression profiling identifies signature genes that are regulated by bendamustine that are distinct from chlorambucil or cyclophosphamide.

[00074] Equitoxic concentrations for bendamustine, chlorambucil, and phosphoramide mustard (the active metabolite of cyclophosphamide) were determined by measuring cell viability after three days exposure to drug. For the assays presented in this study, the concentrations used for bendamustine, phosphoramide mustard, and chlorambucil were selected based on this data (Table 1, below). These concentrations also reflect the clinically achievable levels for each drug. Affymetrix GeneChip analysis was used to

compare the expression levels of over 12,000 genes in drug-treated SU-DHL-1, a non-Hodgkin's lymphoma cell line, cells compared to control cells. SU-DHL-1 cells were incubated with bendamustine at the IC_{50} concentration (25 μM) and at the IC_{90} concentration (35 μM). Chlorambucil and the cyclophosphamide metabolite phosphoramide mustard were tested at IC_{90}, *i.e.*, 5 μM and 50 μM, respectively. Gene expression was monitored following 8 hours treatment with drug to identify the proximal events of this early stress response.

[00075] The genomic analysis revealed that the majority of the genes are similarly regulated between the three tested drugs, as demonstrated by the clustergram of the top 100 modulated genes (Figure 1A). Most genes were upregulated (red color) upon exposure to the drugs. A subset of genes was transcriptionally repressed following drug treatment (blue color). Importantly, a group of genes was identified that displayed differential regulation by bendamustine compared to the other two drugs tested.

[00076] Many of the induced genes (Figure 1B) were known to possess p53-response elements in their promoter regions and are considered p53-dependent. Examples of these genes are: p21 (p53-induced cell division kinase inhibitor); wip1 (p53-induced protein phosphatase 1); NOXA (p53-induced pro-apoptotic Bcl-2 family member); DR5/KILLER (p53-regulated DNA damage-inducible cell death receptor); and BTG2. Interestingly, four members of the tumor necrosis factor receptor superfamily (members 6, 9, 10, and 10b) were identified in the top-100 modulated genes. Several of these genes have been shown to play a critical role in the regulation of the extrinsic apoptotic pathway (REF, TRAIL/TNF apoptosis). Several other genes display an opposite trend between bendamustine and the other two compounds (data not shown). These genes were upregulated by bendamustine, at both concentrations, but were down-regulated by both chlorambucil and phosphoramide mustard.

[00077] To assess the pharmacogenomic differences between bendamustine, chlorambucil, and phosphoramide mustard, the results from the gene profiling were re-analyzed with the GO3 software, an unbiased and unsupervised tool for finding statistically significant terms in the Gene Ontology (GO) database (website: www.geneontology.org) related to the process. Genes significantly up- or down-

regulated in bendamustine-treated cells and at least 1.5-fold above or below levels of expression in control-treated cells were connected to biological process annotations provided by the Gene Ontology (GO) consortium. Based on the hierarchical structure of the GO annotations, the probability that each immediate daughter term (a P value) be linked to the number of selected genes by chance was calculated. The results of the GO analysis comparing the DMSO-treated control and the bendamustine–treated cells (at IC_{90} dose) are reported in Table 2, below. In Table 2, below, the first column represents general categories, the second and third columns are the number and name of the specific biological process, and the last column is the p value for each process. The p value was calculated using the GO3 software. Four major functional groups were found be statistically modulated by bendamustine: (1) DNA-damage, stress response, apoptosis; (2) DNA metabolism, DNA repair, transcription; (3) cell proliferation, cell cycle, mitotic checkpoint; and (4) cell regulation. Each of these groups encompasses several biological processes that were found to be significantly modulated by bendamustine. The biological processes that provided the lowest p values and therefore were the most statistically significant were: response to DNA damage stress (GO6974); DNA metabolism (GO6259); and cell proliferation (GO8283).

[00078] A similar analysis performed with chlorambucil and phosphoramide mustard suggested that little overlap exists between the profile obtained with bendamustine and chlorambucil. Some similarities in gene modulation were observed between bendamustine and phosphoramide mustard, although these were limited to the "DNA metabolism, DNA repair, and transcription" group. These results provided the basis for the selection of specific gene products for the quantitative validation of the gene array results and more definitive differentiation of bendamustine.

b. Validation of genomic analysis by real-time quantitative Q-PCR analysis.

[00079] Confirmation and validation of the array data was performed by real-time quantitative PCR analysis (Q-PCR). Several genes involved in p53-signaling, apoptosis, DNA repair, and cell cycle/mitotic checkpoints were all differentially regulated when comparing bendamustine to the other alkylating agents tested.

[00080] Two examples of "canonical" p53-dependent genes selected for Q-PCR validation were p21 (Cip1/Waf1), the cyclin-dependent kinase inhibitor 1A, and the pro-apoptotic BH3-only Bcl-2 family member, NOXA. Both genes were found to be induced in SU-DHL-1 cells, 8 hours after exposure to bendamustine. Both genes were also induced by equitoxic concentrations of phosphoramide mustard and chlorambucil, but to a much lower extent (Figure 2A).

[00081] One of the most striking results that emerged from the validation analysis was the differential regulation of several mitosis-related genes, including polo-like kinase 1 (PLK-1), the Aurora Kinases A and B, and cyclin B1. These genes are considered to play an important in mitotic checkpoint regulation. Treatment with bendamustine led to a 60 to 80% down-regulation of the mRNA expression of all these genes. In contrast, phosphoramide mustard or chlorambucil only exerted a minor effect on the transcripts of these genes, with possibly the exception of the Aurora kinases (Figure 2B).

[00082] Differences also emerged in the analysis of the mRNA expression of the DNA-repair gene exonuclease-1 (EXO1). Bendamustine induced a slightly stronger (2.5-fold) up-regulation of Exo1 expression (Figure 2C) compared with that observed with phosphoramide mustard (1.5-fold) or chlorambucil (1.8-fold). Fen1 (flap endonuclease 1) was also upregulated by bendamustine, and phosphoramide mustard upregulated this gene to the same level when used at equitoxic concentrations (Figure 2C).

c. Apoptosis signaling by bendamustine in NHL cells.

[00083] To dissect the molecular events involved in bendamustine-induced programmed cell death in NHL cells, expression of key apoptotic proteins was monitored by immunoblot analysis. The results clearly showed that bendamustine can efficiently and rapidly trigger the classical p53-dependent apoptotic pathway. One of the initial or apical events is the induction of p53 phosphorylation, as detected using antibodies that specifically recognize phosphorylation of the serine-15 residue. An 8-fold up-regulation of Ser-15-phosphorylated p53 was observed in SU-DHL-1 cells exposed to bendamustine, while only a minor up-regulation was seen in phosphoramide mustard treated cells, and no changes were observed in chlorambucil-treated cells (Figure 3, top-left panel).

[00084] In parallel with the induction of phosphorylated p53, a strong increase in the expression of total p53 was seen in bendamustine-treated cells. Chlorambucil-treated cells displayed a small increase in total p53, while exposure to phosphoramide mustard induced no change in p53 levels. The changes observed in p21 protein expression were minor for each of the drugs when compared to changes in protein expression levels of p53. An increase in the protein expression of Bax, a key BH3-only pro-apoptotic Bcl-2 family member, was observed only in bendamustine-treated SU-DHL-1 cells (Figure 3, low-left panel).

[00085] The most striking difference observed in comparing the effect of bendamustine with phosphoramide mustard and chlorambucil was found when the expression of PARP, poly-ADP-ribose polymerase-1, was compared. PARP is a critical NAD-requiring enzyme important in DNA-repair mechanisms. PARP is also an "early" substrate of the pro-apoptotic proteolytic caspase enzymes. SU-DHL-1 cells treated with bendamustine showed a dramatic reduction of PARP protein expression (Figure 3, top-right panel). The reason for the reduction of PARP expression was its cleavage by caspases, as demonstrated by the appearance of proteolytic cleavage products recognized by a "cleavage-specific" antibody (Figure 3, middle-right panel). Notably, no changes in the expression of PARP were detected in NHL cells treated by equitoxic concentrations of phosphoramide mustard or chlorambucil. Similar results were observed when using double the equitoxic doses of phosphoramide mustard (40 μM) and chlorambucil (4 μM) while maintaining the dose of bendamustine (50 μM) (data not shown). Thus, an assessment of PARP expression levels can be used for various purposes. For example, a PARP assay can be to provide an indication as to the efficacy of a particular therapeutic regimen, wherein reduced PARP expression (preferably measured at the protein level, for example by PARP activity, for the presence of PARP cleavage products, *etc.*) indicates that the administered drug is having the desired effect. In addition, a PARP assay can be used prognostically to determine, for example, if cells of a tissue (for example, cells derived from a biopsy or other biological sample) are likely to respond to a particular therapy (*e.g.*, bendamustine monotherapy or a combination therapy wherein one of the therapies utilizes bendamustine).

d. Inhibition of base excision repair, but not O^6-methylguanine-DNA methyltransferase repair, blocks bendamustine activity.

[00086] The role of the repair enzyme Ape-1, an apurininc endonuclease that plays a critical role in the base excision repair (BER) pathway in the cytotoxic activity of bendamustine and the cyclophosphamide metabolite, phosphoramide mustard, was assessed using the Ape-1 inhibitor methoxyamine. The IC_{50} of bendamustine was reduced approximately four-fold (from approximately 50 μM to approximately 12 μM) with methoxyamine addition (Figure 4A). In contrast, the IC_{50} of phosphoramide mustard only changed slightly when methoxyamine was added. The results suggest that BER may play an important role in the repair of bendamustine-induced DNA damage, but not in the repair of the damage induced by cyclophosphamide.

[00087] The effect of O^6-benzylguanine, a known inhibitor of O^6-alkylguanine-DNA alkyltransferase (AGT) on the anti-tumor activity of bendamustine, was also tested in the SU-DHL-1 cells. The results demonstrated that the cytotoxic potency of bendamustine was not enhanced by adding O^6-benzylguanine. Opposite results were obtained with cyclophosphamide, suggesting that unlike cyclophosphamide, bendamustine does not rely appreciably on the O^6-methylguanine-DNA methyltransferase DNA repair mechanism (Figure 4B).

e. Bendamustine HCl rapidly induces the formation of double-strand breaks resulting in unique cell cycle alterations.

[00088] To investigate the capacity of bendamustine HCl to induce double-strand breaks (DSBs), two biochemical markers were analyzed: nuclear localization of gamma-H2AX histone by immunofluorescence; and phosphorylation of H2AX at residue Ser139 by immunoblot analysis. Results confirmed that bendamustine HCl potently and rapidly induced DSBs in a variety of tumor cells, including multidrug-resistant and p53 deficient lines. Incubation with 50 μM bendamustine HCl leads to the formation of intranclear foci detectable after as few as 30 minutes. Time-course analysis showed that Ser139 phosphorylation of gamma-H2AX was detectable after 24 hours of continuous exposure to bendamustine HCL as well as after a very short exposure to the drug (30 minutes), followed by drug removal (washout). Bendamustine HCl induced phosphorylation of

H2AX occurred earlier than with other 2-chloroethylamino DNA alkylators such as cyclophosphamide. Cell-cycle analysis of SU-DHL-1 lymphoma cells exposed for eight hours to 50 μM bendamustine HCl showed an average S-phase distribution increase of over 40% without an attendant G2M arrest. Exposure to equitoxic concentrations of chlorambucil and cyclophosamide increased S-phase distribution by approximately 20% and 15% respectively. These findings illustrate that bendamustine HCl can induce DNA double-strand breaks, even after a transient 30 minute exposure.

f. Bendamustine displays a unique profile of activity using the NCI COMPARE analysis.

[00089] Bendamustine cytotoxicity was evaluated in the 60 human cell lines of the National Cancer Institute's preclinical anti-tumor drug discovery screen (NCI screen). The NCI screen is useful for comparing relative potency of potential anti-neoplastic agents with known therapeutic agents from an extensive database of more than 45,000 compounds and natural products. The COMPARE analysis was run using the GI50 results generated with bendamustine as a "seed". Compounds with high Pearson correlation coefficients (PCC) often have similar mechanisms of action. Bendamustine did not demonstrate a strong correlation (>0.8) in the NCI screen with any agent (Table 3, below). Out of the six top matches with bendamustine, only the methylating agent DTIC (dacarbazine) showed approximately an 80% correlative agreement (r value). In contrast, a total of 25 compounds with correlation coefficients over 0.83 were identified for melphalan, chlorambucil, or the active metabolite of cyclophosphamide. In addition, direct comparison of melphalan, chlorambucil, and cyclophosphamide sensitivity patterns in this screen demonstrated high correlation coefficients between the three drugs (0.762-0.934, data not shown). These data show a statistical agreement in sensitivity profile of the agents and a high likelihood of a common mechanism of action. The lack of correlation between bendamustine and other members of the nitrogen mustard class is compelling and reveals that bendamustine has a distinct pattern of anti-tumor activity.

D. Discussion.

[00090] The results of these experiments, obtained using a variety of biological and analytical tools, demonstrate that bendamustine possesses a distinct mechanism of action

when compared to other clinically used compounds that share the same "nitrogen mustard" active moiety, such as cyclophosphamide and chlorambucil.

[00091] One of the tools employed in this study was a pharmacogenomic approach, which allows the simultaneous analysis and monitoring of expression levels of thousands of fully characterized genes upon incubation of target cell lines with a selected drug, has been successfully used to elucidate the mechanism of action of other anticancer drugs. Its major advantage was the generation of unbiased information that led to the identification of a distinct mechanism of action for bendamustine, differentiating it from other DNA-alkylating agents.

[00092] With this approach, a strong classical p53-dependent stress-response "signature" was detected for bendamustine, and present, but at a greatly reduced intensity, in phosphoramide mustard- and chlorambucil-treated cells. Q-PCR analysis confirmed the gene-array analysis, validating the up-regulation of genes containing p53-responsive elements, such as p21 (Waf/Cip1) and NOXA. As an inhibitor for cyclin-dependent kinases, particularly those that function during the G_1 phase of the cell cycle, *p21/Waf1/Cip1* is believed to mediate, at least in part, p53-induced G_1 arrest. The mechanisms leading to p53-induced cell cycle arrest and apoptosis have been extensively investigated and reported. Noxa encodes a Bcl-2 homology 3 (BH3)-only member of the Bcl-2 family of proteins. NOXA was shown to be a target of p53-mediated transactivation and to function as a mediator of p53-dependent apoptosis through mitochondrial dysfunction. Mouse embryonic fibroblasts deficient in Noxa showed notable resistance to oncogene-dependent apoptosis in response to DNA damage.

[00093] Activation of the p53 pro-apoptotic pathway was then confirmed by immunoblot analysis, with the detection of phosphorylated p53 (Ser15), as well as with the up-regulation of Bax. Although other nitrogen mustards have been previously reported to induce a p53-mediated stress response, bendamustine provides a stronger and more rapidly induced signal when compared to equitoxic doses of the cyclophosphamide metabolite (PM) or chlorambucil. Bendamustine was also found to induce a rapid and extensive cleavage of PARP, an enzyme that catalyzes poly(ADP-ribosylation) of a variety of proteins. Although bendamustine induces PARP cleavage, the difference

between the ability of the three drugs to cause PARP cleavage in SU-DHL-1 cells was striking. This rapid induction of PARP cleavage may play a critical role in the mechanism of action of bendamustine, given the importance of PARP for DNA repair mechanisms. Indeed, in response to DNA damage, cells initially activate PARP, resulting in an increase of the accessibility of DNA to DNA repair enzymes and transcription factors. In addition, PARP has been implicated in initiating cell death by either apoptosis or necrosis.

[00094] Another major difference that emerged from the pharmacogenomic profiling of bendamustine and the other tested nitrogen mustards was the effect on expression levels of polo-like kinase 1 (PLK-1), Aurora kinases (A and B), and Cyclin B1. The mitotic checkpoint kinases PLK-1 and Aurora are involved in many aspects of cell cycle regulation, such as activation and inactivation of CDK/cyclin complexes, centrosome assembly and maturation, and activation of the anaphase-promoting complex (APC) during the metaphase-anaphase transition, and cytokinesis. Interestingly, when these checkpoint regulators are inhibited using siRNA or using targeted small molecules, potentiation of the effect of DNA-damaging drugs is observed, together with the appearance of mitotic catastrophe. Mitotic catastrophe is a form of cell death that occurs during metaphase and is morphologically distinct from apoptosis. Mitotic catastrophe can occur in absence of functional p53 or in cells where conventional caspase-dependent apoptosis is suppressed. For this reason, initiation of mitotic catastrophe is an appealing mechanism of tumor cell death, since it may also function in tumor cells that have been selected by several rounds of chemotherapy using conventional chemotherapeutic drugs. The extensive and durable DNA-damage elicited by bendamustine and concomitant inhibition of M-phase-specific checkpoints by bendamustine may trigger mitotic catastrophe in the treated cells. This may explain the clinically documented activity of bendamustine in patients refractory to cyclophosphamide and chlorambucil-containing regimens.

[00095] Efficient DNA-repair mechanisms have been demonstrated to play a critical role in the mechanism of action of DNA-alkylating drugs. Activation of discrete DNA-repair mechanisms may also confer a distinct profile of activity to drugs that share similar chemical features. The pharmacogenomic analysis described herein identified DNA-

repair genes differentially regulated by bendamustine compared to phosphoramide mustard and chlorambucil. One such gene, exonuclease 1 (Exo1), is a 5'-3' exonuclease that interacts with MutS and MutL homologs and has been implicated in the excision step of DNA mismatch repair and in the processing and repair of double-strand breaks. Exo1 has been involved in somatic hypermutation and class-switch recombination and is therefore very important in B cell function and the generation of antibodies.

[00096] To investigate further the differences in the repair mechanisms between bendamustine, cyclophosphamide, and chlorambucil, functional assays were performed. Two major mechanisms were investigated: the DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT); and the apurinic/apyrimidinc endonuclease Ape-1. AGT, a ubiquitous enzyme, removes the O^6-alkylguanine DNA adduct caused by several alkylating agents, including nitrosureas and triazenes. Clinical evidence suggests that brain tumors that express high levels of AGT, and may thus be more resistant to some DNA-alkylators such as temozolomide. The nucleoside O^6-benzylguanine (O^6-BG) provides a means to effectively inactivate the AGT protein. In some cell lines, benzylguanine clearly enhanced the toxicity of the activated from of cyclophosphamide. As shown here, the cytotoxic potency of cyclophosphamide, but not bendamustine, was enhanced by adding O^6-benzylguanine, indicating that bendamustine does not induce O^6-alkylguanine DNA adducts which can be repaired by AGT.

[00097] Ape-1/Ref-1 is an apurinic/apyrimidinic endonuclease that plays a critical role in the base excision repair (BER) pathway. BER is activated by damage induced by a variety of DNA-damaging drugs, including DNA alkylators and DNA-methylating agents, such as temozolomide. The role of Ape-1 was tested using the compound methoxyamine (MX), a specific inhibitor of its enzymatic activity. The cytotoxic activity of bendamustine was enhanced by the inhibition of Ape-1 by MX, indicating a role for BER. No changes were observed using the cyclophosphamide metabolite, underlying a major difference between the DNA-repair mechanisms activated by these drugs.

[00098] The NCI Human Tumor 60 Cell line *In Vitro* Screen is useful in comparing relative potency of potential anti-neoplastic agents with other known therapeutic agents. It has also been demonstrated in many cases that when pairs of compounds are found to

have a high correlation coefficient between their screening results using the panel, as evaluated by the COMPARE statistical analysis program, the agents often have similar mechanisms of action. The high correlation observed for the nitrogen mustards melphalan, chlorambucil, and cyclophosphamide are all with known alkylating agents, confirming the ability of the COMPARE analysis to find common mechanisms of action. Out of the six top matches with bendamustine, only the methylating agent DTIC (dacarbazine) showed approximately an 80% correlative agreement (r value). These results reveal that bendamustine displays a distinct mechanism of action in relationship to other known alkylating agents.

[00099] Based on the results presented in this example, the deduced mechanism of action of bendamustine is illustrated in Figure 5. Bendamustine can efficiently enter tumor cells and induce prolonged and extensive DNA alkylation and fragmentation, probably due to the high chemical stability of the aziridinium transition state ring conferred by bendamustine's benzimidazole ring system. Bendamustine treatment results in the initiation of three main signaling pathways: 1) activation of the "canonical" p53-dependent stress pathway, resulting in strong activation of intrinsic apoptosis, which is mediated by pro-apoptotic BCL-2 family members such as NOXA and Bax; 2) activation of DNA repair mechanisms, such as the base-excision repair machinery, that are not activated by other nitrogen mustards frequently used in NHL or CLL patients; and 3) inhibition of several mitotic checkpoints, such as the kinases PLK-1 and Aurora A and B. The concomitant induction of DNA damage and inhibition of mitotic checkpoints may not allow the tumor cells exposed to bendamustine to efficiently repair the DNA damage before undergoing mitosis. Cells entering mitosis with extensively damaged DNA, or cells that cannot proceed to the "conventional" p53-dependent apoptosis, will undergo death by mitotic catastrophe. This alternative programmed cell death pathway, together with the strong activation of traditional apoptosis, indicates why bendamustine is effective in drug-resistant cells *in vitro*, as well as in patients carrying chemo-refractory tumors. Consequently, bendamustine treatment will represent an important addition to the armamentarium of the clinician for the treatment of patients with indolent non-Hodgkin's lymphoma and other hematologic cancers, among others.

Example 2

Bendamustine Activity in NHL Cells Induces the Mitotic Catastrophe Death Pathway

[00100] As described in Example 1 above, bendamustine is an alkylating agent with a distinct mechanism of action, and is undergoing clinical trials in NHL and CLL patients refractory to traditional DNA-damaging agents. Bendamustine induces unique changes in gene expression in NHL cells and displays a lack of cross-resistance with other 2-chloroethylamine alkylating agents. Quantitative PCR analysis confirmed that the G 2/M checkpoint regulators Polo-like kinase 1 (PLK-1) and Aurora A kinase (AurkA) are down-regulated in the NHL cell line SU-DHL-1 after 8 hours of exposure to clinically relevant concentrations of the drug. No changes in these same genes were observed when cells were exposed to equi-toxic doses of chlorambucil or an active metabolite of cyclophosphamide.

[00101] The ability of bendamustine to induce cytotoxicity in cells unable to undergo classical caspase-mediated apoptosis was investigated. Multi-drug resistant MCF-7/ADR cells and p53 deficient RKO-E6 colon adenocarcinoma cells were exposed for two or three days to either 50 μM bendamustine alone or 50 μM bendamustine and 20 μM pan-caspase inhibitor zVAD-fmk. Although zVAD-fmk was able to inhibit bendamustine-induced increases in Annexin-V-positive cells, microscopic analysis of nuclear morphology using the DNA stain DAPI in cells treated with either bendamustine alone or in combination with zVAD-fmk showed increased incidence of micronucleation. Multi/micro-nucleation and abnormal chromatin condensation are both hallmarks of mitotic catastrophe and have been observed in tumor cells exposed to microtubule-binding drugs such as the vinca alkaloids and taxanes. Activation of mitotic catastrophe may amplify the cytotoxicity of bendamustine and its activity in tumor cells where classical apoptotic pathways were inhibited.

Example 3

Fast-Acting Bendamustine Activates Potent Apoptosis and Cell Death in Lymphoma and Leukemia Cells

[00102] As described above, the alkylating agent bendamustine exhibits chemotherapeutic activity against drug-resistant cancers, among others, and possesses a

unique mechanism of action when compared to other related anti-tumor agents. As is the case with other anti-neoplastic nitrogen mustards, bendamustine has a relatively short serum half-life in humans (approximately 2 hours), and is administered clinically by bolus intravenous infusion. The purpose of the work reported in this example was to assess the capacity of bendamustine to induce cell death and apoptosis when exposed for brief periods to cancer cells in vitro. The activity of bendamustine in such experimental models was compared to other structurally-related agents. The results obtained indicate that bendamustine exerts maximal anti-tumor activity after a brief (30 minute) exposure to cells. To obtain these results, the NHL cell line SU-DHL-1 was exposed to 50 μM bendamustine for brief periods ranging from 30 minutes to 4 hours, washed, and allowed to recover for 20 hours in drug-free media. Cells exposed to bendamustine for as few as 30 minutes displayed extensive loss of viability as measured by a variety of biological assays, including measurement of intracellular ATP and release of adenylate kinase into the supernatant at 48 and 72 hours post drug exposure (Figures 6 and 7). In contrast, cells treated with other members of this class of alkylating agents (here, chlorambucil, melphalan, and the cyclophosphamide metabolite phosphoramide mustard; data shown for chlorambucil and phosphoramide mustard) experienced minimal loss of viability when exposed to these agents for 30, 60, and 120 minutes. These other nitrogen mustards required a much longer exposure period (at least 4 hours) to induce a cytotoxic effect comparable to bendamustine in these assays. These findings were confirmed using an MTT-based assay in which bendamustine had a similar IC_{50} in SU-DHL-1 and HL-60 cells at 72 hours following exposure to drug for 30 minutes, 4 hours, or 72 hours. By comparison, chlorambucil, melphalan, and phosphoramide mustard exhibited 10- to 20-fold higher IC_{50}s when incubated with these same cell lines for 30 minutes compared to continuous (72 hour) exposure.

[00103] Intracellular ATP levels were assayed using the following luciferase-based ATP assay. 10 mL of CellTiter-Glo® reagent was mixed with the appropriate amount of CellTiter-Glo substrate (per the manufacturer's instructions; Promega Corp.), and the mixture was allowed to equilibrate for ten minutes. 100 μL of this solution was then combined with 100 μL of cell-containing culture medium, and the mixture was allowed to incubate for ten minutes. Luminescence was detected using a CCD-based plate reader.

[00104] An adenylate kinase (ADK) assay was selected because as a cell membrane of a treated cell looses integrity, ADK is released into the culture medium (or, in the context of a biological sample, into the extracellular space, blood, etc. To perform the ADK assays in 96-well plates, in each test well 20 μL of supernatant from an aliquot of culture medium briefly centrifuged to pellet cells was mixed with 100 μL of the ADK reagent (20 mL Cambrex ToxiLight reagent plus the appropriate amount of Cambrex ToxiLight substrate per the manufacturer's instructions; Cambrex Corp., NJ) that had just been prepared and allowed to equilibrate for 15 min. The reaction mixture was then incubated for two minutes to allow the kinase reaction to occur. Luminescence from the samples was then read immediately in a plate reader.

[00105] Cell viability was also assessed by mixing 20 μL aliquots of the particular cell culture with180 μL Guava ViaCount Reagent (Guava Technologies, Hayward, CA), diluted 1:10 dilution just prior to use. Each mixture was then incubated for five minutes. A ViaCount cell counting assay was then performed using a Guava PC Flow Cytometer, which allows the number of live cells per 1,000 total cells to be determined. Live versus dead cells were distinguished using the dye 7AAD, which can diffuse into dead or dying cells through their deteriorating cell membranes.

[00106] As described in Example 1, rapid induction of PARP (poly [ADP-ribose] polymerase) cleavage is a hallmark of bendamustine-induced cell death in NHL cells. Maximal PARP cleavage was observed in SU-DHL-1 cells exposed for as few as 30 minutes to 50 μM SDX-105 and, following drug washout, further incubated for 8 hours. No PARP cleavage was observed in cells treated in a similar manner for 30 minutes with 40 μM phosphoramide mustard, 4 μM chlorambucil, or 2 μM melphalan. The concentrations of each drug used represents equitoxic concentrations when compared to 50 μM bendamustine as measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-based assay after a period of 72 hours of drug exposure.

[00107] MTT assays were performed to titrate doses of the various drugs to determine the effective concentrations required to kill 50% of the treated cells. These assays were performed in 96-well plates. Concentrations ranged up to a maximum of 500 μM. In each assay, controls included untreated cells and kill control. For plates used to test cells

in the "wash-out" experiments, plates were centrifuged for 5 minutes to pellet cells. Medium was then removed, the cell pellets were rinsed once with 1X PBS, and then resuspended in fresh medium. Cells were incubated with the particular dosage of drug for 3 days at 37°C in an atmosphere containing 5.0% CO_2. After three days, 10 µL of MTT (12 mM) Reagent (5 mg/mL MTT (Promega) dissolved in fresh culture medium, filter-sterilized, stored at 2-8°C) was added to each well. Following a four-hour incubation, 100 µL of lysis buffer (20% SDS, 0.015M HCl) was added to each well. The mixtures were placed overnight at 37°C in an atmosphere containing 5.0% CO_2 to allow cells to lyse. The next morning, the degree of cell lysis was determined using a multiwell scanning spectrophotometer reading at 595 nm.

[00108] Comparable results were obtained by treating the human cancer cell line HL-60 with 100 µM bendamustine or 12 µM chlorambucil. Periods of exposure to the drug were 30 minutes, 1 hour, or 2.5 hours, wherein the culture medium containing drug was removed after the noted time period and replaced with fresh medium containing no drug.

[00108] Taken together, these results illustrate the unique capacity of bendamustine to activate an irreversible cell death pathway following even brief incubation with cancer cells, which distinguishes it from other related alkylating agents. Such fast-acting cytotoxicity confirms bendamustine's potent clinical activity, and indicates that it will be useful for treating various cancers, including those that are refractory to conventional chemotherapy.

Example 4
Clinical Data

[00109] This study evaluated the efficacy and toxicity of bendamustine in patients with NHL who have relapsed or are refractory to previous chemotherapy regimens. Patients refractory to rituximab had disease progression within 6 months of treatment.

[00110] **Methods:** This Phase II multicenter trial enrolled patients with relapsed indolent or transformed rituximab-refractory B-cell NHL from 17 sites in the US and Canada. Indolent histologic phenotype was seen in 84% of patients, while 16% had transformed disease. Median age of patients was 63 years (range: 38-84) and 88% had

Stage III/IV disease. Patients received bendamustine 120 mg/m^2 IV over 30-60 minutes, days 1 and 2, every 21 days for up to 6 cycles. Response was measured using the International Working Group criteria.

[00111] **Results:** The intent-to-treat (ITT) population consisted of 75 heavily pretreated patients with a median of 2 prior chemotherapies. The overall objective response rate (ORR) in the ITT population was 74%; 25% had a complete response, 49% had a partial response, 12% had stable disease, and 14% had disease progression. Of 15 patients who were refractory to prior alkylator treatment (patients who progressed after at least one prior alkylator-containing therapy), 10 (67%) experienced an objective response to bendamustine. The median duration of response was 6.6 months for all patients, 9.3 months for indolent patients, and 2.4 months for transformed patients.

[00112] **Conclusions:** Single-agent bendamustine produced durable objective responses with acceptable toxicity, despite unfavorable prognostic features, in heavily pretreated rituximab-refractory indolent and transformed NHL patients, including those patients who were also refractory to prior alkylator treatment.

* * *

[00113] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the appended claims.

[00114] All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the spirit and scope of the invention as defined by the appended claims.

[00115] All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents, patent applications, and publications, including those to which priority or another benefit is claimed, are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[00116] The invention illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

What it claimed is:

1. A method of treating cancer, comprising determining that a patient has a cancer characterized by death-resistant cancer cells, followed by administering to the patient a therapeutically effective amount of bendamustine.

2. A method according to claim 1, wherein the cancer is resistant to apoptosis.

3. A method according to claim 1, wherein the death-resistant cancer cells comprise a p53 deficiency.

4. A method according to claim 1, wherein the cancer is selected from the group consisting of non-Hodgkin's lymphoma and chronic lymphocytic leukemia.

5. A method of treating a cancer patient comprising administering bendamustine, waiting for at least about 30 minutes but not longer than about 48 hours, and administering another chemotherapeutic agent or agents that are more active when cells are in the S-phase of the cell cycle.

6. A method according to claim 5, where the chemotherapeutic agent is given about 30 minutes to about 36 hours after the administration of bendamustine.

7. A method according to claim 5, wherein the chemotherapeutic agent is given about 30 minutes to 24 hours after administration of bendamustine.

8. A method according to claim 5, wherein the chemotherapeutic agent is given about 30 minutes to twelve hours after administration of bendamustine.

9. A method according to claim 5, wherein the chemotherapeutic is given about 30 minutes to six hours after administration of bendamustine.

10. A method according to claim 5, wherein the patient has a cancer characterized by death-resistant cancer cells.

11. A method of assessing efficacy of a cancer treatment, comprising determining whether a level of a marker of cancer cell death in a biological sample taken from a cancer patient correlates with treatment efficacy, wherein the determination is made during or following administration of a therapeutic regimen intended to treat the cancer, wherein the therapeutic regimen comprises administration of an alkylating agent.

12. A method according to claim 11, wherein the alkylating agent is bendamustine.

13. A method of assessing efficacy of a cancer treatment, comprising:

a. treating a cancer with a therapeutically effective amount of bendamustine;

b. waiting a sufficient period of time to allow bendamustine to exert a desired therapeutic effect; and

c. determining a level of a marker of cancer cell death to determine if treatment with bendamustine was efficacious.

14. A method of reducing toxicity associated with a cancer therapy that comprises administering a plurality of doses of bendamustine to a cancer patient, comprising administering a first dose of a therapeutically effective amount of bendamustine to the patient, which first bendamustine dose results in an undesired toxicity, and delaying administration of a second dose of a therapeutically effective amount of bendamustine to the patient until after the undesired toxicity begins to subside.

15. A method of assessing whether a patient's cancer is susceptible to bendamustine, comprising:

a. exposing at least a portion of a cell sample from cancerous tissue of a patient to bendamustine under growth conditions which, in the absence of a compound that is toxic to cancer cells, allows the cancer cells to proliferate; and

b. assessing whether the cancer is susceptible to bendamustine exposure.

16. A method according to claim 15 wherein the assessment of whether the cancer is susceptible to bendamustine exposure comprises determining a level of a marker of cancer cell death.

17. A method according to claim 16 wherein the marker of cancer cell death is selected from the group consisting of a level of adenylate kinase activity, , viability of the cells, and a level of a PARP cleavage product.

18. A method of treating cancer, comprising determining that a patient has a cancer characterized as resistant to one or more alkylating agents and an anti-CD20 agent, comprising administering to said patient a therapeutically effective amount of bendamustine.

19. A method according to claim 18 wherein the cancer is Non-Hodgkin's lymphoma.

20. A method according to claim 18, wherein the anti-CD20 agent is rituximab.

21. A method of doing business in connection with the treatment of a cancer characterized by death-resistant cancer cells, comprising promoting bendamustine for use to treat a cancer characterized by death-resistant cancer cells.

22. A method according to claim 21 wherein the cancer is a cancer refractory to a treatment comprises a combination of one or more alkylating agents and an anti-CD20 agent.

23. A method of doing business in connection with the treatment of a refractory cancer, comprising promoting bendamustine use to treat a refractory cancer.

24. A method according to claim 23 wherein the refractory cancer is a cancer refractory to treatment with a combination of one or more alkylating agents and an anti-CD20 agent.

25. Use of bendamustine in the manufacture of a medicament for treatment of a cancer characterized by death-resistant cancer cells.

26. Use of bendamustine in the manufacture of a medicament for treatment of a refractory cancer.

27. A use according to claim 26 wherein the refractory cancer is a cancer refractory to treatment with a combination of one or more alkylating agents and an anti-CD20 agent.

Figure 1A Bendamustine gene expression profile clustering: top 100 modulated genes

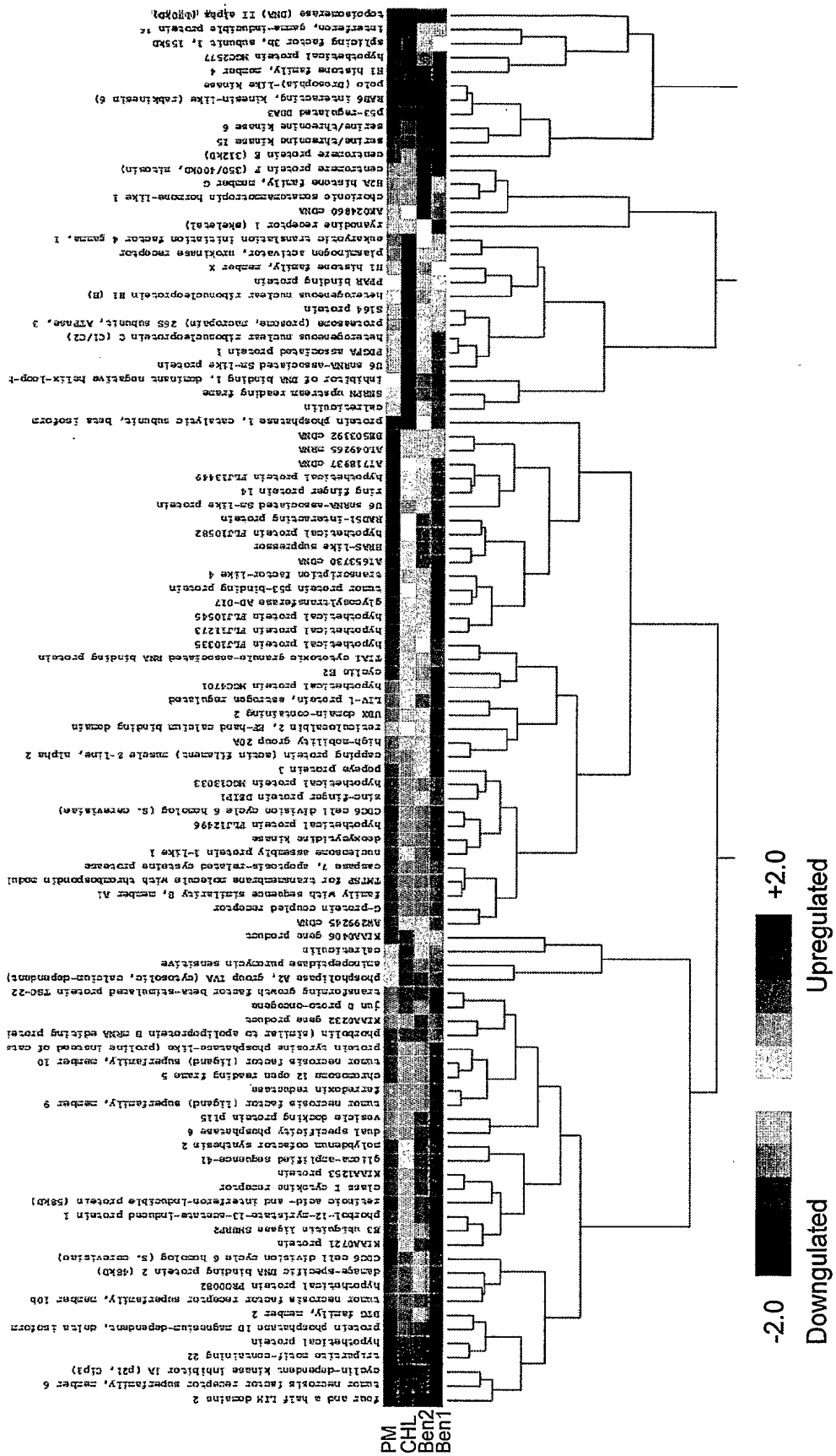
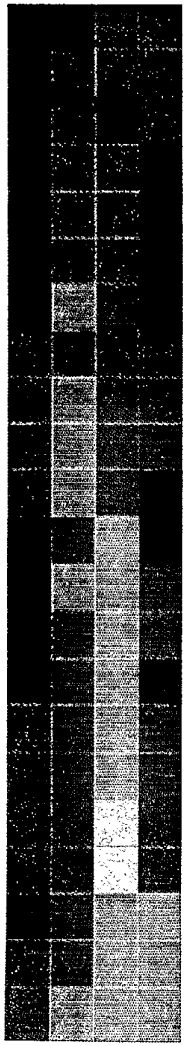


Figure 1B: Top genes up-regulated by the three drugs tested



-2.0 Downgulated +2.0 Upregulated

NM_001450	four and a half LIM domains 2
AA164751	tumor necrosis factor receptor superfamily, member 6
NM_000389	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
AA083478	tripartite motif-containing 22
NM_016617	hypothetical protein
NM_003620	protein phosphatase 1D magnesium-dependent, delta isoform
NM_006763	BTG family, member 2
AF016266	tumor necrosis factor receptor superfamily, member 10b
NM_018590	hypothetical protein PRO0082
NM_000107	damage-specific DNA binding protein 2 (48kD)
NM_001254	CDC6 cell division cycle 6 homolog (S. cerevisiae)
AL050331	KIAA0721 protein
AY014180	E3 ubiquitin ligase SMURF2
NM_021127	phorbol-12-myristate-13-acetate-induced protein 1
N47725	retinoic acid- and interferon-inducible protein (58kD)
AI983115	class I cytokine receptor
AF164794	KIAA1253 protein
NM_006530	glioma-amplified sequence-41
NM_004531	molybdenum cofactor synthesis 2
BC003143	dual specificity phosphatase 6
BE875592	vesicle docking protein p115
NM_003811	tumor necrosis factor (ligand) superfamily, member 9

Figure 2A: Q-PCR validation of selected p53-dependent and pro-apoptotic genes

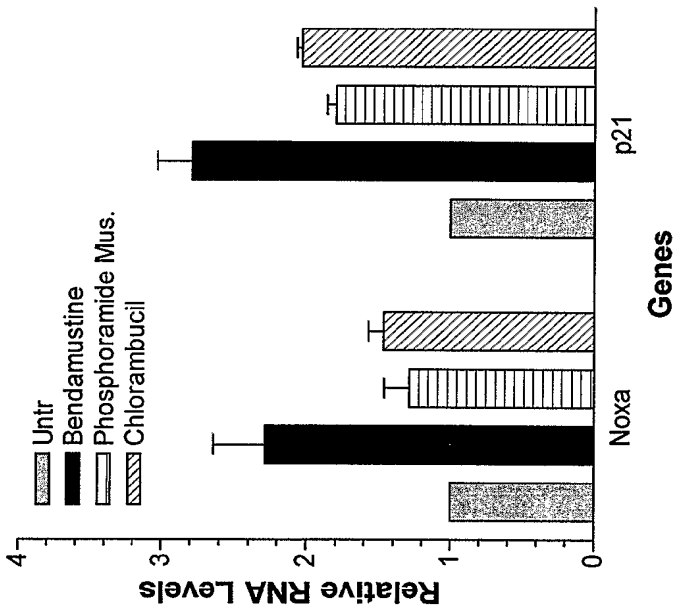


Figure 2B: Q-PCR validation of selected mitotic checkpoint genes

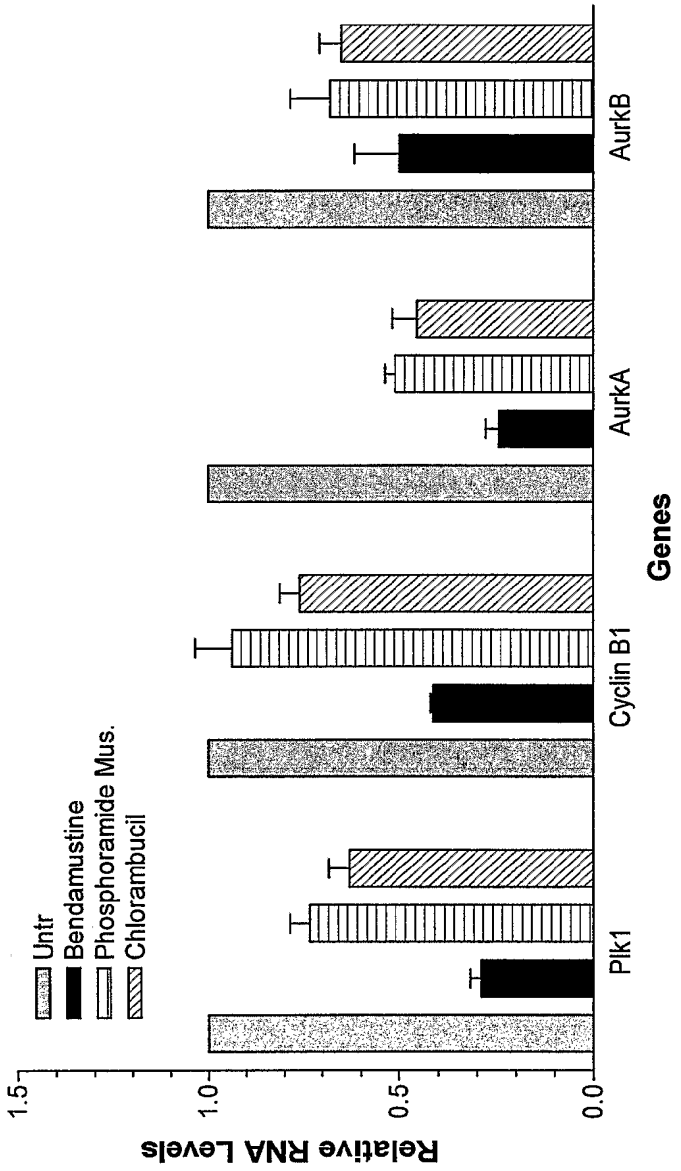


Figure 2C: Q-PCR validation of selected DNA-repair genes

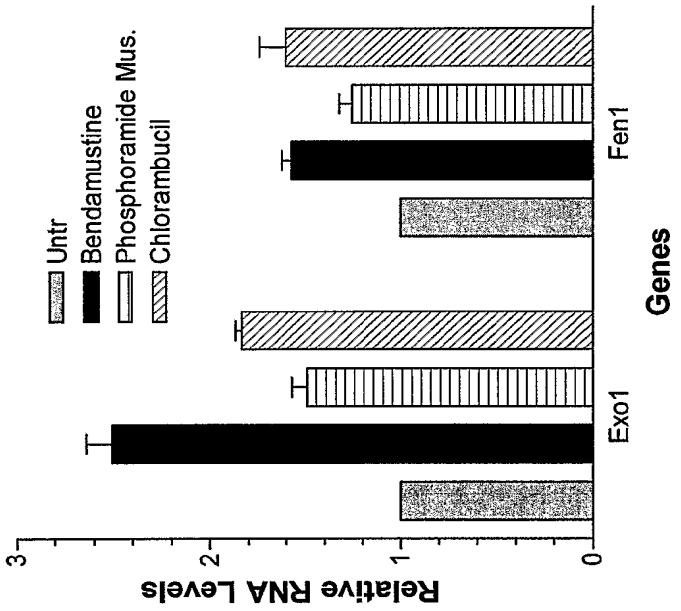


Figure 3

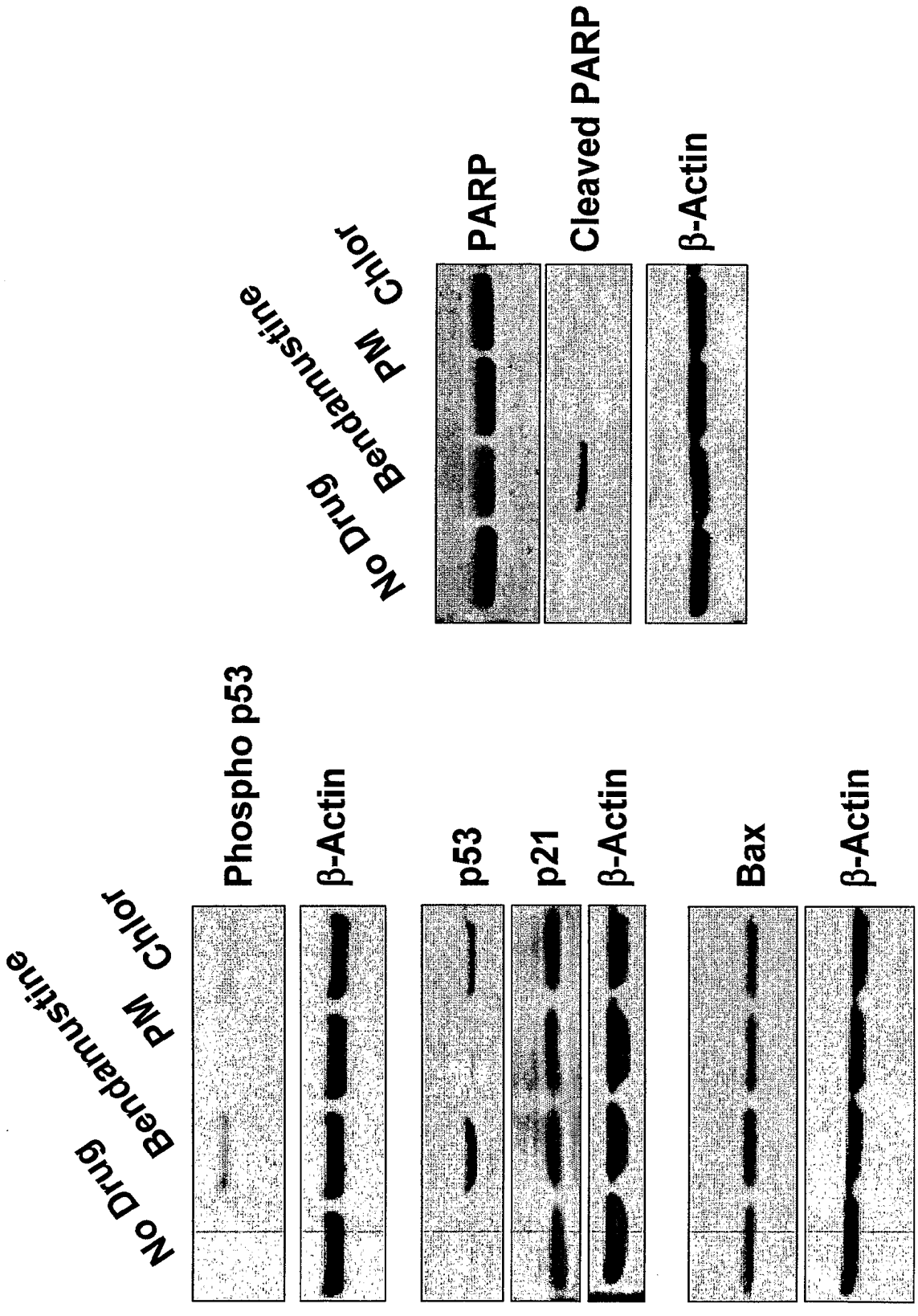

No Drug
Bendamnustine
PM Chlor
Phospho p53
β-Actin
p53
p21
β-Actin
Bax
β-Actin
No Drug
Bendamnustine
PM Chlor
PARP
Cleaved PARP
β-Actin

Figure 4A: Effect of MX (Ape-1 inhibitor) on bendamustine activity vs. cyclophosphamide activity

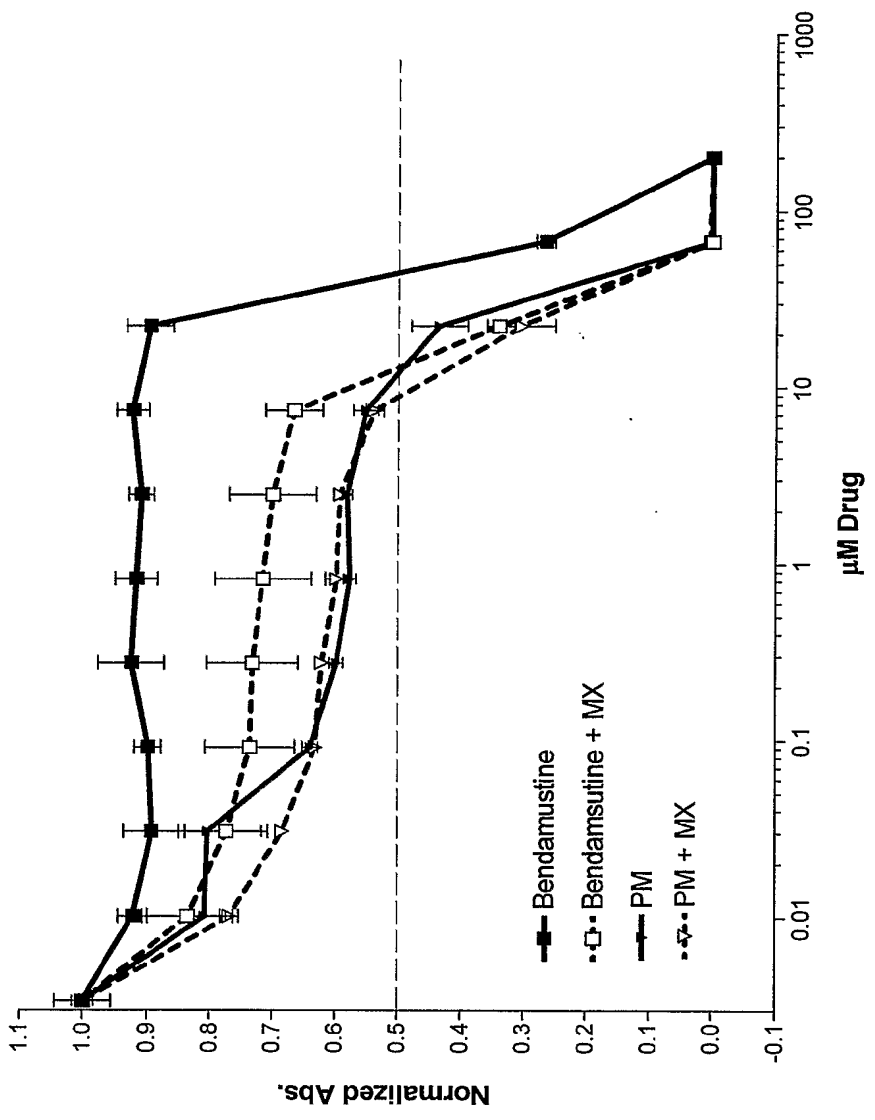


Figure 4B: Effect of O6-Benzylguanine on bendamustine, cyclophosphamide, and carmustine

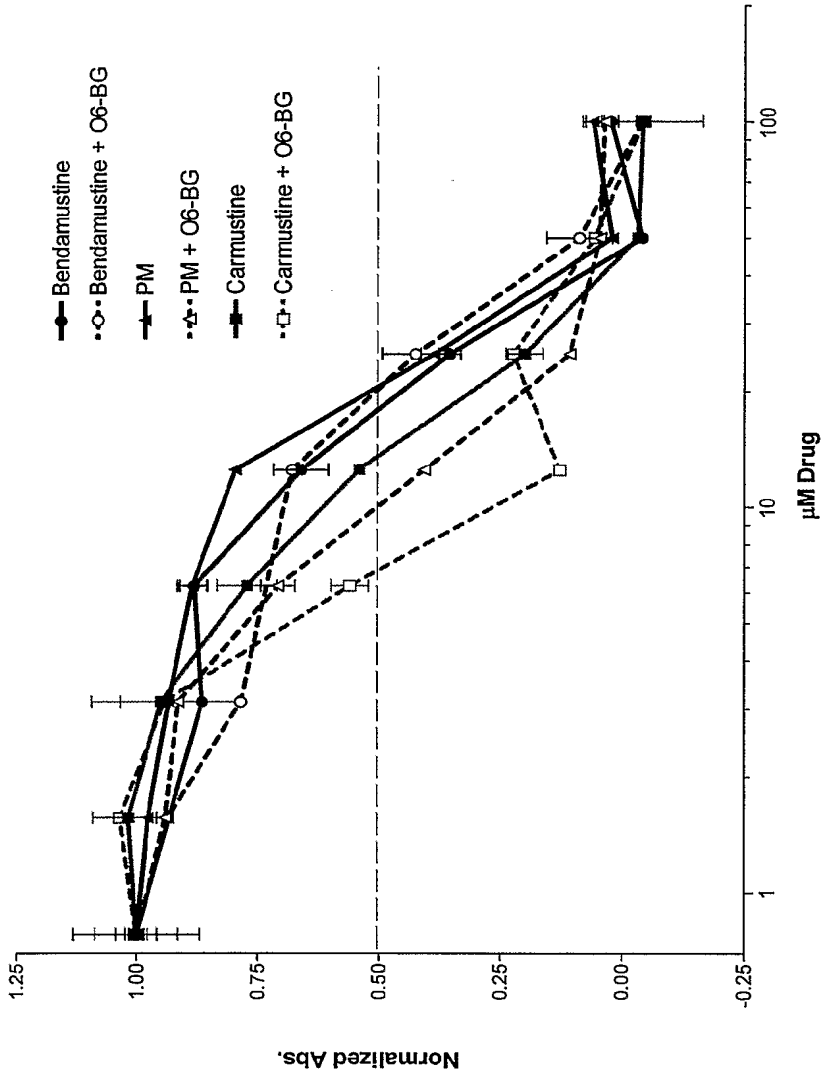


Figure 5

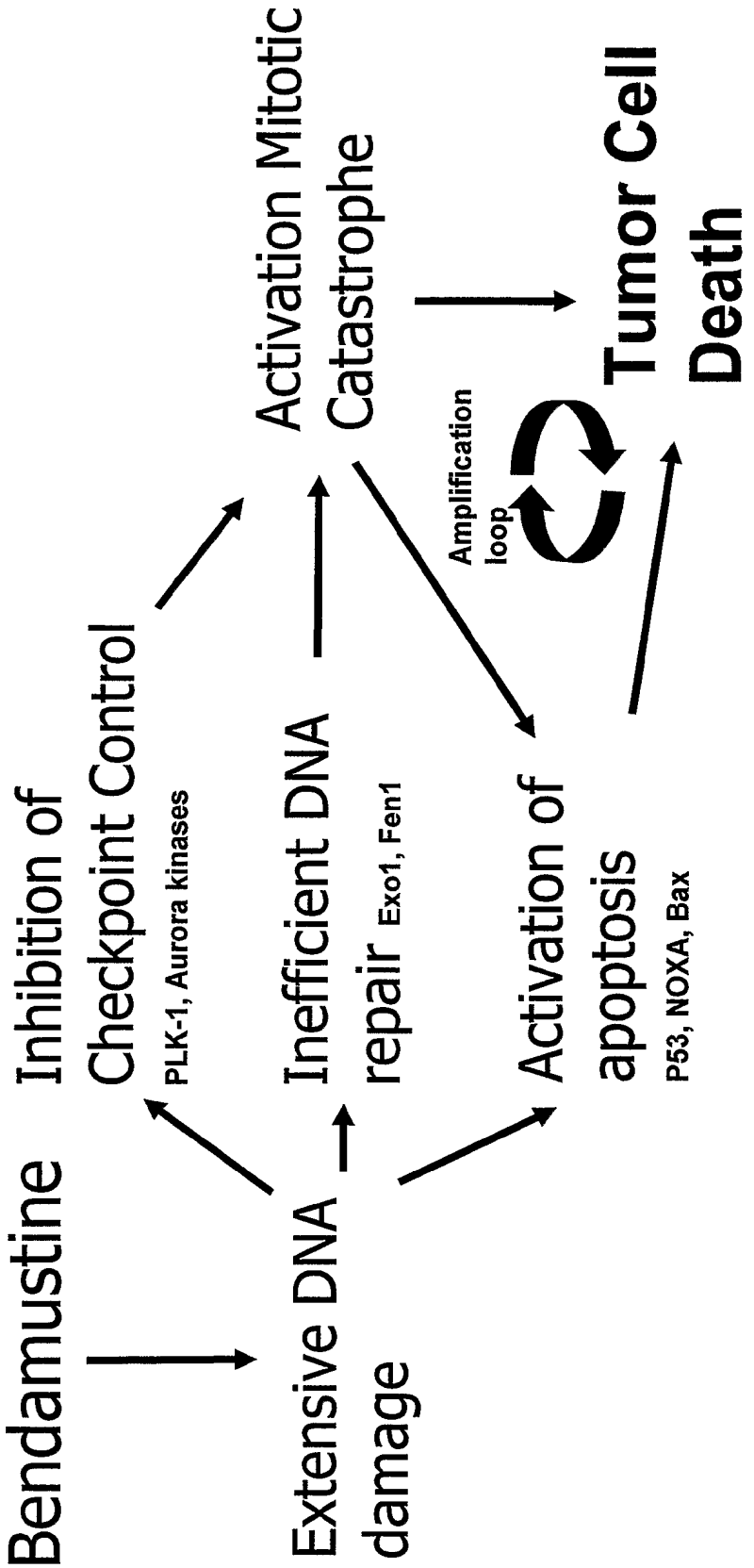

Bendamustine
Extensive DNA damage
Inhibition of Checkpoint Control
PLK-1, Aurora kinases
Inefficient DNA repair Exo1, Fen1
Activation of apoptosis
P53, NOXA, Bax
Activation Mitotic Catastrophe
Amplification loop
Tumor Cell Death

Figure 6

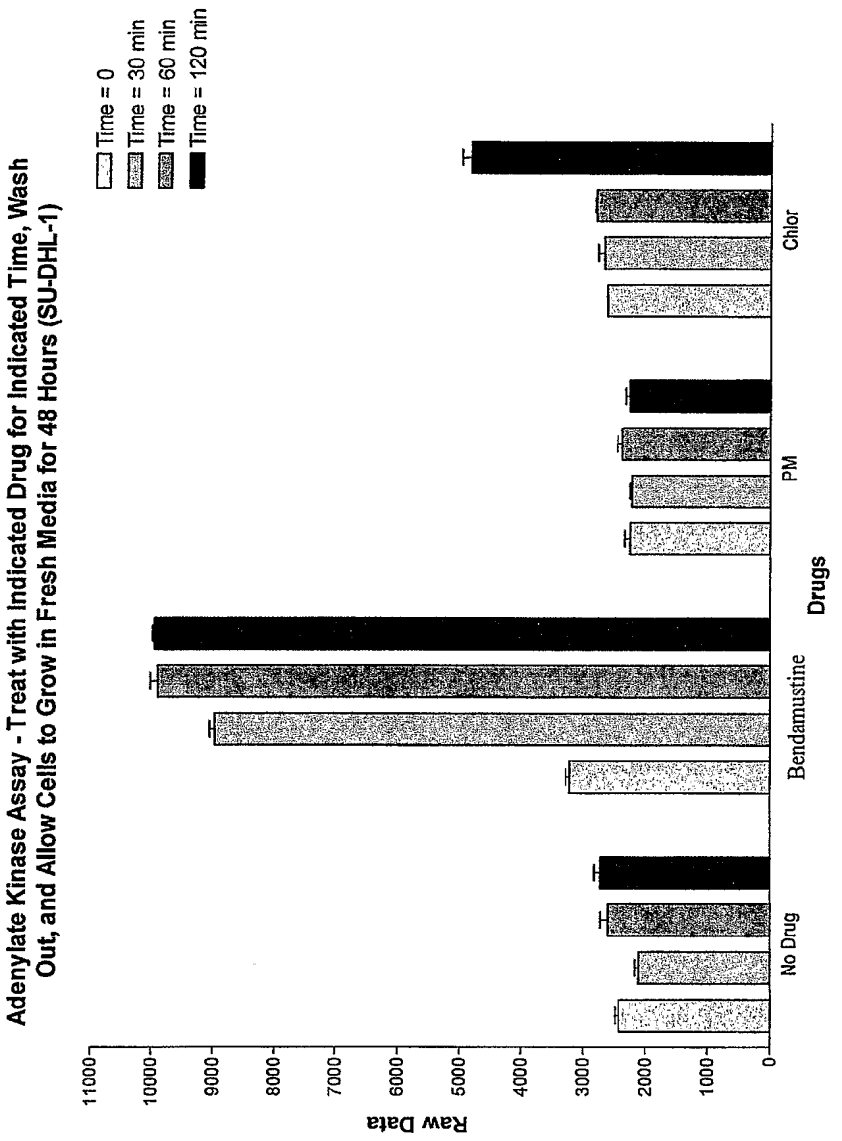

Adenylate Kinase Assay - Treat with Indicated Drug for Indicated Time, Wash Out, and Allow Cells to Grow in Fresh Media for 48 Hours (SU-DHL-1)
Time = 0
Time = 30 min
Time = 60 min
Time = 120 min
Raw Data
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
No Drug
Bendamustine
PM
Chlor
Drugs

Figure 7

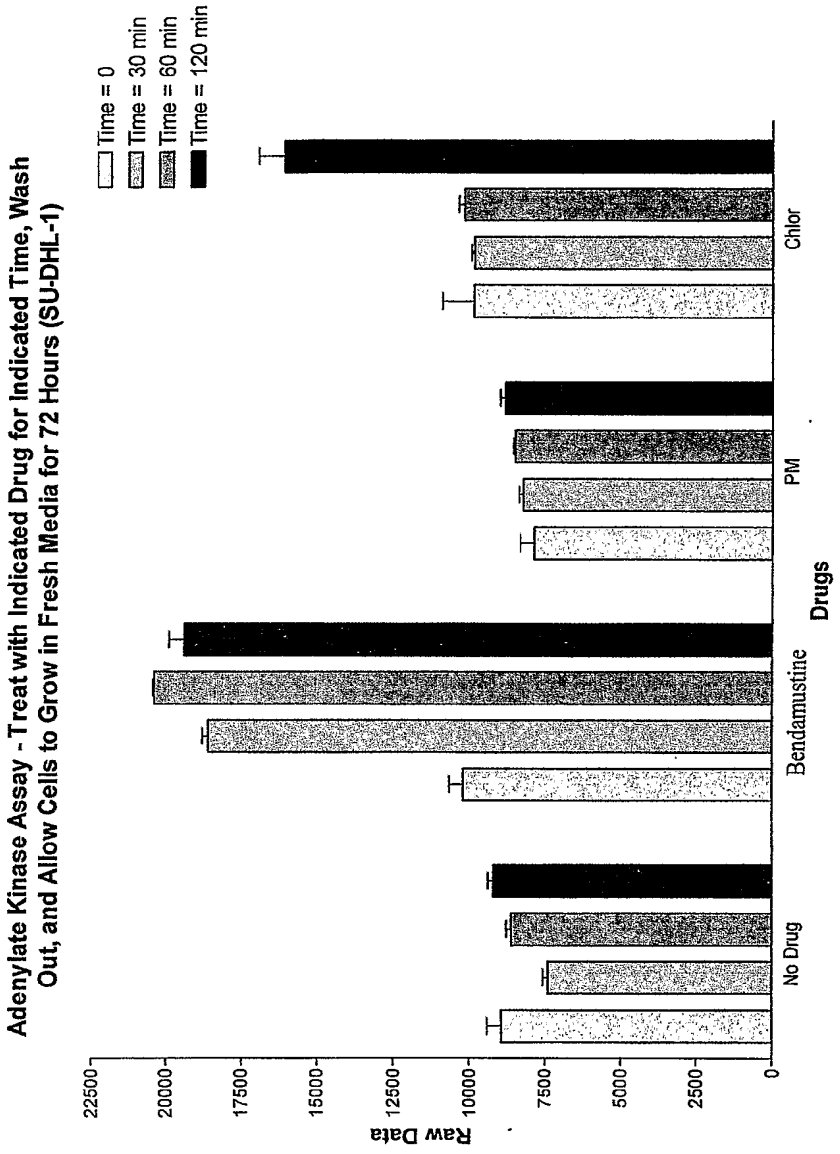

Adenylate Kinase Assay - Treat with Indicated Drug for Indicated Time, Wash Out, and Allow Cells to Grow in Fresh Media for 72 Hours (SU-DHL-1)
Time = 0
Time = 30 min
Time = 60 min
Time = 120 min
Raw Data
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
No Drug
Bendamustine
PM
Chlor
Drugs

Table 1: IC50s of Bendamustine, PM, Chlorambucil, in SU-DHL-1 cells

Cell Line	Drug	Ave IC50 (μM)	STDV	Ave IC90 (μM)	STDV
SU-DHL-1	**Bendamustine**	33.2	10.6	56.3	16.1
	Chlorambucil	3.4	1.1	6.2	1.3
	Phosporamide Mustard	21.3	7.6	33.0	6.2

Table 2: Results from GO-clustering analysis from bendamustine-indced gene changes in SU-DHL-1 cells (see Figure 2C)

Functional Groups	GO number	GO Description: Biological Process	P value
DNA-damage, stress response, apoptosis	**6974**	**Response to DNA damage stress**	**0.00001**
	6950	Response to stress	0.0003
	16265	Death	0.0482
DNA metabolism, DNA repair, transcription	**6259**	**DNA metabolism**	**0.00003**
	6139	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	0.0004
	6357	Regulation of transcription from Pol II promoter	0.0003
	6366	Transcription from Pol II promoter	0.0068
Cell proliferation, cell cycle, mitotic checkpoint	**8283**	**Cell proliferation**	**0.00001**
	8151	Cell growth and/or maintenance	0.0041
	6275	Regulation of DNA replication	0.0101
	278	Mitotic cell cycle	0.0334
	79	Regulation of CDK activity	0.0192
	7078	Mitotic metaphase plate congression	0.0470
	50790	Regulation of enzyme activity	0.0363
Cell regulation	**50789**	**Regulation of biological process**	**0.00004**
	50794	Regulation of cellular process	0.0035
	9987	Cellular process	0.0379

GO clustering analysis performed as described in Methods section. The table represents the terms identified from the Gene Ontology database (http://www.geneontology.org/) that are the most statistically-significantly modulated between untreated control and SU-DHL-1 treated with IC50 dose of bendamustine.

Table 3: Closest compounds to bendamustine by NCI COMPARE Analysis

Compound	Mechanism of Action	Correlation (PCC) GI50, TGI, or LC50
0 compounds show a PCC>0.800		
DTIC, Dacarbazine	DNA Alkylator, Methylating agent	0.792 (LC50)
TOPO1B	Topoisomerase I inhibitor	0.619 (TGI)
Daunomycin analog	Anthracycline, DNA intercalator	0.574 (TGI)
Melphalan	DNA Alkylator, Nitrogen mustard	0.550 (GI50)
YOSHI 864	DNA Alkylator	0.542 (GI50)
Ara-AC (Fazarabine)	Antimetabolite, DNA methylation inhibitor	0.524 (TGI)

Electronic Patent Application Fee Transmittal

Application Number:	11330868
Filing Date:	12-Jan-2006
Title of Invention:	BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS
First Named Inventor/Applicant Name:	Jason Edward Brittain
Filer:	Stephanie A. Barbosa/D. McCarty
Attorney Docket Number:	CP391

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
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Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for continued examination	1801	1	930	930
		Total in USD ($)		**930**

Electronic Acknowledgement Receipt

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Application Number:	11330868
International Application Number:	
Confirmation Number:	9998
Title of Invention:	BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS
First Named Inventor/Applicant Name:	Jason Edward Brittain
Customer Number:	46347
Filer:	Stephanie A. Barbosa/D. McCarty
Filer Authorized By:	Stephanie A. Barbosa
Attorney Docket Number:	CP391
Receipt Date:	15-NOV-2012
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Payment was successfully received in RAM	$930
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Deposit Account	233050
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1	Request for Continued Examination (RCE)	CEPH-4391-Request-for-Continued-Examination.PDF	697815 3651fc99edcb3775e7feeebcece8d5c39e1a459f	no	3
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	CEPH-4391-1449-SB08.PDF	118926 3294cc37639a83da6d151771c4d769cdea8247c3	no	1
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3	Non Patent Literature	CEPH-4391-WO2006065392.PDF	3615895 fc5629117211880284 3abd382a57c479043dc2b3	no	63
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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.

Substitute for 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	11/330,868
				Filing Date	January 12, 2006
				First Named Inventor	Jason Edward Brittain
				Art Unit	1617
(use as many sheets as necessary)				**Examiner Name**	Soroush, Ali
Sheet	1	of	1	**Attorney Docket Number**	CEPH-4391 / CP391

FOREIGN PATENT DOCUMENTS					
Examiner Initials	Cite No.	Foreign Patent Document Country Code- Number -Kind Code (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	T
	95	EP 0780386	06-25-1997	F. Hoffmann-La Roche AG	
	96	WO 97/08174	03-06-1997	Smithkline Beecham Corporation	

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials	Cite No.	Include name of the author, title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), Volume-issue Number(s), publisher, city and/or country where published.	T
	97	Department of Health and Human Services, Food and Drug Administration, "International Conference on Harmonisation; Guidance on Impurities: Residual Solvents," Federal Register, December 24, 1997, 62(247), 67377-67388	

Examiner Signature		**Date Considered**	

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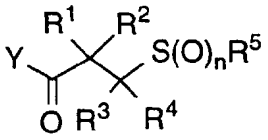
(54) Matrix metalloprotease inhibitors

(57) Compounds of the formula:



I

wherein:

n is 0, 1 or 2;

Y is hydroxy or XONH-, where X is hydrogen or lower alkyl;

R^1 is hydrogen or lower alkyl;

R^2 is hydrogen, lower alkyl, heteroalkyl, aryl, aralkyl, arylheteroalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaralkyl, heteroarylheteroalkyl, heterocyclo, heterocylo-lower alkyl, heterocyclo-lower heteroalkyl or $-NR^6R^7$, wherein:

R^6 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl, aryl, heteroaryl and heteroaralkyl;

R^7 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, $-C(O)R^8$, $-C(O)NR^8R^9$, $-SO_2NR^8R^9$, $-SO_2R^{10}$, aryloxycarbonyl, or alkoxycarbonyl; or R^6 and R^7 together with the nitrogen atom to which they are attached represent a heterocyclo group; wherein

R^8 and R^9 are independently hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl or heteroalkyl; and

R^{10} is lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heteroalkyl or heterocyclo; or

R^1 and R^2 together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group;

R^3 is hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl,

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heteroaralkyl, heteroalkyl or lower alkoxy;

R^4 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl; or

R^2 and R^3 together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or

R^3 and R^4 together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and

R^5 is lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

or pharmaceutically acceptable salts or esters thereof exhibit useful pharmacological properties, in particular for use as matrix metalloprotease inhibitors, particularly for interstitial collagenases.

Description

The present invention relates to compounds of formula I and their pharmaceutically acceptable salts and esters thereof, that inhibit matrix metalloproteases, particularly interstitial collagenases, and are therefore useful in the treatment of mammals having disease states alleviated by the inhibition of such matrix metalloproteases.

Matrix metalloproteases ("MMPs") are a family of proteases (enzymes) involved in the degradation and remodeling of connective tissues. Members of this family of endopeptidase enzymes are present in various cell types that reside in or are associated with connective tissue, such as fibroblasts, monocytes, macrophages, endothelial cells, and invasive or metastatic tumor cells. MMP expression is stimulated by growth factors and cytokines in the local tissue environment, where these enzymes act to specifically degrade protein components of the extracellular matrix, such as collagen, proteoglycans (protein core), fibronectin and laminin. These ubiquitous extracellular matrix components are present in the linings of joints, interstitial connective tissues, basement membranes, and cartilage. Excessive degradation of extracellular matrix by MMPs is implicated in the pathogenesis Of many diseases, including rheumatoid arthritis, osteoarthritis, multiple sclerosis, chronic obstructive pulmonary disease, cerebral hemorrhaging associated with stroke, periodontal disease, aberrant angiogenesis, tumor invasion and metastasis, corneal ulceration, and in complications of diabetes. MMP inhibition is, therefore, recognized as a good target for therapeutic intervention.

The MMPs share a number of properties, including zinc and calcium dependence, secretion as zymogens, and 40-50% amino acid sequence homology. The MMP family currently consists of at least eleven enzymes, and includes collagenases, stromelysins, gelatinases, matrilysin, metalloelastase, and membrane-type MMP, as discussed in greater detail below.

Interstitial collagenases catalyze the initial and rate-limiting cleavage of native collagen types I, II, and III. Collagen, the major structural protein of mammals, is an essential component of the matrix of many tissues, for example, cartilage, bone, tendon and skin. Interstitial collagenases are very specific matrix metalloproteases which cleave these collagens to give two fragments which spontaneously denature at physiological temperatures and therefore become susceptible to cleavage by less specific enzymes. Cleavage by the collagenases results in the loss of structural integrity of the target tissue, essentially an irreversible process. There are currently three known human collagenases. The first is human fibroblast-type collagenase (HFC, MMP-1, or collagenase-1) that is produced by a wide variety of cells including fibroblasts and macrophages. The second is human neutrophil-type collagenase (HNC, MMP-8, or collagenase-2) that has so far only been demonstrated to be produced by neutrophils. The most recently discovered member of this group of MMPs is human collagenase-3 (MMP-13) which was originally found in breast carcinomas, but has since shown to be produced by chondrocytes. The only collagenase known to exist in rodents is the homolog of human collagenase-3.

The gelatinases include two distinct, but highly related, enzymes: a 72-kD enzyme (gelatinase A, HFG, MMP-2) secreted by fibroblasts and a wide variety of other cell types, and a 92-kD enzyme (gelatinase B, HNG, MMP-9) released by mononuclear phagocytes, neutrophils, corneal epithelial cells, tumor cells, cytotrophoblasts and keratinocytes. These gelatinases have been shown to degrade gelatins (denatured collagens), collagen types IV (basement membrane) and V, fibronectin and insoluble elastin.

Stromelysins 1 and 2 have been shown to cleave a broad range of matrix substrates, including laminin, fibronectin, proteoglycans, and collagen types IV and IX in their non-helical domains.

Matrilysin (MMP-7, PUMP-1) has been shown to degrade a wide range of matrix substrates including proteoglycans, gelatins, fibronectin, elastin, and laminin. Its expression has been documented in mononuclear phagocytes, rat uterine explants and sporadically in tumors. Other less characterized MMPs include macrophage metalloelastase (MME, MMP-12), membrane type MMP (MMP-14), and stromelysin-3 (MMP-11).

Inhibitors of MMPs provide useful treatments for diseases associated with the excessive degradation of extracellular matrix, such as arthritic diseases (rheumatoid arthritis and osteoarthritis), multiple sclerosis, bone resorptive diseases (such as osteoporosis), the enhanced collagen destruction associated with diabetes, chronic obstructive pulmonary disease, cerebral hemorrhaging associated with stroke, periodontal disease, corneal or gastric ulceration, ulceration of the skin, tumor invasion and metastasis, and aberrant angiogenesis. The involvement of individual collagenases in the degradation of tissue collagens probably depends markedly on the tissue. The tissue distribution of human collagenases suggests that collagenase-3 is the major participant in the degradation of the collagen matrix of cartilage, while collagenase-1 is more likely to be involved in tissue remodeling of skin and other soft tissues. Thus, inhibitors selective for collagenase-3 over collagenase-1 are preferred for treatment of diseases associated with cartilage erosion, such as arthritis, etc.

Inhibitors of MMP also are known to substantially inhibit the release of tumor necrosis factor (TNF) from cells, and which therefore may be used in the treatment of conditions mediated by TNF. Such uses include, but are not limited to, the treatment of inflammation, fever, cardiovascular effects, hemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, restinosis, aneurysmal disease, graft versus host reactions and autoimmune disease.

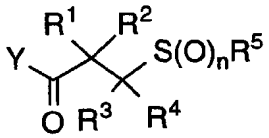
In addition to these effects on the release of TNF from cells, MMP inhibitors have also been shown to inhibit the

release of other biologically active molecules from cells, including soluble receptors (CD30 and receptors for TNF (p55 and p75), IL-6, IL-1 and TSH), adhesion molecules (*e.g.,* L-selection, ICAM-1, fibronectin) and other growth factors and cytokines, including Fas ligand, TGF-a, EGF, HB-EGF, SCF and M-CSF. Inhibition of the release or shedding of such proteins may be of benefit in a number of disease states, including rheumatoid arthritis, multiple sclerosis, vascular disease, Type II diabetes, HIV, cachexia, psoriasis, allergy, hepatitis, inflammatory bowel disease, and cancer.

Since non-specific inhibition of the shedding enzymes (sheddases) may have opposite pharmacological effects, selectivity will be a particular advantage, *e.g.,* the inhibition of TNF release without the concurrent inhibition of TNF receptor release.

The design and uses of MMP inhibitors is described, for example, in *J. Enzyme Inhibition,* **2**, 1-22 (1987); *Drug News & Prospectives,* **3**(8), 453-458 (1990); *Arthritis and Rheumatism,* **36**(2), 181-189 (1993); *Arthritis and Rheumatism,* **34**(9), 1073-1075 (1991); *Seminars in Arthritis and Rheumatism,* **19**(4), Supplement 1 (February), 16-20 (1990); *Drugs of the Future,* **15**(5), 495-508 (1990); and *J. Enzyme Inhibition,* **2**, 1-22 (1987). MMP inhibitors are also the subject of various patents and patent applications, for example, U.S. Patent Nos. 5,189,178 and 5,183,900, European Published Patent Applications 438 223, 606 426, and 276 436, and published Patent Cooperation Treaty International Applications WO 92/21360, WO 92/06966, WO 92/09563, and WO 94/25434.

One aspect of the invention concerns compounds represented by Formula I:



I

wherein:

n is 0, 1 or 2;
Y is hydroxy or XONH-, where X is hydrogen or lower alkyl;
R^1 is hydrogen or lower alkyl;
R^2 is hydrogen, lower alkyl, heteroalkyl, aryl, aralkyl, arylheteroalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaralkyl, heteroarylheteroalkyl, heterocyclo, heterocylo-lower alkyl, heterocyclo-lower heteroalkyl or $-NR^6R^7$, wherein:

R^6 is hydrogen, lower alkyl, cycloalkyl or cycloalkyl alkyl, aryl, heteroaryl and heteroaralkyl;
R^7 is hydrogen, lower alkyl, cycloalkyl or cycloalkyl alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, $-C(O)R^8$, $-C(O)NR^8R^9$, $-SO_2NR^8R^9$, $-SO_2R^{10}$, aryloxycarbonyl, or alkoxycarbonyl; or
R^6 and R^7 together with the nitrogen atom to which they are attached represent a heterocyclo group; wherein

R^8 and R^9 are independently hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl or heteroalkyl; and
R^{10} is lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heteroalkyl or heterocyclo; or

R^1 and R^2 together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group;
R^3 is hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heteroalkyl or lower alkoxy;
R^4 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl; or
R^2 and R^3 together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or
R^3 and R^4 together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and
R^5 is lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl; or heteroaralkyl;

or a pharmaceutically acceptable salt or ester thereof.

A second aspect of this invention relates to pharmaceutical compositions containing a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt or ester thereof admixed with at least one pharmaceutically acceptable excipient.

A third aspect of this invention relates to methods for treating mammals having a disease state alleviated by the inhibition of matrix metalloproteases, by administering an effective amount of a compound of Formula I, or a pharmaceutical composition thereof, to the mammal. Such disease states include arthritic diseases, multiple sclerosis, bone

resorption disease (such as osteoporosis), the enhanced collagen destruction associated with diabetes, chronic obstructive pulmonary disease, cerebral hemorrhaging associated with stroke, periodontal disease, corneal or gastric ulceration, ulceration of the skin, and tumor metastasis.

A fourth aspect of this invention relates to methods for preparing compounds of Formula I.

Among the family of compounds of the present invention as defined above, a particular family of compounds of formula I consists of n is 0, 1 or 2; Y is hydroxy or XONH-, where X is hydrogen or lower alkyl; R^1 is hydrogen or lower alkyl; R^2 is hydrogen, lower alkyl, aralkyl, cycloalkyl, cycloalkylalkyl, heterocyclo, or $-NR^6R^7$; or R^1 and R^2 together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group; in which R^6 is hydrogen, lower alkyl, or phenyl; and R^7 is hydrogen, lower alkyl, benzyl, $-C(O)R^8$, $-C(O)NR^8R^9$, $-SO_2NR^8R^9$, $-SO_2R^{10}$, benzyloxycarbonyl, or alkoxycarbonyl; or R^6 and R^7 together with the nitrogen atom to which they are attached represent a heterocyclo group; wherein R^8 and R^9 are independently hydrogen or lower alkyl; and R^{10} is lower alkyl, aryl, heteroaryl, or heterocyclo; R^3 is hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, heteroaralkyl, or lower alkoxy; R^4 is hydrogen or lower alkyl; or R^2 and R^3 together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or R^3 and R^4 together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and R^5 is lower alkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl.

Within these families a preferred category includes compounds where n is 2 and Y is -NHOH.

Within this category, one preferred group includes the compounds where R^1 is hydrogen and R^5 is aryl. One preferred subgroup within this group includes the compounds where R^2 is hydrogen and R^3 is aralkyl, especially benzyl, and R^4 is hydrogen and R^5 is optionally substituted phenyl or naphthyl, more especially where R^5 is 4-methoxyphenyl, phenylthiophenyl, phenoxyphenyl, or biphenyl.

Another preferred subgroup within this group includes the compounds where R^3 and R^4 together with the carbon to which they are attached form a cycloalkyl group, especially cyclopentyl and cyclcohexyl, more especially in combination where R^5 is 4-methoxyphenyl or 4-phenoxyphenyl.

Yet another preferred subgroup within this group includes the compounds where R^3 and R^4 together with the carbon to which they are attached form a heterocyclo group, in particular optionally substituted piperidinyl or tetrahydropyranyl, especially piperidin-4-yl, 1-methylpiperidin-4-yl, 1-(cyclopropylmethyl)piperidin-4-yl, or tetrahydropyranyl, more especially in combination where R^5 is 4-phenoxyphenyl, 4-(4-chlorophenoxy)phenyl, 4-bromophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

Another preferred group within this category includes the compounds where R^2 is $-NR^6R^7$, R^1, R^3 and R^4 are hydrogen, and R^5 is aryl. One preferred subgroup within this group includes the compounds where R^5 is 4-phenoxyphenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl, especially where R^6 is hydrogen and R^7 is CBZ-valinamido, valinamido or dimethylaminosulfonyl.

Another preferred group within this category includes the compounds where R^1 and R^2 together with the carbon to which they are attached form a heterocyclo group. A preferred subgroup within the group includes compounds where R^3 and R^4 are hydrogen and R^1 and R^2 together with the carbon to which they are attached form a heterocyclo group, in particular optionally substituted piperidinyl or tetrahydropyranyl, especially piperidin-4-yl, 1-methylpiperidin-4-yl, 1-(cyclopropylmethyl)piperidin-4-yl, or most preferably tetrahydropyranyl, more especially in combination where R^5 is 4-phenoxyphenyl, 4-(4-chlorophenoxy)phenyl, 4-(4-bromophenoxy)phenyl, 4-(4-fluorophenoxy)phenyl, 4-(thiophen-2-yl)phenoxy)phenyl, 4-(thiophen-3-yl)phenoxy)phenyl, 4-(thiazol-2-yl)phenoxy)phenyl, 4-(2-pyridyloxy)phenyl, or 4-(5-chloro-2-pyridyloxy)phenyl.

Another preferred group within this category includes compounds wherein R^1 and R^2 are both alkyl, R^3 and R^4 are hydrogen. One preferred subgroup includes compounds wherein R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

Another group within this category includes compounds wherein R^2 and R^3 together with the carbons to which they are attached form a cycloalkyl group and R^5 is aryl. Preferably, the cycloalkyl group is cyclopentyl or cyclohexyl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

Preferred compounds are:

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide;
2-{4-[4-(4-chlorophenoxy)-phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide;
2-{4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-[1-methyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide;
N-hydroxy-2-{1-methyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-acetamide;
N-hydroxy-2-{1-methyl-4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-acetamide;
2-[1-cyclopropylmethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-*N*-hydroxyacetamide;
2-{1-cyclopropylmethyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;

2-{1-cyclopropylmethyl-4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-[4-(4-phenoxyphenylsulfinyl)-tetrahydropyran-4-yl]-acetamide;
2-{4-[4-(4-chlorophenoxy)-phenylsulfinyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)-phenylsulfinyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetamide;
2-{4-[4-(4-chlorophenoxy)-phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)-phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-bromophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-fluorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
3-[4-(4-chlorophenoxy)phenylsulfonyl]-2,2-dimethyl-*N*-hydroxypropionamide;
4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-(cyclopropylmethyl)piperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-(nicotinoyl)piperidine-4-(*N*-hydroxycarboxamide);
4-[4-(phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(thiophen-2-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(thiophen-3-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(furan-2-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(benzofuran-2-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(thiazol-2-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(thiazol-4-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(thiazol-5-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(imidazol-1-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-(imidazol-2-yl)-phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(5-chloro-2-pyridyloxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide);
3-[4-(5-chloro-2-pyridyloxy)phenylsulfonyl]-2,2-dimethyl-*N*-hydroxypropionamide;
(R)-2-(CBZ-valinamido)-N-hydroxy-3-(4-phenoxyphenylsulfonyl)propionamide;
(R)-N-hydroxy-2-valinamido-3-(4-phenoxyphenylsulfonyl)-propionamide;
(R)-2-dimethylamino-N-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide;
(R)-2-dimethylaminosulfonamido-N-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide

and pharmaceutically acceptable salts thereof.

Definitions

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

"Alkyl" means a branched or unbranched saturated hydrocarbon chain containing 1 to 8 carbon atoms, such as methyl, ethyl, propyl, *tert*-butyl, *n*-hexyl, *n*-octyl and the like.

"Lower alkyl" means a branched or unbranched saturated hydrocarbon chain containing 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, *tert*-butyl, *n*-butyl, *n*-hexyl and the like, unless otherwise indicated.

The term "heteroalkyl" refers to a branched or unbranched, cyclic or acyclic saturated organic radical containing carbon, hydrogen and one or more heteroatom containing substituents independently selected from OR^a, NR^aR^b, and $S(O)_nR^a$ (where n is 0, 1 or 2) and R^a is hydrogen, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl or acyl, R^b is hydrogen, alkyl, cycloalkyl, aryl, aralkyl, acyl, alkylsulfonyl, carboxamido, or mono- or di-alkylcarbamoyl. Representative examples include hydroxyalkyl, aminoalkyl, alkoxyalkyl, aryloxymethyl, N-acylaminoalkyl, thienylthiomethyl and the like.

"Acyl" refers to the group -C(O)-R', where R' is lower alkyl.

"Alkylene" refers to a straight chain or branched chain divalent radical consisting solely of carbon and hydrogen, containing no unsaturation and having from one to six carbon atoms, *e. g.,* methylene, ethylene, propylene, 2-methylpropylene, butylene, 2-ethylbutylene, hexylene, and the like.

"Lower alkoxy" means the group -O-R', where R' is lower alkyl.

"Alkoxycarbonyl" means the group RO-C(O)- where R is alkyl as herein defined.

"Alkoxycarbonylalkyl" means the group $ROC(O)(CH_2)_n$- where R is alkyl as herein defined and n is 1, 2 or 3.

"Aryl" refers to a monovalent aromatic carbocyclic radical having a single ring (*e.g.,* phenyl) or two condensed rings (*e.g.,* naphthyl), which can optionally be mono-, di- or tri-substituted, independently, with hydroxy, carboxy, lower alkyl, cycloalkyl, cycloalkyloxy, lower alkoxy, chloro, fluoro, trifluoromethyl and/or cyano. The ring(s) can alternatively be optionally monosubstituted with the group R^a-Z-, where Z is oxygen, sulfur, -CH=CH-, $-CH_2$, carbonyl, a covalent bond, or nitrogen optionally substituted with lower alkyl, and R^a is a monovalent aromatic carbocyclic, heteroaryl or heterocyclo radical, or a combination thereof, having 1 or 2 rings, for example phenyl, pyridyl, thienyl, imidazolyl, furanyl, pyrimidinyl, benzothiophene, azanaphthalene, indolyl, phenyl-(furan-2-yl), phenyl-(thien-2-yl), phenyl-(thien-3-yl), phenyl-

(imidazol-2-yl), phenyl-(thiazol-2-yl), phenyl-(morpholin-2-yl), and phenyl-(oxazol-2-yl), (the ring(s) represented by R^a being optionally mono-or disubstituted by hydroxy, carboxy, lower alkyl, lower alkoxy, halo, trifluoromethyl and/or cyano). Examples of aryl substituted by R^a-Z- are benzoyl, diphenylmethane, biphenyl, 6-methoxybiphenyl, 4-(4-methylphenoxy)phenyl, 4-phenoxyphenyl, 2-thiophenoxyphenyl, 4-pyridethenylphenyl, 4-(thiophen-2-yl)phenoxyphenyl, 4-(thiophen-3-yl)phenoxyphenyl, 4-(2-pyridyloxy)phenyl, 4-(5-chloro-2-pyridyloxy)phenyl, 4-(thiazol-5-yl)phenoxyphenyl, 4-(imidazol-2-yl)phenoxyphenyl, and the like.

"Heteroaryl" refers to a monovalent aromatic carbocyclic radical having one or two rings incorporating one, two or three heteroatoms (chosen from N, O or S) within the ring(s), such as thiazole, oxazole, imidazole, thiophene, quinolyl, benzofuranyl, pyridyl, and indolyl, which can optionally be mono-, di- or tri-substituted, independently, with OH, COOH, lower alkyl, lower alkoxy, halo, trifluoromethyl and/or cyano.

"Aralkyl" refers to a radical of the formula R^b-R^c-, wherein R^b is aryl as defined above and R^c is alkylene as defined above, for example benzyl, phenylethylene, 3-phenylpropyl, biphenylpropyl.

"Benzyloxycarbonyl" refers to a radical of the formula $R^dCH_2OC(O)$-, where R^d is phenyl. "Benzyloxycarbonylamino" refers to a radical of the formula $R^dCH_2OC(O)NH$-, where R^d is phenyl.

"Cycloalkyl" means a saturated monovalent monocyclic hydrocarbon radical containing 3-8 carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

"Cycloalkylalkyl" means cycloalkyl as defined above attached to an alkylene radical as defined above.

"Halo" refers to bromo, chloro or fluoro.

"Heteroaralkyl" refers to a radical of the formula R^eR^c-, where R^e is heteroaryl as defined above and R^c is alkylene as defined above.

"Heterocyclo" refers to a monovalent saturated carbocyclic radical, consisting of either a 5 to 7 membered monocyclic ring or a 9 to 14 membered bicyclic ring, substituted by one, two or three heteroatoms chosen from N, O, or S, optionally fused to a substituted or unsubstituted benzene ring. Examples of heterocyclo radicals are morpholino, piperazinyl, piperidinyl, pyrrolidinyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl-1,1-dioxide, tetrahydropyranyl, and the like, which can be optionally substituted by one or more substituents independently selected from lower alkyl, lower alkoxy, alkylamino, alkylaminoalkyl, acyl valyl, alkylsulfonyl, dialkylamino, heteroaroyl, alkoxycarbonylalkyl, and an amino protecting group where appropriate (*e.g.* CBZ, for example, 1-CBZ-piperidin-4-yl). However, the definition "R^6 and R^7 together with the nitrogen to which they are attached represent a heterocyclo group" clearly can refer only to a heterocyclo group containing at least one nitrogen atom.

"Hydroxylamino" refers to the group -NHOH.

"BOC" refers to *tert*-butoxycarbonyl.

"CBZ" refers to benzyloxycarbonyl.

"DCC" refers to 1,3-dicyclohexylcarbodiimide.

"Valine amide" refers to the radical $(CH_3)_2CHCH(NH_2)C(O)NH$-.

"Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted phenyl or aryl" means that the phenyl or aryl moiety may or may not be substituted and that the description includes both substituted and unsubstituted phenyl. The phrase "optional pharmaceutical excipients" indicates that a composition or dosage form so described may or may not include pharmaceutical excipients other than those specifically stated to be present, and that the formulation or dosage form so described includes instances in which optional excipients are present and instances in which they are not.

"Amino-protecting group" as used herein refers to those organic groups intended to protect nitrogen atoms against undesirable reactions during synthetic procedures, and includes, but is not limited to, benzyl, acyl, benzyloxycarbonyl (carbobenzyloxy), *p*-methoxybenzyloxy-carbonyl, *p*-nitrobenzyloxycarbonyl, *tert*-butoxycarbonyl, trifluoroacetyl, and the like.

"Base" as used here includes both strong inorganic bases such as sodium hydroxide, lithium hydroxide, ammonium hydroxide, potassium carbonate and the like, and organic bases such as pyridine, diisopropylethylamine, 4-methylmorpholine, triethylamine, dimethylaminopyridine and the like.

"Pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the free bases or free acids and which are not biologically or otherwise undesirable. If the compound exists as a free base, the desired acid salt may be prepared by methods known to those of ordinary skill in the art, such as treatment of the compound with an inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like; or with an organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, and the like. If the compound exists as a free acid, the desired base salt may also be prepared by methods known to those of ordinary skill in the art, such as the treatment of the compound with an inorganic base or an organic base. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of

primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like.

"Pharmaceutically acceptable ester" as used herein refers for example to those non-toxic esters of a compound of Formula I where R^1 is hydroxy, and are formed by reaction of such compounds, by means well known in the art, with an appropriate alkanol of 1-8 carbon atoms, for example methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, *tert*-butanol, *i*-butanol (or 2-methylpropanol), *n*-pentanol, *n*-hexanol, and the like.

The terms "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith, including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), *N,N*-dimethylformamide ("DMF"), chloroform ("$CHCl_3$"), methylene chloride (or dichloromethane or "CH_2Cl_2"), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, *tert*-butanol, dioxane, pyridine, and the like. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

The compounds of this invention may possess one or more asymmetric centers; such compounds can therefore be produced as mixtures of stereoisomers or as individual (*R*)- or (*S*)- stereoisomers. The individual enantiomers may be obtained by resolving a racemic or non-racemic mixture of an intermediate at some appropriate stage of the synthesis. It is understood that the individual (*R*)- or (*S*)-stereoisomers as well as racemic mixtures and other mixtures of stereoisomers are encompassed within the scope of the present invention.

The use of the symbol "(*R*)" or "(*S*)" preceding a substituent designates the absolute stereochemistry of that substituent according to the Cahn-Ingold-Prelog rules [see Cahn et al., *Angew. Chem. Inter. Edit.,* **5**, 385 (1966), ertata p. 511; Cahn et al., *Angew. Chem.,* **78**, 413 (1966); Cahn and Ingold, *J. Chem. Soc.,* (London), 612 (1951); Cahn et al., *Experientia,* **12**, 81 (1956); Cahn J., Chem. Educ., **41**, 116 (1964)]. Because of the interrelation of the designated substituent with the other substituents in a compound having a or β prefixes, the designation of the absolute configuration of one substituent fixes the absolute configuration of all substituents in the compound and thus the absolute configuration of the compound as a whole.

"Stereoisomers" are isomers that differ only in the way the atoms are arranged in space.

"Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other. Enantiomers rotate the plane of polarized light in opposite directions. The enantiomer that rotates the plane to the left is called the levo isomer, and is designated (-). The enantiomer that rotates the plane to the right is called the dextro isomer, and is designated (+).

"Diastereoisomers" are stereoisomers which are not mirror-images of each other.

"Racemic mixture" means a mixture containing equal parts of individual enantiomers. "Non-racemic mixture" is a mixture containing unequal parts of individual enantiomers.

"Mammal" includes humans and all domestic and wild animals, including, without limitation, cattle, horses, swine, sheep, goats, dogs, cats, and the like.

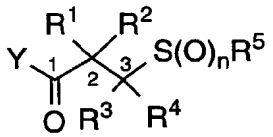
"Treating" or "treatment" as used herein cover the treatment of a disease-state in a mammal, particularly in a human, and include:

(i) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it;
(ii) inhibiting the disease-state, *i.e.,* arresting its development; or
(iii) relieving the disease-state, *i.e.,* causing regression of the disease-state.

The term "therapeutically effective amount" refers to that amount of a compound of Formula I that is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending on the subject and disease state being treated, the severity of the affliction and the manner of administration, and may be determined routinely by one of ordinary skill in the art.

Nomenclature

The compounds of Formula I, illustrated below, will be named using the indicated numbering system:



A compound of Formula I wherein is Y is *N*-hydroxylamino; R^1 and R^2 are hydrogen; R^3 is benzyl; R^4 is hydrogen; R^5 is 4-methoxyphenyl; and n is 2, is named 3-benzyl-3-(4-methoxyphenylsulfonyl)-*N*-hydroxypropionamide.

A compound of Formula I wherein Y is *N*-hydroxylamino; R^1 and R^2 are hydrogen; R^3 and R^4 together with the carbon to which they are attached represent tetrahydropyran-4-yl; R^5 is 4-(4-fluorophenoxy)phenyl; and n is 2, is named as an acetic acid derivative, *i.e.,* 2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxy-acetamide.

A compound of Formula I wherein Y is hydroxy; R^1 is hydrogen; R^2 is methyl; R^3 and R^4 together with the carbon to which they are attached represent 1-methylpiperidin-4-yl; R^5 is biphenyl; and n is 1, is named 2-[4-(biphenyl-4-sulfinyl)-1-methylpiperidin-4-yl]-propionic acid.

A compound of Formula I wherein Y is *N*-hydroxylamino; R^1 and R^2 together with the carbon to which they are attached represent tetrahydropyran-4-yl, R^3 and R^4 are hydrogen, R^5 is 4-(4-chlorophenoxy)-phenyl; and n is 2, is named 4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide).

Synthetic Reaction Parameters

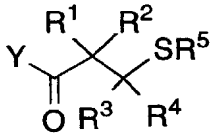
Unless specified to the contrary, the reactions described herein take place at atmospheric pressure within a temperature range from 5°C to 100°C (preferably from 10°C to 50°C; most preferably at "room" or "ambient" temperature, *e.g.,* 20°C). Further, unless otherwise specified, the reaction times and conditions are intended to be approximate, *e.g.,* taking place at about atmospheric pressure within a temperature range of about 5°C to about 100°C (preferably from about 10°C to about 50°C; most preferably about 20°C) over a period of about 1 to about 10 hours (preferably about 5 hours). Parameters given in the Examples are intended to be specific, not approximate.

Amide couplings used to form the compounds of Formula I are generally performed by the carbodiimide method with reagents such as 1,3-dicyclohexylcarbodiimide or *N'*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride or alternatively 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), in the presence of 1-hydroxybenzotriazole hydrate (HOBT) in an inert solvent such as *N,N*-dimethylformamide (DMF) or methylene chloride (CH_2Cl_2). Other methods of forming the amide or peptide bond include, but are not limited to, synthetic routes via an acid chloride, acyl azide, mixed anhydride or activated ester such as a *p*-nitrophenyl ester. Typically, solution phase amide couplings with or without peptide fragments are performed.

The selection of amino protecting groups used in the preparation of compounds of Formula I is dictated in part by the particular amide coupling conditions, and in part by the components involved in the coupling. Amino-protecting groups commonly used include those which are well-known in the art, for example, benzyloxycarbonyl (carbobenzyloxy) (CBZ), *p*-methoxybenzyloxycarbonyl, *p*-nitro-benzyloxycarbonyl, *N-tert*-butoxycarbonyl (BOC), and the like. It is preferred to use either BOC or CBZ as the protecting group for the a-amino group because of the relative ease of removal by mild acids in the case of BOC, *e.g.,* by trifluoroacetic acid (TFA) or hydrochloric acid in ethyl acetate; or removal by catalytic hydrogenation in the case of

PREPARATION OF COMPOUNDS OF FORMULA I

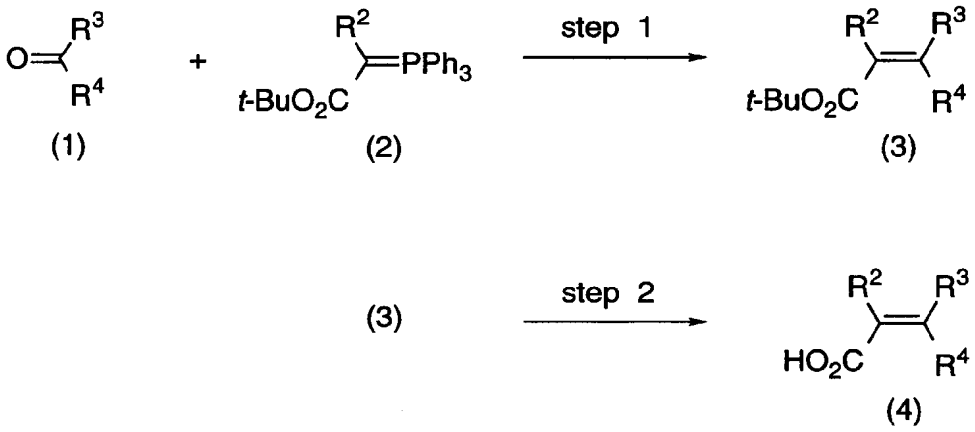
One method for preparing a compound of the Formula I, in particular wherein n is 1 or 2; Y is hydroxy or XONH-, where X is hydrogen or lower alkyl; R^1 is hydrogen or lower alkyl; R^2 is hydrogen, lower alkyl, aralkyl, cycloalkyl, cycloalkylalkyl, or heterocyclo; or R^1 and R^2 together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group; R^3 is hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, heteroaralkyl, or lower alkoxy; R^4 is hydrogen or lower alkyl; or R^2 and R^3 together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or R^3 and R^4 together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and R^5 is lower alkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; comprises contacting a compound of the Formula:



with an oxidizing agent. Suitable oxidation conditions are outlined in the description of reaction scheme VIII below.

One method of preparing compounds of Formula I where n is 0, R^1 is hydrogen and R^2 is not $-NR^6R^7$ is from the corresponding unsaturated acid of Formula (4), the preparation of which is shown below in Reaction Scheme I:

REACTION SCHEME I



Starting Materials

Aldehydes and ketones of Formula (1) are commercially available, for example from Aldrich Chemical Co., or may be prepared as shown below, or prepared according to methods well known to those skilled in the art. The ylides of Formula (2) are commercially available, for example, (*tert*-butoxycarbonylmethylene)triphenylphosphorane is available from Aldrich, or may be prepared by standard methods known to those skilled in the art, for example by reacting the appropriate bromo derivative of formula $R^2CHBrCO_2$-(*tert*-butyl) with triphenylphosphine, and reacting the resulting triphenylphosphonium bromide derivative with a strong base.

Step 1 - Preparation of Compounds of Formula (3)

In general, a solution of an aldehyde or ketone compound of Formula (1) is reacted in an inert organic solvent, for example benzene, with a compound of Formula (2) (or alternatively, the corresponding phosphonate, for example trimethyl phosphonoacetate) for a period of 8 to 48 hours at 15°C to 30°C (aldehydes), preferably 20°C, or 70°C to 90°C (ketones), preferably 80°C, until starting material is consumed. The reaction product, an enoic ester of Formula (3), is isolated and purified by conventional means.

Step 2 - Preparation of Compounds of Formula (4)

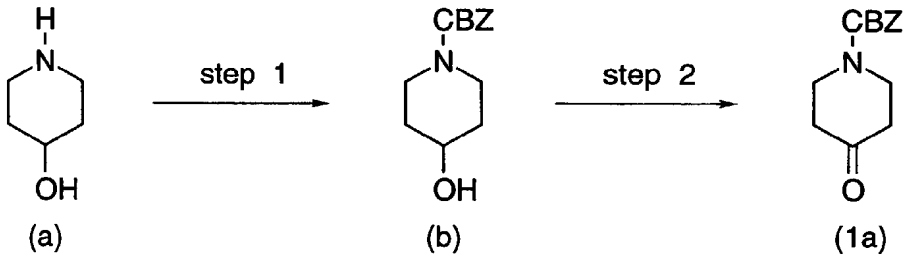
The compound of Formula (3) is then hydrolyzed under acidic conditions, optionally in the presence of an inert solvent, *e.g.,* treatment with trifluoroacetic acid in methylene chloride for about 20 minutes to 3 hours. The reaction is carried out at a temperature range from about 0°C to 40°C, preferably at about room temperature. In the case where trimethyl phosphonoacetate is used in Step 1, a methyl ester is produced which may be hydrolyzed conventionally

under basic conditions, for example sodium hydroxide in aqueous methanol or ethanol. The reaction product, an enoic acid of Formula (4), is isolated and purified by conventional means.

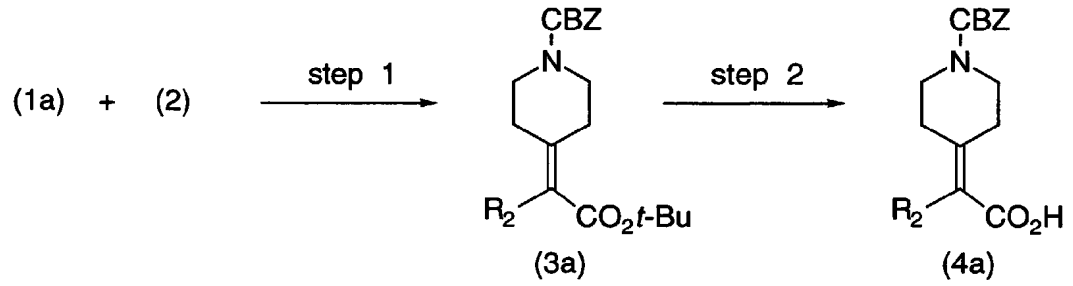
Preparation of Compounds of Formula (4) where R^3 and R^4 together with the Carbon to which they are attached represent a Piperidine Derivative

The preparation of compounds of Formula (4) where R^3 and R^4 together with the carbon to which they are attached represent a piperidine derivative, represented below as a compound of Formula (4a), in general requires the protection of the NH group. An example is shown below in Reaction Scheme II.

REACTION SCHEME II



(a) (b) (1a)



(3a) (4a)

Step 1 - Preparation of Compounds of Formula (b)

In general, a solution of a hydroxypiperidine compound of Formula (a) is protected by reaction of (a) in an inert organic solvent, for example tetrahydrofuran, in the presence of an excess of a tertiary base, for example triethylamine, with an equimolar amount of benzyl chloroformate. The reaction is carried out in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 10 to 30 hours, preferably about 18 hours. The reaction product of Formula (b) is isolated and purified by conventional means.

Step 2 - Preparation of Compounds of Formula (1a)

A compound of Formula (1a) is a compound of Formula (1) where R^3 and R^4 together with the carbon to which they are attached represent a protected piperidine derivative.

In general, a solution of a compound of Formula (b) is oxidized to a ketone of Formula (1a) by reaction of (b) in an inert organic solvent, for example methylene chloride, with an oxidizing agent, for example pyridinium chlorochromate, preferably in the presence of an inert support, for example Celite. The reaction is carried out in the temperature range

from about 0°C to 40°C, preferably at about 25°C, for about 10 to 30 hours, preferably about 18 hours. The reaction product of Formula (1a) is isolated and purified by conventional means.

Alternatively, reaction of commercially available 4-piperidone monohydrate hydrochloride with benzyl chloroformate under Schotten-Baumann conditions gives a compound of Formula (1a) in a single step.

Preparation of Compounds of Formula (4) where R^3 and R^4 Together with the Carbon to which they are attached Represent a Piperidine Derivative

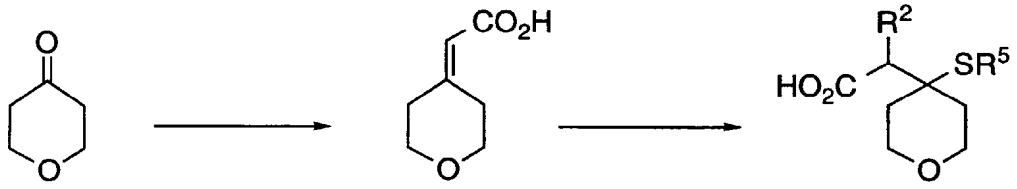
A compound of Formula (4) where R^3 and R^4 together with the carbon to which they are attached represent a piperidine derivative is represented as a compound of Formula (4a).

The protected piperidine ketone of Formula (1a) is converted to (3a), which is hydrolyzed to (4a) as described in Reaction Scheme I, Steps 1 and 2. The compound of Formula (4a) is then converted to a compound of Formula I where n is 0 as described in Reaction Scheme III below. The benzyloxycarbonyl (CBZ) protecting group is removed by catalytic hydrogenation, to give a compound of Formula I where R^3 and R^4 together with the carbon to which they are attached represent piperidine.

Preparation of Compounds of Formula (4) where R^3 and R^4 Together with the Carbon to which they are attached Represent a Pyran Derivative

Compounds of Formula (4) where R^3 and R^4 together with the carbon to which they are attached represent a tetrahydropyran derivative, represented as Formula (4b), are prepared similarly to the procedure shown above, starting from the corresponding 4-oxotetrahydropyran. The reaction is shown below in Reaction Scheme III and described in Example 3.

REACTION SCHEME III



The tetrahydropyran derivative of Formula (4b) is then converted to the corresponding compound of Formula I, *i.e.*, a compound of Formula I where n is 0, as described in Reaction Scheme VII.

Preparation of Compounds of Formula (4) where R^3 and R^4 Together with the Carbon to which they are Attached represent a Tetrahydrothiopyran-1,1-dioxide Derivative

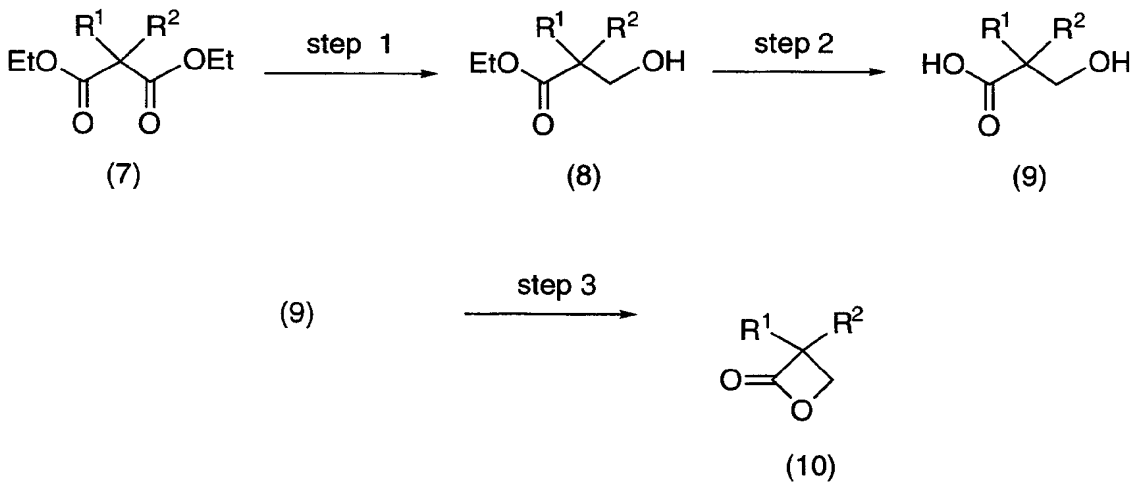
Compounds of Formula (4) where R^3 and R^4 together with the carbon to which they are attached represent a tetrahydrothiopyran1,1-dioxide derivative are prepared similarly to the procedure shown above, starting from the corresponding 4-oxotetrahydrothiopyran.

The tetrahydrothiopyran-1,1-dioxide derivative of Formula (4) is then converted to the corresponding compound of Formula I where n is 0 as described in Reaction Scheme III.

Alternative Preparation of Compounds of Formula I

Another method of preparing compounds of Formula I where R^2 is not $-NR^6R^7$ and R^3 and R^4 are both hydrogen is from the corresponding lactone of Formula (10), the preparation of which is shown below in Reaction Scheme IV.

REACTION SCHEME IV



Step 1 - Preparation of Compounds of Formula (8)

The starting compounds of Formula (7) are commercially available, or may be prepared by means well known in the art starting from diethyl malonate, *e.g.*, Gibson and Johnson, *J. Chem. Soc.*, p2525 (1930), (other diesters may be employed in place of the diethyl ester if desired). In general, a solution of a compound of Formula (7) is dissolved in an inert aromatic solvent, preferably benzene or toluene, and cooled to about -40° to -20°C, preferably about -30°C. To this cold solution is added a suitable hindered reducing agent, preferably diisobutylaluminum hydride in an inert aromatic solvent, maintaining the temperature at no higher than about 25°C. After the addition is complete, the reaction is maintained at about 15°C until all the starting material is consumed. After about 10 minutes the reaction is quenched by addition of a protic solvent, preferably ethanol, maintaining the temperature at no higher than about -15°C. Sodium borohydride is optionally added, but preferably the reaction is simply allowed to warm to about room temperature. The reaction product of Formula (8) is isolated and purified by conventional means.

Step 2 - Preparation of Compounds of Formula (9)

In general, the compound of Formula (8) is hydrolysed with a base to form the hydroxymethyl acid of Formula (9).

The compound of Formula (8) is dissolved in an aqueous protic solvent, preferably aqueous methanol, and reacted with about 3 molar equivalents of a base, for example potassium hydroxide or lithium iodide, followed by sodium cyanide. The reaction is carried out in the temperature range from about 80°C to 120°C, preferably at about the reflux temperature of the solvent mixture, for about 8 hours. The reaction product of Formula (9) is isolated and purified by conventional means.

Step 3 - Preparation of Compounds of Formula (10)

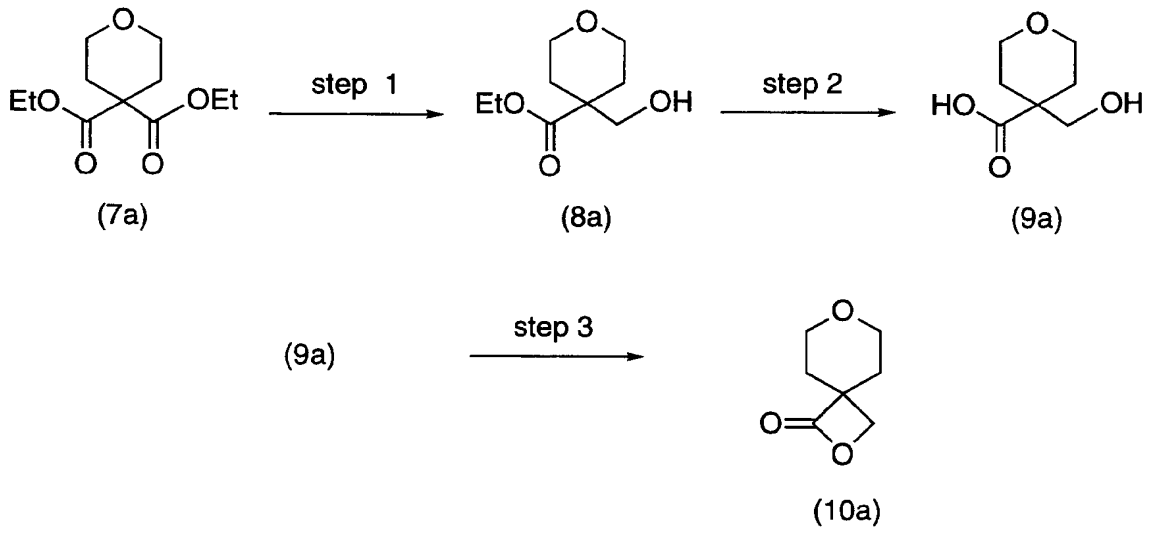
In general, the compound of Formula (9) is dehydrated to form a lactone of Formula (10).

To a mixture of the compound of Formula (9) and about 2 molar equivalents of a tertiary base, preferably triethylamine, optionally in the presence of 4-dimethylaminopyridine, in an inert solvent, for example, diethyl ether or dichloromethane, at about -20°C, is added about 1 molar equivalent of a dehydrating agent, for example trifluoromethanesulfonic anhydride, methanesulfonic anhydride, methanesulfonyl chloride, *p*-toluenesulfonyl chloride, benzenesulfonyl chloride, preferably benzenesulfonyl chloride. The reaction is carried out at about -10°C, for about 10 minutes to 4 hours, preferably about 30 minutes. The reaction product of Formula (10) is isolated by conventional means synthesis without further purification.

Preparation of Compounds of Formula (10) where R^1 and R^2 together with the Carbon to which they are attached Represent a Tetrahydropyran Derivative

To give a specific example, the preparation of a compound of Formula (10) where R^1 and R^2 together with the carbon to which they are attached represent a tetrahydropyran derivative (represented as Formula (10a)) is shown below in Reaction Scheme V, and described in Example 5.

REACTION SCHEME V

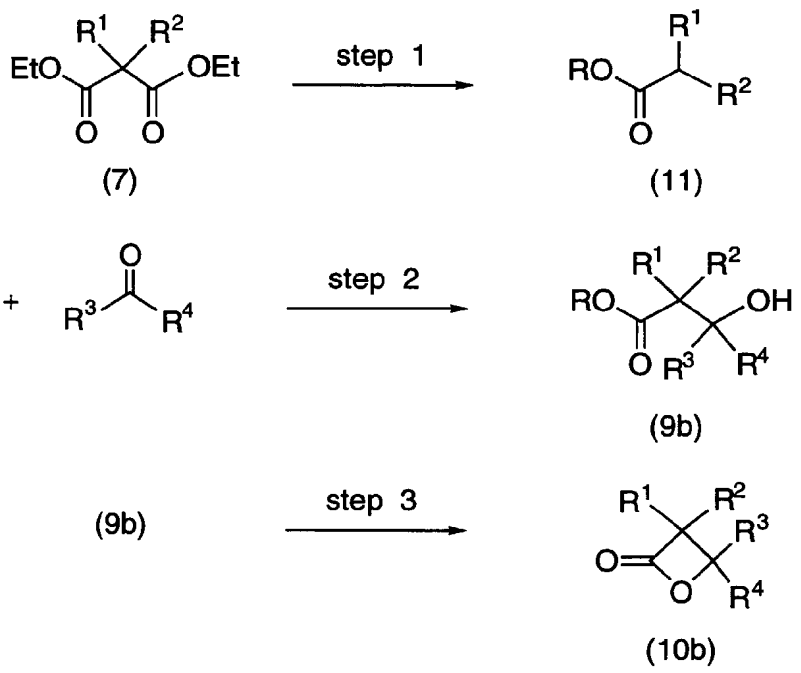


The starting compound of Formula (7a) is either commercially available or may be prepared as shown in Example 31A. Steps 1-3 are carried out in the same manner as shown in Reaction Scheme IV.

Preparation of Compounds of Formula (10) where R^3 and R^4 are as Defined in the compounds of formula I

The preparation of a compound of Formula (10) where R^3 and R^4 are as defined in the compounds of formula I, represented as Formula (10b), is shown below in Reaction Scheme VI, and described in Example 5.

REACTION SCHEME VI



Step 1 - Preparation of Compounds of Formula (11)

The compound of Formula (11), where R is Et, may be prepared from the compound of Formula (7) by decarboxylation. In general, the diester is reacted with a mixture of lithium iodide and sodium cyanide at about 130° to 140°C in a suitable solvent, for example *N, N*-dimethylformamide, for about 24 hours.

Step 2 - Preparation of Compounds of Formula (9b)

In general, an anion of a compound of Formula (11), where R is H or lower alkyl, is reacted with a compound of the formula $R^3R^4C=O$ to form a hydroxy acid or hydroxy ester, respectively, of Formula (9b).

A solution of the compound of Formula (11) in an anhydrous ethereal solvent, preferably tetrahydrofuran, is added to about 1.1 molar equivalent (when R is lower alkyl) or about 2 molar equivalents (when R is hydrogen) of a hindered base, preferably lithium diisopropylamide, in an anhydrous ethereal solvent, preferably tetrahydrofuran, at about 0°C. When the addition is complete, a small quantity of a polar solvent is optionally added, preferably hexamethylphosphoramide. To this mixture is added an excess of a compound of the formula $R^3R^4C=O$. The addition is carried out at a temperature range of about -78 to 10°C, preferably at about -78°C when R^3 and R^4 are hydrogen, or preferably 0°C for ketones, followed by reaction at room temperature for about 2-24 hours, preferably about 10 hours. Where R in the starting material of Formula (11) is hydrogen, the reaction product of Formula (9b) is isolated and purified by conventional means. Where R in the starting material of Formula (11) is lower alkyl, the reaction product of Formula (9b), where R = H, is obtained by hydrolyzing the ester product using a base, preferably lithium hydroxide, as described above, then isolating and purifying (9b) by conventional means.

Step 3 - Preparation of Compounds of Formula (10b)

The compound of Formula (9b) is then converted to a compound of Formula (10b) in the same manner as described in Reaction Scheme IV.

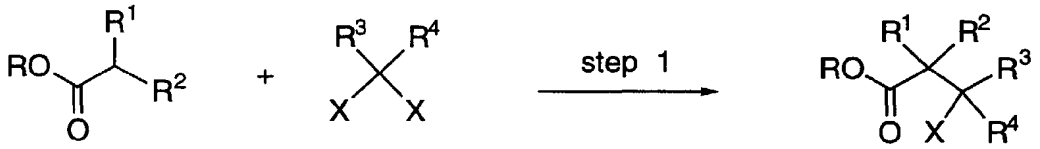
The method of Reaction Scheme VI can be used, for example, to prepare compounds of Formula (10) where R^1 and R^2 taken together with the carbon to which they are attached is tetrahydropyran-4-yl, by starting with 4-carboxytet-

rahydropyran or an ester thereof, for example, the ethyl ester. Similarly, compounds of Formula (10) where R^1 and R^2 taken together with the carbon to which they are attached is piperidin-4-yl or derivatives thereof, may be prepared by starting with 1-benzyloxycarbonyl-4-carboxypiperidine, *N*-(*tert*-butoxycarbonyl)-4-carboxypiperidine, or an ester thereof, for example, the ethyl ester.

Alternative Preparation of Compounds of Formula I

Compounds of Formula I can also be prepared from compounds of Formula (13), the preparation of which is shown below in Reaction Scheme VIa, and described in Example 5A.

REACTION SCHEME VIA



(11) (13)

where R is hydrogen or lower alkyl, and X is halo or -*p*-tosyl.

Step 1 - Preparation of Compounds of Formula (13) from (11)

The starting compounds of Formula (13) are commercially available, for example, an ester of commercially available chloropivalic acid may be prepared conventionally, or compounds of Formula (13) may be prepared by means well known in the art, for example, Gibson and Johnson, *J. Chem. Soc.*, p2525 (1930). In general, an anion of a compound of Formula (11) is reacted with an alkyl dihalide to form a halo-substituted hydroxy acid ester of Formula (13).

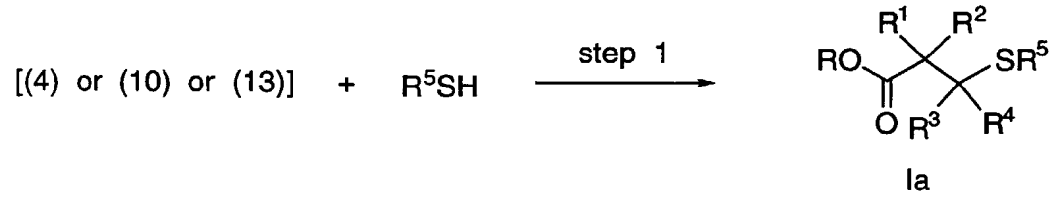
A solution of the compound of Formula (11) in an anhydrous ethereal solvent, preferably tetrahydrofuran, is added to about 1.1 molar equivalent (when R is lower alkyl) or about 2 molar equivalents (when R is hydrogen) of a hindered base, preferably lithium diisopropylamide, in an anhydrous ethereal solvent, preferably tetrahydrofuran, at about -100 to 0°C, preferably at about -78°C. To this mixture is added an excess of an alkyl dihalide, preferably diiodomethane. The addition is carried out a temperature range of about -5° to 50°C for about 1-5 hours. The reaction product of Formula (13) is isolated by conventional means, and preferably used in the next step of the synthesis without further purification.

It should be noted that a compounds of Formula (13) where X is *p*-tosyl, are obtained by tosylation by conventional means of compounds of Formula (8) or (9b).

Preparation of Compounds of Formula I

The intermediates of Formulae (4), (10), and (13) may be converted to compounds of Formula I where Y is hydroxy and n is 0, designated as compounds of Formula Ia, as shown in Reaction Scheme VII below.

REACTION SCHEME VII



Ia

where R is hydrogen or lower alkyl.

Compounds of Formula (4) are either commercially available, for example from Aldrich, or may be prepared according to methods known to those skilled in the art, for example, as described by Mannich and Rister, *Chem. Ber.*, **57**, 1116

(1924) for acids where R^3 and R^4 are each hydrogen, or may be prepared as described above, or as described in Example 3. Compounds of Formula (5) are commercially available, for example from Aldrich, Fluka, etc.), or may be prepared according to methods known to those skilled in the art, *e.g.,* as described below in Example 4.

Step 1 - Preparation of Compounds of Formula Ia from (4)

Compounds of Formula I where n is 0 and Y is hydroxy, designated as compounds of Formula Ia, may be prepared by heating an enoic acid of Formula (4) with an equimolar amount of a thiol of Formula (5) in the presence of an approximately equimolar amount of a secondary amine, preferably piperidine. The reaction is carried out in the temperature range from about 70°C to 120°C, preferably at about 100°C, for about 1 to 24 hours, preferably about 3 hours. The sulfide reaction product, a compound of Formula Ia, is isolated and purified by conventional means.

Step 1 - Preparation of Compounds of Formula Ia from (10)

Compounds of Formula I where n is 0 and Y is hydroxy, designated as compounds of Formula Ia, may be prepared by reacting a lactone of Formula (10) with about 1.1 molar equivalents of an anion of a thiol of Formula (5) (generated by reaction of (5) with an alkaline metal hydride, preferably sodium hydride in a polar solvent, preferably *N, N*-dimethylformamide). The reaction is carried out in a polar solvent, preferably *N, N*-dimethylformamide, at a temperature range of about 0°C to 70°C, preferably at about 0° to 25°C. The sulfide reaction product, a compound of Formula Ia, is isolated and purified by conventional means.

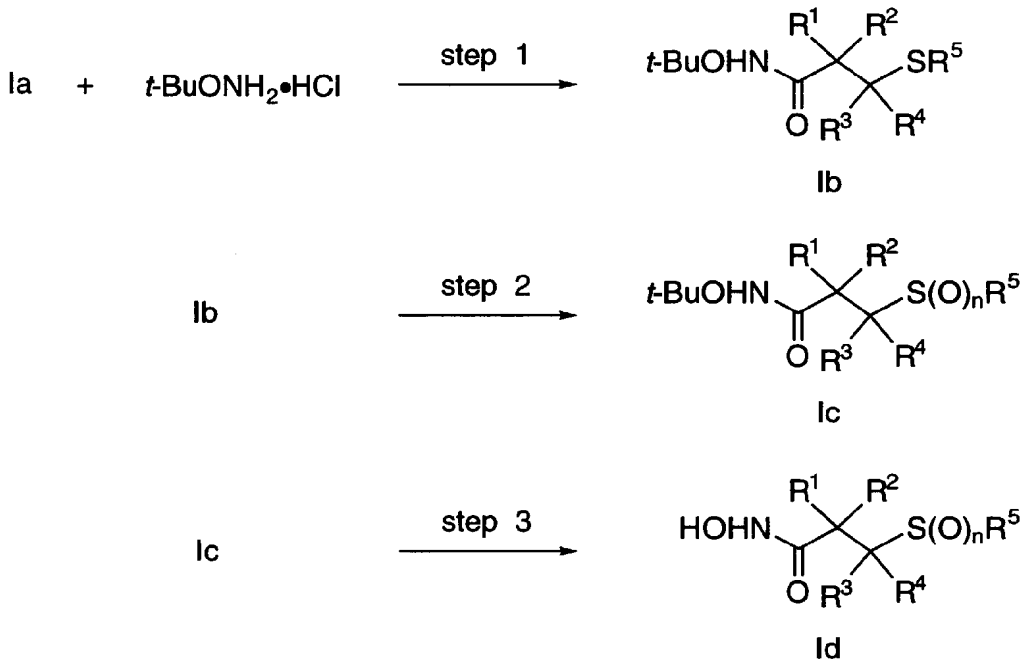
Step 1 - Preparation of Compounds of Formula Ia from (13)

Compounds of Formula I where n is 0 and Y is hydroxy or lower alkoxy, designated as compounds of Formula Ia, may be prepared by reacting an enoic acid ester of Formula (13) with about 1.1 molar equivalents of an anion of a thiol of Formula (5) (generated by reaction of (5) with an alkaline metal hydride, preferably sodium hydride in a polar solvent, preferably *N,N*-dimethylformamide). The reaction is carried out in a polar solvent, preferably *N,N*-dimethylformamide, at a temperature range of about 30°C to 120°C, preferably at about 80°C, for about 10 minutes. The sulfide reaction product, a compound of Formula Ia, is isolated and purified by conventional means.

Conversion of Compounds of Formula Ia to other Compounds of Formula I

One method of converting compounds of Formula Ia to other compounds of Formula I is shown below in Reaction Scheme VIII.

REACTION SCHEME VIII



Step 1 - Preparation of Compounds of Formula Ib

In general, compounds of Formula I where n is 0 and Y is *tert*-BuONH-, designated as compounds of Formula Ib, are prepared by reacting a compound of Formula Ia with an excess of a *O*-(*tert*-butyl)-hydroxylamine hydrochloride and *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (or other carbodiimide derivatives, for example 1,3-dicyclohexylcarbodiimide), in the presence of 1-hydroxybenzotriazole hydrate and a tertiary base, for example dimethylaminopyridine, triethylamine, 4-methylmorpholine, pyridine, or a mixture of such bases. The reaction is carried out in an inert solvent, preferably methylene chloride, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 10 to 30 hours, preferably about 18 hours. The *N*-*tert*-butoxy reaction product, a compound of Formula Ib, is isolated and purified by conventional means.

Step 2 - Preparation of Compounds of Formula Ic where n is 1

In general, compounds of Formula I where n is 1 and Y is *tert*-BuONH-, (*i.e.,* sulfoxides), designated as compounds of Formula Ic, are prepared from compounds of Formula Ib by reaction with a mild oxidizing agent, for example sodium periodate or one equivalent of "OXONE"™ (potassium peroxymonosulfate, Aldrich Chemical Co.), until starting material can no longer be detected. The reaction is carried out in an inert solvent, preferably aqueous acetone, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 10 minutes to 4 hours, preferably about 30 minutes. The sulfoxide product, a compound of Formula Ic where n is 1, is isolated and purified by conventional means.

Step 2 - Preparation of Compounds of Formula Ic where n is 2

In general, compounds of Formula I where n is 2, Y is *tert*-BuONH-, and R^1 is hydrogen (*i.e.,* sulfones), designated as compounds of Formula Ic, are prepared from compounds of Formula Ib by reaction with about 1-3 molar equivalents, preferably about 1.5 molar equivalents, of a strong oxidizing agent, for example, *m*-chloroperbenzoic acid or OXONE. The reaction is carried out in an inert solvent, preferably a protic solvent, preferably aqueous methanol, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 10 minutes to 4 hours, preferably about 2 hours. The sulfone product, a compound of Formula Ic where n is 2, is isolated and purified by conventional means.

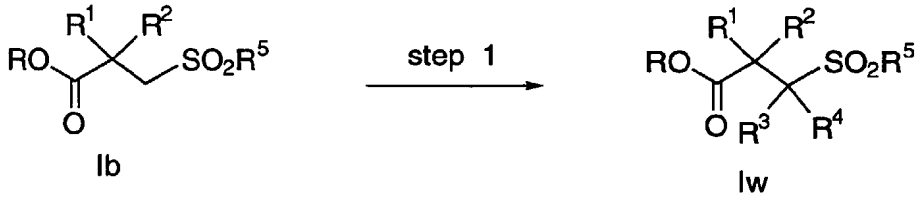
Step 3 - Preparation of Compounds of Formula Id

In general, compounds of Formula I where Y is HONH-, designated as compounds of Formula Id, are prepared by hydrolysing an *N-tert*-butoxy compound of Formula Ib or Ic under acid conditions under conditions similar to that shown for the preparation of compounds of Formula (4) above, or using hydrochloric acid gas in a sealed tube in an inert solvent, for example, 1,2-dichloroethane. The hydroxyamino reaction product, a compound of Formula Id where Y is HONH-, is isolated and purified by conventional means.

Alternative Method of Introduction of R^3 and R^4 into Compounds of Formula I

An alternative method of introducing the groups R^3 and R^4 into compounds of Formula I is shown below in Reaction Scheme VIIIA.

REACTION SCHEME VIIIA



Ib Iw

where R is hydrogen or lower alkyl.

Step 1- Preparation of Compounds of Formula I where n is 2, and R^3 is as defined in the compounds of formula I but is other than Hydrogen

The compounds of Formula I where n is 2, Y is hydroxy or alkoxy, R^3 is as defined in the compounds of formula I other than hydrogen, and R^1, R^2, and R^4 are defined in the compounds of formula I, designated as compounds of Formula Iw are prepared by the alkylation of compounds of Formula I where both R^3 and R^4 are hydrogen.

A solution of the compound of Formula Iw in an anhydrous ethereal solvent, preferably tetrahydrofuran, is added to a hindered base, preferably lithium diisopropylamide, in a manner similar shown above in Reaction Scheme VIA. To this mixture is added about 1 molar equivalent of an alkyl or aralkyl halide. The reaction addition is stirred for about 1-3 hours, then stirred stirred for an additional 1-5 hours, preferably 3 hours, at about room temperature. The reaction product is isolated and purified by conventional means.

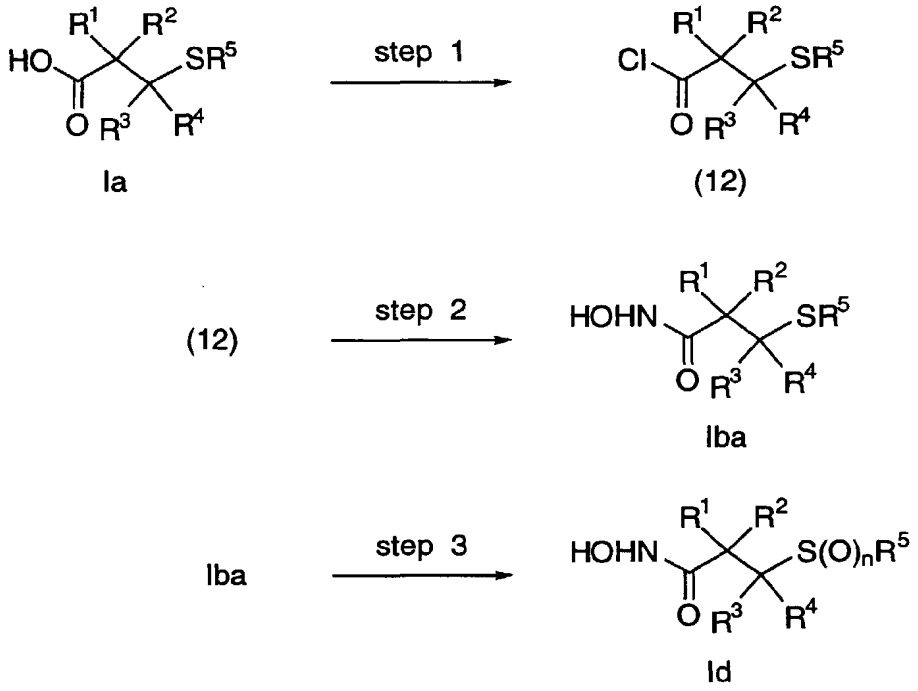
R^4 may be introduced in the same manner as shown above.

Compounds of Formula Iw can be converted to other compounds of Formula I as shown previously.

Preferred Procedure for Preparing Compounds of Formula Id from Compounds of Formula Ia

A preferred method of converting compounds of Formula Ia to other compounds of Formula I is shown below in Reaction Scheme IX.

REACTION SCHEME IX



Step 1 - Preparation of Compounds of Formula Iba

In general, an acid halide of a compound of Formula Ia, designated as compounds of Formula (12), is prepared by reacting a compound of Formula Ia with a halogenating agent.

The compound of Formula Ia is reacted with an excess of a halogenating agent, for example oxalyl chloride, oxalyl bromide, phosphorous oxychoride, phosphorous trichloride, phosphorous pentachloride, thionyl chloride, preferably oxalyl chloride in the presence of a small amount of *N, N*-dimethylformamide as a catalyst. The reaction is carried out in an inert solvent, preferably methylene chloride, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 10 to 30 hours, preferably about 18 hours. The acid halide reaction product, a compound of Formula (12), is isolated by conventional means.

Step 2 - Preparation of Compounds of Formula Iba

Compounds of Formula I where n is 0 and Y is HONH-, designated as compounds of Formula Iba, may be prepared by reacting a compound of Formula (12) with about 1-5 molar equivalents, preferably about 3.5 molar equivalents, of *N, O*-bis(trimethylsilyl)-hydroxylamine, or more preferably aqueous hydroxylamine dissolved in a suitable solvent, for example a mixture of *tert*-butanol/tetra-hydrofuran. The reaction is carried out in an inert solvent, preferably methylene chloride, in the temperature range from about 0°C to 25°C, preferably at about 25°C, for about 1-10 hours, preferably about 3 hours for *N, O*-bis(trimethylsilyl)hydroxylamine, or about 1.5 hours for aqueous hydroxylamine. The *N*-hydroxamic acid product, a compound of Formula Iba, is isolated and purified by conventional means.

Step 3 - Preparation of Compounds of Formula Id

The compound of Formula Iba is converted to a compound of Formula Id where n is 1 or 2 in the same manner as shown in Reaction Scheme VIII, steps 2 or 3, above.

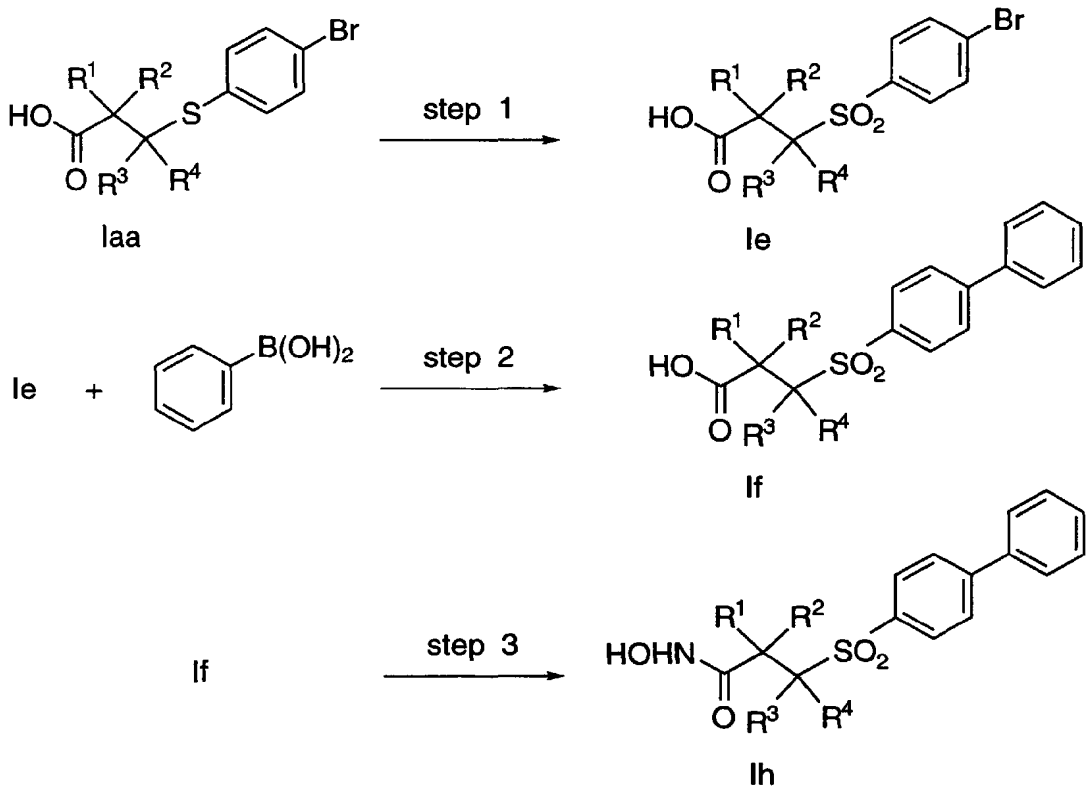
Alternative Preparation of Compounds of Formula I

It should be noted that the sequence of the steps in the above Reaction Schemes for the preparation of compounds of Formula Id may be changed. That is, a compound of Formula Ia may be oxidized first to a sulfone, followed by conversion of the carboxy group to hydroxyamino as shown above, if so desired.

Preparation of Compounds of Formula I where R^5 is Biphenyl

Compounds of Formula I where R^5 is optionally substituted biphenyl are preferably prepared from compounds of Formula Ia where R^5 is optionally substituted bromophenyl. For example, compounds where R^5 is 4-biphenyl can be prepared from compounds of Formula Ia where R^5 is 4-bromophenyl, represented below as a compound of Formula Iaa, as shown below in Reaction Scheme X.

REACTION SCHEME X



Step 1 - Preparation of Compounds of Formula Ie

In general, compounds of Formula I where n is 2, Y is hydroxy, R^5 is 4-bromophenyl, and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula Ie, are prepared from compounds of Formula Iaa by reaction with a strong oxidizing agent in the same manner as shown above in Reaction Scheme VIII, Step 2.

Step 2 - Preparation of Compounds of Formula If

In general, compounds of Formula I where n is 2, Y is hydroxy, R^5 is biphenyl, and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula If, are prepared by reacting a compound of Formula Ie with phenylboronic acid and zero-valent palladium catalysts, preferably tetrakis(triphenylphosphine)palladium.

The reaction is carried out in a protic solvent, preferably a mixture of ethanol and benzene, in the temperature range from about 30°C to 100°C, preferably at about 80°C. When the desired temperature is reached, aqueous 2M sodium carbonate is added, and refluxing continued for about 1-8 hours, preferably about 2 hours. The reaction product, a compound of Formula If, is isolated by conventional means and preferably purified using preparative TLC.

Step 3 - Preparation of Compounds of Formula Ih

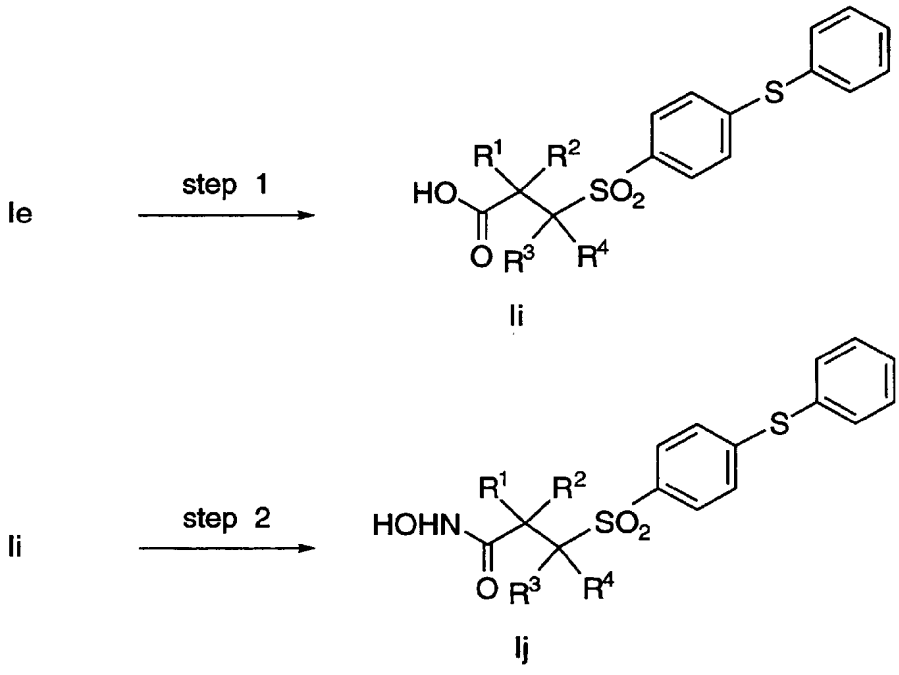
In general, compounds of Formula I where n is 2, Y is HONH-, R^5 is biphenyl, and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula Ih, may be prepared from the corresponding compounds of Formula If in the same manner as shown above in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

To prepare compounds of Formula I where R^5 is substituted biphenyl, a compound of Formula Iaa optionally substituted on the 4-bromophenyl ring is reacted with an optionally substituted boronic acid in the same manner as shown above.

Preparation of Compounds of Formula I where R^5 is Diphenylsulfide

Compounds of Formula I where R^5 is optionally substituted diphenylsulfide are preferably prepared from the corresponding compounds of Formula Ie, *i.e.,* compounds of Formula I in which R^5 is optionally substituted 4-bromophenyl, prepared as in Reaction Scheme X. For example, compounds where R^5 is 4-diphenylsulfide can be prepared from compounds of Formula Ie as shown below in Reaction Scheme XI.

REACTION SCHEME XI



Step 1 - Preparation of Compounds of Formula Ii

In general, compounds of Formula I where n is 2, Y is hydroxy, R^5 is 4-diphenylsulfide, and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula Ii, are prepared from compounds of Formula Ie by heating an anion of thiophenol (preferably prepared *in situ,* for example, by treatment of thiophenol with sodium or potassium hydride, preferably potassium hydride, in a polar solvent, preferably *N,N*-dimethylformamide. The

reaction is carried out in a polar solvent, preferably *N,N*-dimethylformamide, in the temperature range from about 30°C to 100°C, preferably at about 75°C, for about 4-48 hours, preferably about 18 hours. The reaction product, a compound of Formula Ii, is isolated by conventional means and preferably purified using preparative TLC.

Step 2 - Preparation of Compounds of Formula Ij

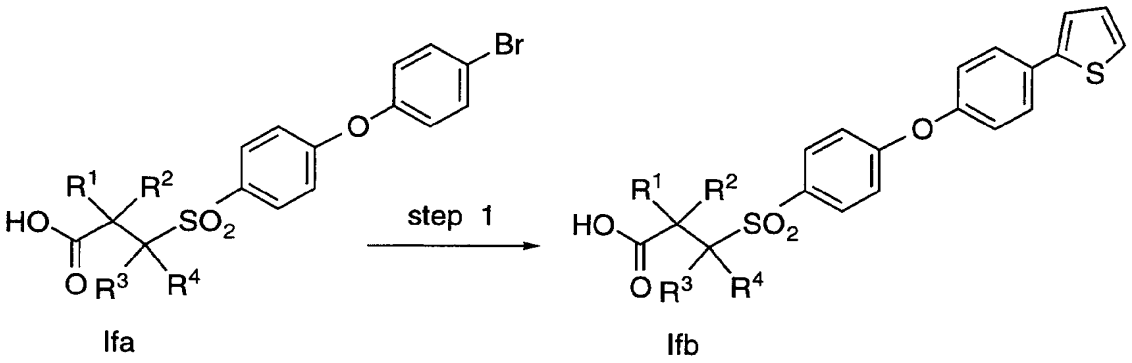
In general, compounds of Formula I where n is 2, Y is HONH-, R^5 is 4-diphenylsulfide, and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula Ij, are prepared from the corresponding compounds of Formula Ii in the same manner as shown above in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

To prepare compounds of Formula I where R^5 is substituted 4-diphenylsulfide, a compound of Formula Ie optionally substituted on the 4-bromophenyl ring is reacted with an optionally substituted anion of thiophenol in the same manner as shown above.

Preparation of Compounds of Formula I where R^5 is 4-[4-(thiophen-2-yl)phenoxy]phenyl

Compounds of Formula I where R^5 is optionally substituted 4-[4-(4-thiophen-2-yl)phenoxy]phenyl are prepared from the corresponding compounds of Formula I where R^5 is optionally substituted 4-(4-bromophenoxy)phenyl. This reaction is shown in Reaction Scheme XIA.

SCHEME XIA



Preparation of Compounds of Formula Ifb

The 4-bromo group of the compound of Formula (Ifa), which may be prepared by methods analogous to those previously shown, or as described in Example 16D, is displaced to give a compound of Formula Ifb, using the same procedure as described in Reaction Scheme X, step 2.

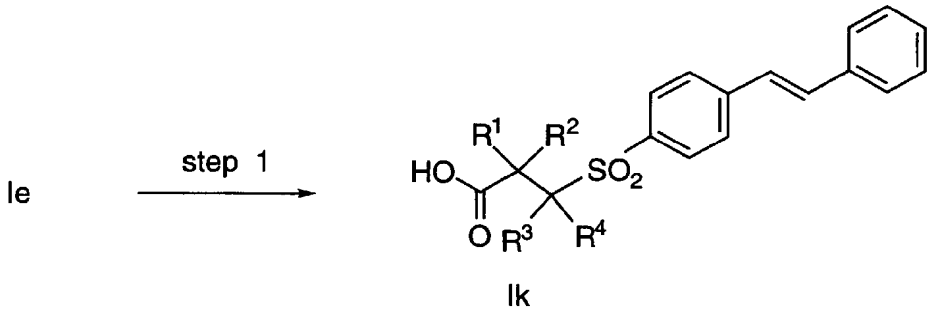
The compound of Formula (Ifa) is reacted similarly in order to introduce other aryl or heteroaryl groups.

Reduction of a compound of Formula Ifa with palladium and hydrogen replaces the bromo group by hydrogen.

Preparation of Compounds of Formula I where R^5 is 1,2-Diphenylethene

Compounds of Formula I where R^5 is optionally substituted 1,2-diphenylethene are preferably prepared from the corresponding compounds of Formula I where R^5 is optionally substituted 4-bromophenyl, as prepared in Reaction Scheme X. For example, compounds where R^5 is 4-diphenylethene can be prepared from compounds of Formula Ie as shown below in Reaction Scheme XII.

REACTION SCHEME XII



Step 1 - Preparation of Compounds of Formula Ik

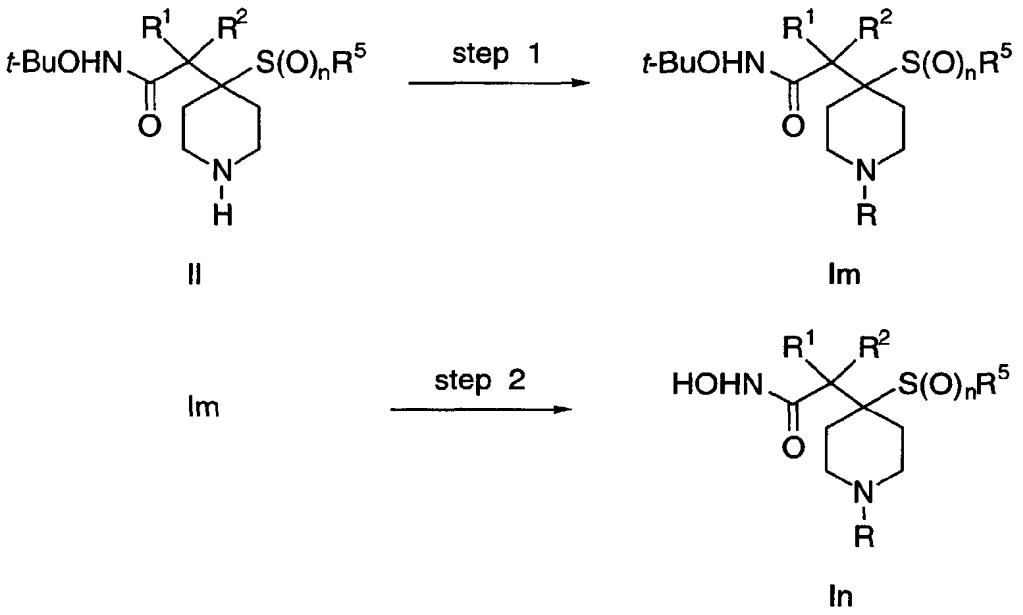
In general, compounds of Formula I where Y is hydroxy, R^5 is 4-(1,2-diphenylethene), and R^1, R^2, R^3, and R^4 are as defined in the compounds of formula I, designated as compounds of Formula Ik, are prepared by reacting a compound of Formula Ie with an optionally substituted styrene in the presence of a hindered tertiary organic base, for example diisopropylethylamine, and palladium diacetate, and trimethylphenylphosphine or other triphenylphosphine derivatives, preferably trimethylphenylphosphine or tetrakis(triphenylphosphine)-palladium(0). The reaction is carried out in the absence of solvent, in the temperature range from about 30°C to 100°C, preferably at about 80°C, for about 4-48 hours, preferably about 16 hours. The reaction product, a compound of Formula Ik, is isolated by conventional means and preferably purified using preparative TLC.

Conversion of the carboxylic acid of Formula Ik to its hydroxyamino equivalent is carried out in the same manner as shown above in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

Preparation of Compounds of Formula I where R^3 and R^4 together with the Carbon to which they are attached represent an *N*-Substituted Piperidine Derivative

The preparation of compounds of Formula I where R^1 and R^2 or R^3 and R^4 together with the carbon to which they are attached represent an *N*-substituted piperidine derivative are prepared from the corresponding unsubstituted piperidine derivative. This procedure is exemplified by reference to a compound of Formula I where R^3 and R^4 together with the carbon to which they are attached represent an *N*-substituted piperidine derivative, designated as compounds of Formula II, as shown below in Reaction Scheme XIII.

REACTION SCHEME XIII



Step 1 - Preparation of Compounds of Formula Im

Compounds of Formula I where Y is *t*-BuONH-, R^1 and R^2 are as defined in the compounds of formula I, and R^3 and R^4 together with the carbon to which they are attached represent an *N*-substituted piperidine derivative, are designated as compounds of Formula Im.

In general, compounds of Formula Im are prepared by reacting a compound of Formula II with a compound of the formula RX, where R is lower alkyl, cycloalkylalkyl, acyl, alkoxycarbonylalkyl, picolyl, $-SO_2R^a$, where R^a is lower alkyl or $-NR^bR^c$, where R^b and R^c are independently hydrogen or lower alkyl; and the like, and X is chloro, bromo or iodo; for example, RX may be methyl iodide, cyclopropylmethyl bromide, 3-picolyl chloride, ethyl bromoacetate, bromoacetamide, acetyl chloride, dimethylaminosulfonyl chloride, in the presence of a base, for example triethylamine or potassium carbonate. The reaction is carried out in a polar solvent, preferably *N,N*-dimethylformamide, in the temperature range from about 0°C to 50°C, preferably at about 25°C, for about 4 to 48 hours, preferably about 16 hours. The reaction product, a compound of Formula Im, is isolated by conventional means and preferably used with no further purification.

Alternatively, a reductive alkylation may be carried out on a compound of Formula II to give a compound of Formula Im. For example, reducing a compound of Formula II in acetone in the presence of a catalyst, for example palladium on carbon, under hydrogen gives an *N*-isopropyl derivative of Formula Im.

Step 2 - Preparation of Compounds of Formula In

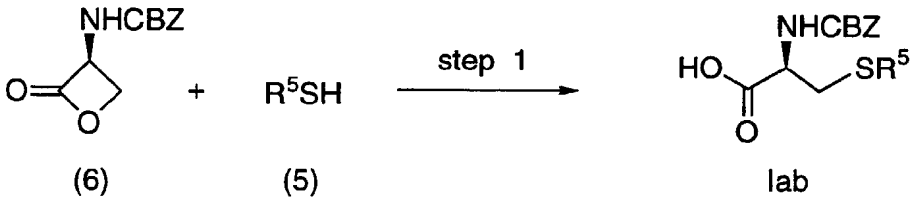
Compounds of Formula I where Y is HONH-, R^1 and R^2 are as defined in the compounds of formula I, and R^3 and R^4 together with the carbon to which they are attached represent an *N*-substituted piperidine derivative, are designated as compounds of Formula In.

In general, compounds of Formula In are prepared from a compound of Formula Im by reaction with a strong acid, preferably hydrochloric acid. The reaction is carried out in a sealed tube in an inert solvent, preferably 1,2-dichloroethane, in the temperature range from about 0°C to 45°C, preferably at about 20°C, for about 10 to 72 hours, preferably about 48 hours. The reaction product, a compound of Formula In, is isolated and purified by conventional means, preferably by chromatography.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

Compounds of Formula I where R^2 is $-NR^6R^7$, in which R^6 is hydrogen and R^7 is CBZ, where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, shown below, for example, as Formulae Ip and Iq, are prepared by a different route, as shown in Reaction Schemes XIV, XV, and XVI. This route provides compounds of Formula Iab, optically pure or as racemic mixtures, depending upon the chirality of the starting lactone.

REACTION SCHEME XIV



(6) (5) Iab

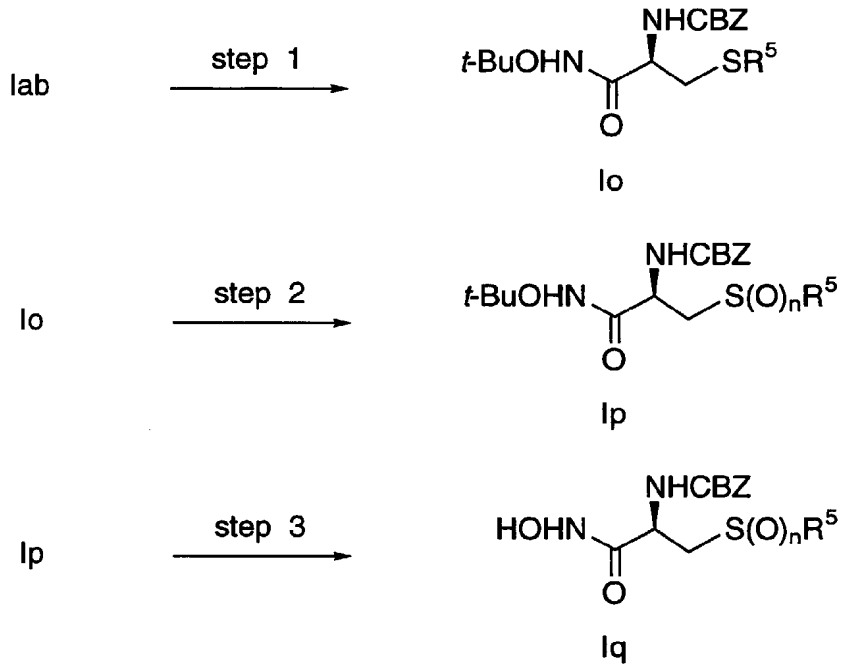
Step 1 - Preparation of Compounds of Formula Iab

In general, compounds of Formula Ia where Y is hydroxy, R^2 is $-NR^6R^7$, in which R^6 is hydrogen and R^7 is CBZ, where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula Iab, are prepared by treating an anion of a thiol of Formula (5) (preferably prepared *in situ,* for example, by treatment of Formula (5) with sodium or potassium hydride, preferably potassium hydride, in a polar solvent, preferably *N, N*-dimethylformamide) with a lactone of Formula (6). The reaction is carried out in a polar solvent, preferably *N, N*-dimethylformamide, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 5 minutes to 10 hours, preferably about 30 minutes to 6 hours. The sulfide reaction product, a compound of Formula Iab, is isolated by conventional means and preferably used directly in the next step.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

Compounds of Formula I where R^2 is $-NR^6R^7$, in which R^6 is hydrogen and R^7 is CBZ, where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, are prepared from compounds of Formula Iab as shown below in Reaction Scheme XV.

REACTION SCHEME XV



Step 1 - Preparation of Compounds of Formula Io

Compounds of Formula I where Y is *tert*-BuONH-, R^2 is -NHCBZ where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula Io, are prepared as shown in the same manner as shown in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

Step 2 - Preparation of Compounds of Formula Ip

Compounds of Formula Ip where n is 2, Y is *tert*-BuONH-, R^2 is -NHCBZ where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of the Formula Ip, are prepared in the same manner as shown in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

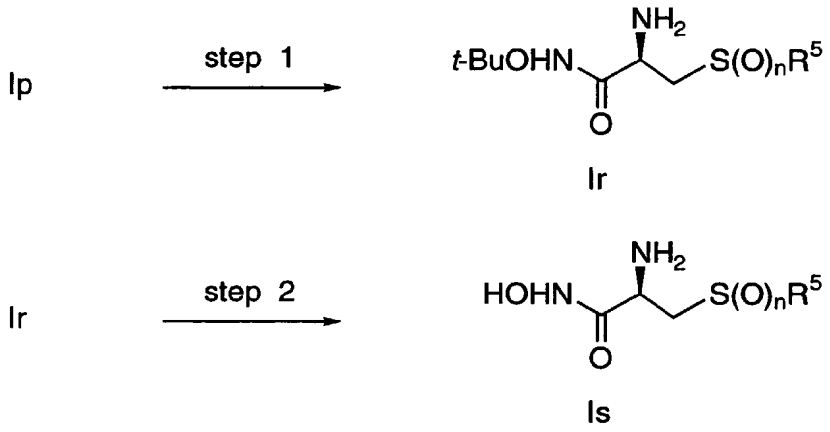
Step 3 - Preparation of Compounds of Formula Iq

Compounds of Formula I where n is 2, Y is HONH-, R^2 is -NHCBZ where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are as defined in the compounds of formula I, designated as compounds of the Formula Iq, are prepared by hydrolyzing a compound of Formula Ip in the same manner as shown above in Reaction Scheme VIII, or preferably as shown in Reaction Scheme IX or X.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

Compounds of Formula I where R^2 is $-NR^6R^7$, in which R^6 and R^7 are both hydrogen, and R^1, R^3 and R^4 are hydrogen, are prepared from compounds of Formula Ip as shown below in Reaction Scheme XVI.

REACTION SCHEME XVI



Step 1 - Preparation of Compounds of Formula Ir

In general, compounds of Formula I where n is 2, Y is *tert*-BuONH-, R^2 is $-NH_2$, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula Ir, are prepared by reducing a compound of Formula Ip using a metal catalyst, preferably palladium on carbon. The reaction is carried out under hydrogen at about 1 atmosphere, in a protic solvent, preferably ethanol, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 4 to 48 hours, preferably about 18 hours. The *N-tert*-butoxy reaction product, a compound of Formula Ir, is isolated and purified by conventional means.

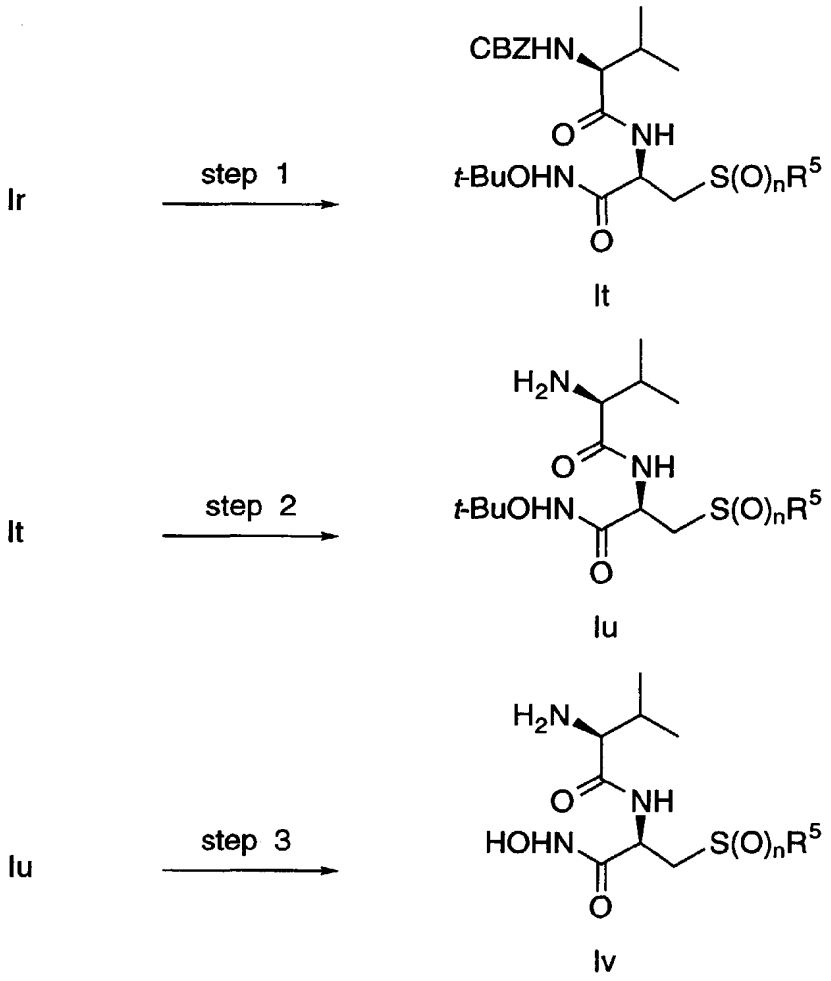
Step 2 - Preparation of Compounds of Formula Is

In general, compounds of Formula I where n is 2, Y is HONH-, R^2 is $-NH_2$, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula Is, are prepared by reacting a compound of Formula Ir with a strong acid, preferably hydrochloric acid. The reaction is carried out in a sealed tube in an inert solvent, preferably 1,2-dichloroethane, in the temperature range from about -10°C to 40°C, preferably at about 25°C, for about 4 to 48 hours, preferably about 18 hours. The hydroxyamino reaction product, a compound of Formula Is, is isolated and purified by conventional means, preferably as its hydrochloride salt.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

Alternatively, the compound of Formula Ir can be used to produce other compounds of Formula I where R^6 and/or R^7 are as defined in the Summary of the invention, but not both hydrogen. For example, the preparation of a compound of Formula I where R^2 is valine amide is shown below in Reaction Scheme XVII.

REACTION SCHEME XVII



Step 1 - Preparation of Compounds of Formula It

In general, compounds of Formula I where n is 2, Y is *tert*-BuONH-, R^2 is 2-(*S*)-CBZ-valine amide, *i.e.,* where R^6 is hydrogen and R^7 is 2-(*S*)-CBZ-3-methyl-1-butanoyl, where CBZ represents benzyloxycarbonyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula It, are prepared by reacting a compound of Formula Ir with CBZ-(*S*)-valine in the presence of *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide and 1-hydroxybenzotriazole and a slight excess of a tertiary amine, preferably triethylamine. The reaction is carried out in an inert solvent, preferably methylene chloride, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 6-48 hours, preferably about 16 hours. The reaction product, a compound of Formula It, is isolated by conventional means, and is preferably used in the next step without further purification.

Step 2 - Preparation of Compounds of Formula Iu

In general, compounds of Formula I where n is 2, Y is *tert*-BuONH-, R^2 is 2-(*S*)-amino-valine amide, *i.e.,* where R^6 is hydrogen and R^7 is 2-(*S*)-amino-3-methyl-1-butanoyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula It, are prepared by reducing a compound of Formula It using a metal catalyst, preferably palladium on carbon. The reaction is carried out under hydrogen at about 1 atmosphere, in a protic solvent, preferably a mixture of methanol

and ethanol, in the temperature range from about 0°C to 40°C, preferably at about 25°C, for about 1 to 8 hours, preferably about 3 hours. The reaction product, a compound of Formula Iu, is isolated and purified by conventional means, preferably chromatography.

Step 3 - Preparation of Compounds of Formula Iv

In general, compounds of Formula I where n is 2, Y is HONH-, R^2 is 2-(*S*)-amino-valine amide, *i.e.,* where R^6 is hydrogen and R^7 is 2-(*S*)-amino-3-methyl-1-butanoyl, and R^1, R^3 and R^4 are hydrogen, designated as compounds of Formula Iv, are prepared by reacting a compound of Formula Iu with a strong acid, preferably hydrochloric acid. The reaction is carried out in a sealed tube in an inert solvent, preferably 1,2-dichloroethane, in the temperature range from about -20°C to 40°C, preferably at about 25°C, for about 4 to 48 hours, preferably about 24 hours. The hydroxyamine reaction product, a compound of Formula Iv, is isolated and purified by conventional means, preferably as its hydrochloride salt.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

In a manner similar to that shown above, compounds of Formula I where R^2 is $-NR^6R^7$, in which R^6 and R^7 are both methyl, are prepared by reacting a compound of Formula Ir in a polar solvent, preferably *N,N*-dimethylformamide, with about two equivalents of methyl iodide in the presence of a base, preferably potassium carbonate, then treating the product with hydrochloric acid gas as shown in Step 3 above.

Preparation of Compounds of Formula I where R^2 is $-NR^6R^7$

In a manner similar to that shown above, compounds of Formula I where where R^2 is $-NR^6R^7$, in which R^6 is hydrogen and R^7 is $-NHSO_2N(CH_3)_2$, are prepared by reacting a compound of Formula Ir with about one equivalent of dimethylsulfamoyl chloride in an inert solvent, preferably methylene chloride, in the presence of a base, preferably pyridine, then treating the product with hydrochloric acid gas as shown in Step 3 above.

Similarly, the compound of Formula Ir can be used to produce other compounds of Formula I where R^6 and/or R^7 are as defined in the Summary of the invention, but not both hydrogen, in the same manner as shown in Reaction Scheme XVII above.

Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the Examples hereinbelow. However, other equivalent separation or isolation procedures could, of course, also be used.

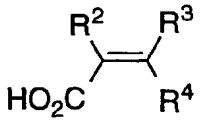
Salts of Compounds of Formula I

Some of the compounds of Formula I may be converted to a corresponding acid addition salt by virtue of the presence of basic nitrogen atoms. The conversion is accomplished by treatment with at least a stoichiometric amount of an appropriate acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid and the like. Typically, the free base is dissolved in an inert organic solvent such as diethyl ether, ethyl acetate, chloroform, ethanol or methanol and the like, and the acid added in a similar solvent. The temperature is maintained at 0° to 50°C. The resulting salt precipitates spontaneously or may be brought out of solution with a less polar solvent.

In summary, the compounds of the present invention are made by the procedures outlined below:

1. A process for preparing compounds of Formula I where R^1 is hydrogen comprises:

reacting a compound of the formula:



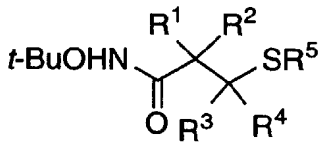
(4)

where R^2, R^3 and R^4 are as defined in the compounds of formula I, except that R^2 cannot be $-NR^6R^7$;

with a compound of the formula R^5SH, where R^5 is as defined in the compounds of formula I, in the presence of a secondary base.

2. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

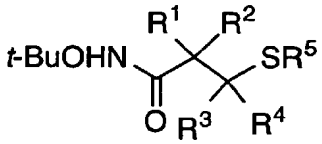


where R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

with a mild oxidizing agent, for example, sodium periodate.

3. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

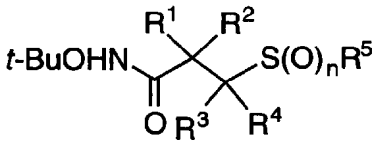


where R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

with a strong oxidizing agent, for example, OXONE or m-chloroperbenzoic acid.

4. Alternatively, a process for preparing compounds of Formula I where n is 2 comprises:

reacting a compound of the formula:

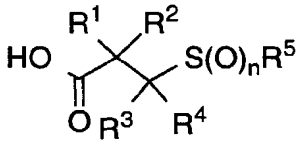


where R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

with a strong oxidizing agent, for example, OXONE or m-chloroperbenzoic acid.

5. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

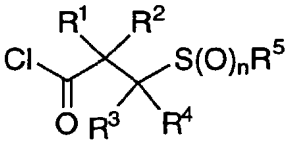


where n, R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

with *O*-(*tert*-butyl)hydroxylamine hydrochloride in the presence of a carbodiimide, for example, *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride, and a tertiary amine.

6. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

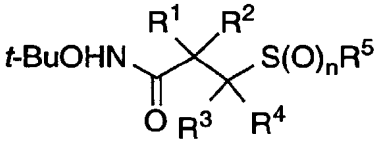


where n, R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

with hydroxylamine or *N,O*-bistrimethylsilyl hydroxylamine.

7. Alternatively, a process for preparing compounds of Formula I comprises:

hydrolysing a compound of the formula:

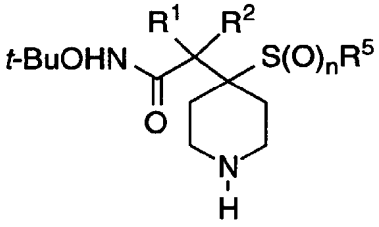


where n, R^1, R^2, R^3, R^4 and R^5 are as defined in the compounds of formula I,

under acid conditions, for example, with hydrochloric acid or trifluoroacetic acid.

8. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

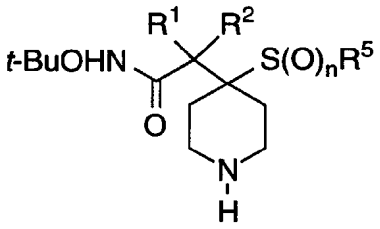


where n, R^1, R^2 and R^5 are as defined in the compounds of formula I, except that R^2 cannot be $-NR^6R^7$;

with a compound of the formula RX, where R is lower alkyl, cycloalkylalkyl, acyl, alkoxycarbonylalkyl, acetamido, picolyl, $-SO_2R^a$, where R^a is lower alkyl or NR^bR^c, where R^b and R^c are independently hydrogen or lower alkyl; and X is chloro, bromo or iodo.

9. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

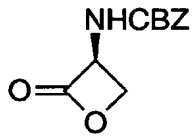


where n, R^1, R^2 and R^5 are as defined in the compounds of formula I, except that R^2 cannot be $-NR^6R^7$;

with acetone under hydrogen in the presence of a catalyst, for example, palladium on carbon, to give the *N*-isopropyl derivative.

10. Alternatively, a process for preparing compounds of Formula I comprises:

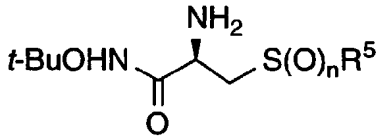
reacting a compound of the formula:



with an anion of a compound of the formula R^5 SH, where R^5 is as defined in the compounds of formula I.

11. Alternatively, a process for preparing compounds of Formula I comprises:

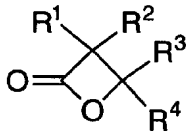
reacting a compound of the formula:



where R^5 is as defined in the compounds of formula I, with an acylating agent, for example CBZ-(*S*)-valine in the presence of *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide and 1-hydroxybenzotriazole and a tertiary amine, or an alkylating agent, for example, methyl iodide in the presence of a base or a sulfamoyl halide, such as dimethylsulfamoyl chloride in the presence of a base.

12. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:

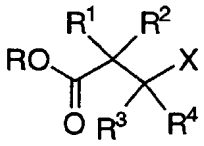


where R^1, R^2, R^3 and R^4 are as defined in the compounds of formula I, except that R^2 cannot be $-NR^6R^7$;

with a compound of the formula R^5SH, where R^5 is as defined in the compounds of formula I, in the presence of a secondary base.

13. Alternatively, a process for preparing compounds of Formula I comprises:

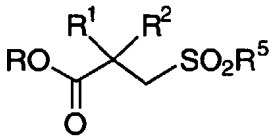
reacting a compound of the formula:



with an anion of a compound of the formula R^5SH, where R^5 is as defined in the compounds of formula I.

14. Alternatively, a process for preparing compounds of Formula I comprises:

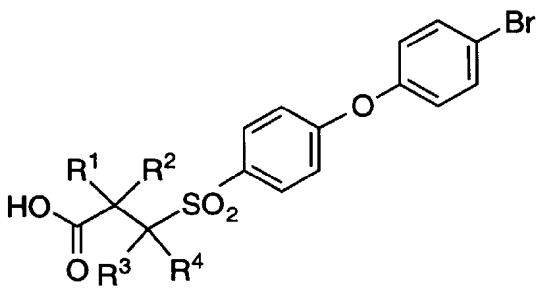
reacting a compound of the formula:



with an alkyl or aralkyl halide in the presence of a hindered base.

15. Alternatively, a process for preparing compounds of Formula I comprises:

reacting a compound of the formula:



with a compound of the formula $R^{11}B(OH)_2$ or $R^{11}SnMe_3$, where R^{11} is aryl or heteroaryl, in the presence of tetrakis(triphenylphosphine)-palladium(0).

The compounds of Formula I inhibit mammalian matrix metalloproteases, such as the stromelysins, gelatinases, matrilysin and collagenases, and are therefore useful as therapeutically active substances, especially for treating diseases associated with the MMP-induced excessive degradation of matrix and connective tissue within the mammal, for example, arthritic diseases (rheumatoid arthritis and osteoarthritis), multiple sclerosis, bone resorptive diseases (such as osteoporosis), the enhanced collagen destruction associated with diabetes, chronic obstructive pulmonary disease, cerebral hemorrhaging associated with stroke, periodontal disease, corneal ulceration, ulceration of the skin, tumor invasion and metastasis, and aberrant angiogenesis.

The compounds of Formula I substantially inhibit the release of tumor necrosis factor (TNF) from cells, and are therefore useful for the treatment of conditions mediated by TNF, for example inflammation, fever, cardiovascular effects, hemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, restinosis, aneurysmal disease, graft versus host reactions and autoimmune disease.

The compounds of Formula I also inhibit the release of other biologically active molecules from cells, including soluble receptors (CD30 and receptors for TNF (p55 and p75), IL-6, IL-1 and TSH), adhesion molecules (*e.g.,* L-selection, ICAM-1, fibronectin) and other growth factors and cytokines, including Fas ligand, TGF-α, EGF, HB-EGF, SCF and M-CSF. Inhibition of the release or shedding of such proteins, and are therefore useful for treating a number of disease states, for example rheumatoid arthritis, multiple sclerosis, vascular disease, Type II diabetes, HIV, cachexia, psoriasis, allergy, hepatitis, inflammatory bowel disease, and cancer.

The ability of the compounds of Formula I to inhibit matrix metalloprotease activity, such as the activity of collagenase-1, -2 and -3, stromelysin-1, gelatinases A and B, and matrilysin may be demonstrated by a variety of *in vitro* assays known to those of ordinary skill in the art, such as the assay described in the MMP Enzymatic Assay described in *FEBS,* **296**, 263 (1992) or modifications thereof. The ability of the compounds of Formula I to inhibit MMP mediated processes *in vivo* may be tested using the interleukin-1 stimulated cartilage explant assay and cartilage plug implantation assay.

The ability of the compounds of Formula I to inhibit the release of TNF as shown in Examples 45 to 47.

The present invention also relates to a pharmaceutical composition comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of a compound of formula I.

Administration of the compounds of Formula I or their pharmaceutically acceptable salts, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration or agents for serving similar utilities. Thus, administration can be, for example, orally, nasally, parenterally, topically, transdermally, or rectally, in the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as for example, tablets, suppositories, pills, soft elastic and hard gelatin capsules, powders, solutions, suspensions, or aerosols, or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and a compound of Formula I as the/an active agent, and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, *etc.*

Generally, depending on the intended mode of administration, the pharmaceutically acceptable compositions will contain about 1% to about 99% by weight of a compound(s) of Formula I, or a pharmaceutically acceptable salt thereof, and 99% to 1% by weight of a suitable pharmaceutical excipient. Preferably, the composition will be about 5% to 75% by weight of a compound(s) of Formula I, or a pharmaceutically acceptable salt thereof, with the rest being suitable pharmaceutical excipients.

The preferred route of administration is oral, using a convenient daily dosage regimen which can be adjusted according to the degree of severity of the disease-state to be treated. For such oral administration, a pharmaceutically acceptable composition containing a compound(s) of Formula I, or a pharmaceutically acceptable salt thereof, is formed by the incorporation of any of the normally employed excipients, such as for example, pharmaceutical grades of mannitol, lactose, starch, pregelatinized starch, magnesium stearate, sodium saccharine, talcum, cellulose ether derivatives, glucose, gelatin, sucrose, citrate, propyl gallate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations, and the like.

Preferably such compositions will take the form of capsule, caplet or tablet and therefore will also contain a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant, such as croscarmellose sodium or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as a starch, gum acacia, polyvinylpyrrolidone, gelatin, cellulose ether derivatives, and the like.

The compounds of Formula I, or their pharmaceutically acceptable salts, may also be formulated into a suppository using, for example, about 0.5% to about 50% active ingredient disposed in a carrier that slowly dissolves within the body, *e.g.,* polyoxyethylene glycols and polyethylene glycols (PEG), *e.g.,* PEG 1000 (96%) and PEG 4000 (4%).

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, *etc.,* a compound(s) of Formula I (about 0.5% to about 20%), or a pharmaceutically acceptable salt thereof, and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

If desired, a pharmaceutical composition of the invention may also contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, antioxidants, and the like, such as, for example, citric acid,

sorbitan monolaurate, triethanolamine oleate, butylated hydroxytoluene, *etc.*

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences,* 18th Edition, Mack Publishing Company, Easton, Pennsylvania (1990). The composition to be administered will, in any event, contain a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof, for treatment of a disease-state alleviated by the inhibition of matrix metalloprotease activity in accordance with the teachings of this invention.

The compounds of Formula I or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount which will vary depending upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of the compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular disease-state, and the host undergoing therapy. Generally, a therapeutically effective daily dose is from about 0.014 mg to about 14.3 mg/kg of body weight per day of a compound of Formula I or a pharmaceutically acceptable salt thereof; preferably, from about 0.07 mg to about 5 mg/kg of body weight per day; and most preferably, from about 0.14 mg to about 1.4 mg/kg of body weight per day. For example, for administration to a 70 kg person, the dosage range would be from about 1 mg to about 1.0 gram per day of a compound of Formula I or a pharmaceutically acceptable salt thereof, preferably from about 5 mg to about 300 mg per day, and most preferably from about 10 mg to about 100 mg per day.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

EXAMPLE 1

Preparation of Compounds of Formula (1)

1A. Preparation of (1) where R^3 and R^4 when taken together with the Carbon to which they are attached represent *N*-CBZ-piperidine

1. A solution of benzyl chloroformate (35 ml, 247 mmol) in tetrahydrofuran (70 ml) was added to an ice-cold solution of 4-hydroxypiperidine (25 g, 247 mmol) and triethylamine (45 ml, 321 mmol) in tetrahydrofuran (350 ml). The mixture was stirred overnight at room temperature and the solvent removed under reduced pressure. The residue was partitioned between 5% hydrochloric acid and ethyl acetate, and the organic layer washed with brine, dried over magnesium sulfate, and the solvent removed under reduced pressure to give 4-hydroxy-*N*-CBZ-piperidine as a pale yellow oil.

2. Celite (66 g) was added to a solution of 4-hydroxy-*N*-CBZ-piperidine (18 g, 76.5 mmol) in methylene chloride (500 ml), followed by pyridinium chlorochromate (33 g, 153 mmol). The mixture was stirred overnight, and then isopropyl alcohol (12 ml) was added over a period of 3 hours. The reaction mixture was filtered through silica gel and the filter cake was repeatedly rinsed with methylene chloride and ethyl acetate. The combined filtrates were evaporated under reduced pressure. Silica gel chromatography using 50% ethyl acetate/hexane, gave 4-oxo-*N*-CBZ-piperidine as a yellow oil.

EXAMPLE 2

Preparation of Compounds of Formula (3)

2A. Preparation of (3) where R^2 is Hydrogen, and R^3 and R^4 when taken together with the Carbon to which they are attached represent *N*-CBZ-piperidine

tert-(Butoxycarbonylmethylene)triphenylphosphorane (28 g, 74.4 mmol) was added to 4-oxo-*N*-CBZ-piperidine (14.2 g, 61.3 mmol) in benzene (150 ml), and the solution was stirred at reflux overnight. The solution was concentrated, and the residue triturated with hexane (500 ml). Filtration and concentration of the filtrate gave 4-*tert*-butoxycarbonyl-methylene-*N*-CBZ-piperidine as a colorless oil.

2B. Preparation of (3), varying R^2, R^3, and R^4

Similarly, following the procedures of Example 2A above, but replacing 4-oxo-*N*-CBZ-piperidine with:

formaldehyde;
acetone;
propionaldehyde;
cyclopentanone;
cyclohexanone;
1,4-cyclohexanedione mono-ethylene ketal;
4-methylcyclohexanone;
phenylacetaldehyde;
4-(biphen-4-yl)butyraldehyde;
cyclopentylacetaldehyde;
tetrahydropyranone; and
tetrahydrothiopyran;

and optionally replacing *tert*-(butoxycarbonylmethylene)triphenylphosphorane with:

tert-butyl-3-phenylpropionate-2-triphenylphosphorane;
tert-butyl-propionate-2-triphenylphosphorane; and
tert-butyl-3-methylpropionate-2-triphenylphosphorane;

the following compounds of Formula (3) were prepared:

1-(*tert*-butoxycarbonyl)-1-benzylethene;
1-(*tert*-butoxycarbonyl)-2,2-dimethylethene;
1-(*tert*-butoxycarbonyl)-1-methyl-2-ethylethene;
tert-butoxycarbonylmethylenecyclopentane;
tert-butoxycarbonylmethylenecyclohexane;
tert-butoxycarbonylmethylene-4-methylcyclohexane;
1-(*tert*-butoxycarbonyl)-2-benzylethene;
1-(*tert*-butoxycarbonyl)-1-isopropyl-2-benzylethene;
1-(*tert*-butoxycarbonyl)-2-[3-(biphen-4-yl)]propylethene;
1-(*tert*-butoxycarbonyl)-2-cyclopentylmethylethene;
4-(*tert*-butoxycarbonylmethylene)-tetrahydropyran; and
4-(*tert*-butoxycarbonylmethylene)-tetrahydrothiopyran.

2C. Preparation of (3), varying R^2, R^3, and R^4

Similarly, following the procedures of Example 2A above, but optionally replacing 4-oxo-*N*-CBZ-piperidine with other compounds of Formula (1), and optionally replacing (*tert*-butoxycarbonylmethylene)triphenyl-phosphorane with other compounds of Formula (2), other compounds of Formula (3) are prepared.

EXAMPLE 3

Preparation of Compounds of Formula (4)

3A. Preparation of (4) where R^2 is Hydrogen, and R^3 and R^4 when taken together with the Carbon to which they are attached represent *N*-CBZ-piperidine, a Compound of Formula (4a)

Trifluoroacetic acid (10 ml) was added to 4-*tert*-butoxycarbonylmethylene-*N*-CBZ-piperidine (20 g, 60.3 mmol) in methylene chloride (30 ml) and the solution was stirred at room temperature for 1.5 hours. After evaporation of the solvent, the residue was triturated with diethyl ether to give 4-carboxymethylene-*N*-CBZ-piperidine as a crystalline white solid.

3B. Preparation of (4) where R^2 is Hydrogen, and R^3 and R^4 when taken together with the Carbon to which they are attached represent Tetrahydropyran, a Compound of Formula (4b)

Methanol (204 ml) was slowly added to a suspension of sodium hydride (5.48 g, 228.2 mmol) in tetrahydrofuran (204 ml) at 0°C. When addition was complete, trimethylphosphonoacetate (34.22 ml, 211.4 mmol) was added to the mixture at such a rate as to maintain the temperature below 12°C. Stirring was continued for a further 10 minutes. To this reaction mixture was added a solution of 2,3,5,6-tetrahydropyran-4-one (16.28 g, 163.0 mmol) in tetrahydrofuran

(20 ml), keeping the temperature below 30°C. After the addition was complete, stirring was continued for 30 minutes at room temperature, then methanol (100 ml) and 2M sodium hydroxide (326 ml) was added, and the mixture stirred overnight at room temperature. The resulting solution was concentrated to one half of the original volume, and acidified to pH 1.2 with 6M hydrochloric acid (108 ml). The reaction mixture was partitioned between ethyl acetate and water, the combined organic extracts dried over magnesium sulfate, and solvent removed under reduced pressure to give 4-(carboxymethylene)-2,3,5,6-tetrahydropyran (22.62 g), which was used with no further purification.

3C. Preparation of (4), varying R^2, R^3, and R^4

Similarly, following the procedures of Example 3A above, but replacing 4-(*tert*-butoxycarbonylmethylene)-*N*-CBZ-piperidine with other compounds of Formula (3), the following compounds of Formula (4) were prepared:

1-benzyl-1-carboxyethene;
1-carboxy-2,2-dimethylethene;
1-carboxy-2-ethyl-1-methylethene;
carboxymethylenecyclopentane;
carboxymethylenecyclohexane;
carboxymethylene-(4-methylcyclohexane);
4-carboxymethylenecyclohexanone mono-ethylene ketal;
2-benzyl-1-carboxyethene;
2-[3-(biphen-4-yl)propyl]-1-carboxyethene;
2-benzyl-1-carboxy-1-isopropylethene;
1-carboxy-2-cyclopentylmethylethene;
4-carboxymethylene-tetrahydrothiopyran; and
4-carboxymethylene-(tetrahydrothiopyran-1,1-dioxide).

3D. Preparation of (4), varying R^2, R^3, and R^4

Similarly, following the procedures of Example 3A above, but replacing 4-(*tert*-butoxycarbonylmethylene)-*N*-CBZ-piperidine with other compounds of Formula (3), other compounds of Formula (4) are prepared, or may be prepared by means well known to those skilled in the art. Alternatively, they are commercially available, for example, 1-cyclopentene carboxylic acid and 1-cyclohexene carboxylic acid are available from Lancaster Synthesis Inc.

EXAMPLE 4

Preparation of Compounds of Formula (5)

4A. Preparation of (5) where R^5 is 4-Phenoxyphenyl

A solution of sodium thiomethoxide (25 g) and 4-bromodiphenyl ether (25 g) in *N,N*-dimethylformamide (DMF) (150 ml) was refluxed overnight. The mixture was cooled and added to dilute aqueous sodium hydroxide. The water layer was washed with ether to remove by-products and acidified with hydrochloric acid. The product, 4-(phenoxy)thiophenol, was extracted with ether, and the ether layer dried and evaporated to give 4-(phenoxy)thio-phenol (19-20 g) as a red oil. This material can be used without further purification.

4B. Alternative Preparation of (5) where R^5 is 4-(4-Bromophenoxy)phenyl

A solution of 4-bromodiphenyl ether (50 g, 200.7 mmol) in methylene chloride (118 ml) was cooled to 0°C and chlorosulfonic acid (14.7 ml, 220.8 mmol) was added dropwise over a 20 minute period. The solution was stirred an additional 10 minutes, warmed to room temperature and stirred an additional 1 hour. To this mixture was added oxalyl chloride (23.6 ml, 270.9 mmol), followed by *N,N*-dimethylformamide (1.5 ml) as a catalyst, and the mixture refluxed for 2 hours. The mixture was cooled to room temperature, and additional oxalyl chloride (23.6 ml, 270.9 mmol) was added, the mixture refluxed for 3 hours, cooled to room temperature and stirred 12 hours more. The solution was concentrated to an oil, azeotroped several times using methylene chloride and put under high vacuum (1 torr) for several hours until the mixture had completely solidified. This mixture was immediately dissolved in methylene chloride (160 ml) which was added dropwise to a solution of triphenylphosphine (157.0 g, 602 mmol) in methylene chloride (160 ml) containing *N,N*-dimethylformamide (4 ml, 52.2 mmol). The mixture was stirred 2 hours, diluted with 1M aqueous hydrochloric acid (300 ml) and stirred for 1 hour. The aqueous layer was separated, extracted with methylene chloride (200 ml), and the organic layers were combined, washed with 200 ml of brine, dried ($MgSO_4$) and concentrated *in vacuo.* The resulting

solid was further purified through trituration with 750 ml of hexane. The solid was then dissolved in 750 ml of diethyl ether, extracted with 2M aqueous sodium hydroxide (2 x 350 ml), and the basic aqueous layer back extracted using diethyl ether (2 x 400 ml). The aqueous layer was adjusted to pH 2, extracted with diethyl ether (3 x 200 ml) and the combined organic layers dried ($MgSO_4$) and concentrated to afford 4-(4-bromophenoxy)thiophenol (45.6 g, 81%). ^{1}HNMR ($CDCl_3$) δ 3.43 (s, 1H), 6.86 (d, J = 8.9 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 8.9 Hz, 2H).

The corresponding 4-chloro and 4-fluoro analogues were obtained in similar fashion from the corresponding commercially available 4-halodiphenylethers, respectively.

4-(4-chlorophenoxy)thiophenol: ^{1}HNMR ($CDCl_3$) δ 3.43 (s, 1H), 6.90 (m_c, 4H), 7.27 (m_c, 4H).
4-(4-fluorophenoxy)thiophenol: ^{1}HNMR ($CDCl_3$) δ 3.41 (s, 1H), 6.85 (d, J = 8.7 Hz, 2H), 7.00 (m_c, 4H), 7.26 (d, J = 8.7 Hz, 2H).
4-(4-pyridyloxy)thiophenol: ^{1}HNMR ($CDCl_3$) δ 7.05 (d, J =9.0 Hz, 2H), 7.29 (d, J = 7.3 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 8.70 (d, J = 7.3 Hz, 2H); EIMS (M^+): 203.
4-(5-chloro-2-pyridyloxy)thiophenol: ^{1}HNMR ($CDCl_3$) δ 6.87 (d, J =8.5 Hz, 1H), 7.01 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 2.8 Hz, 1H).

EXAMPLE 5

Preparation of Compounds of Formula (10)

5A. Preparation of a Compound of Formula (8) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran, a Compound of Formula (8a)

A solution of 1.5M diisobutylaluminum hydride (DIBAL-H) (419 ml, 629 mmol) in toluene was added to a 3-L Morton flask equipped with a nitrogen gas inlet, mechanical stirrer, low temperature thermometer, 500 ml pressure equalizing funnel, and containing tetrahydropyran-4,4-dicarboxylic acid diethyl ester (70.78 g, 307.4 mmol) in toluene (600 ml) at -40°C, at a rate to maintain an internal temperature no higher than -25°C. The mixture was stirred an additional 10 minutes and anhydrous ethanol (595 ml) was added dropwise over 20 minutes maintaining an internal temperature no higher than -15°C. Solid sodium borohydride (11.6 g, 307.4 mmol) was added in three portions over a 15 minute period, the cooling bath was removed, the mixture allowed to warm to room temperature over 1 hour, and saturated aqueous sodium sulfate (325 ml) added over 15 minutes. The mixture was cooled to -15°C, ethyl acetate (250 ml) was added, and the flocculent white precipitate filtered over a pad of celite. The celite pad was washed with ethyl acetate (7 x 450 ml), the filtrate washed with brine (200 ml), dried over magnesium sulfate, and concentrated *in vacuo*. The residue was dissolved in the minimum amount of ethyl acetate, filtered through a sintered glass funnel containing silica gel (40 g), eluting with ethyl acetate, and the filtrate concentrated *in vacuo* to afford the hydroxyester, 4-(hydroxymethyl)tetrahydropyran-4-carboxylic acid ethyl ester, as a pale yellow oil (48.5 g, 84%).

5B. Alternative Preparation of a Compound of Formula (8) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran

1. To a solution of tetrahydropyran-4,4-dicarboxylic acid diethyl ester (400 mg, 1.74 mmol) in *N,N*-dimethylformamide (4 ml), was added lithium iodide (1.16 g, 8.66 mmol), followed by sodium cyanide (94 mg, 1.91 mmol). The mixture was heated at 130°C for 7 hours, 140°C for 25 hours, after which GC analysis indicated the reaction to be >95% complete. The mixture was partitioned between 33% diethyl ether/hexanes (100 ml) and brine (25 ml). The organic layer was washed with additional brine (25 ml), dried ($MgSO_4$) and concentrated *in vacuo* to afford the tetrahydropyran-4-carboxylic acid ethyl ester (253 mg, 92%). Note: Substitution of 2 equivalents of sodium acetate for 1.1 equivalents of sodium cyanide in this reaction and heating 12 hours longer provides identical results.

2. Lithium diisopropylamide was prepared by the addition of 2.5M *N*-butyl lithium (30.3 ml, 75.6 mmol) in hexanes to a solution of diisopropylamine (10.6 ml, 75.6 mmmol) in tetrahydrofuran (244 ml) at 0°C and stirring for 20 minutes. Then a solution of tetrahydropyran-4-carboxylic acid ethyl ester (10 g, 63.2 mmol) in tetrahydrofuran (50 ml) was added to the solution of lithium diisopropylamide over 15 minutes at -78°C. The resulting solution was stirred an additional 50 minutes, and solid paraformaldehyde (10 g) was added in one portion. The mixture was slowly allowed to warm to room temperature over 9 hours, diluted with 2M aqueous hydrochloric acid (100 ml), and filtered over a pad of celite pad which was washed with diethyl ether (2 x 200 ml). The aqueous layer of the filtrate was washed with additional portions of diethyl ether (2 x 200 ml). The combined organic layers were washed once with 2M aqueous hydrochloric acid (100 ml), saturated aqueous sodium bicarbonate (100 ml), dried over magnesium sulfate, and concentrated *in vacuo* to afford a slightly impure product 4-(hydroxymethyl)tetrahydropyran-4-carbox-

ylic acid ethyl ester (11.5 g, 97%), which was taken into the next reaction without further purification. IR (neat) 3433 (br), 1726 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.30 (t, J = 7.1 Hz, 3H), 1.57 (ddd, J = 13.8, 10.1, 4.4 Hz, 2H), 2.07 (dm, J = 13.8 Hz, 2H), 2.30-2.45 (br s, 1H), 3.56 (ddd, J = 11.9, 10.3, 2.7 Hz, 2H), 3.66 (s, 2H), 3.82 (dt, J = 11.9, 4.2 Hz, 2H), 4.24 (q, J = 7.2 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 14.25 (q), 30.54 (t), 46.63 (s), 61.04 (t), 64.79 (t), 69.02 (t), 175.24 (s); HRMS Calcd for $C_9H_{16}O_4$: 188.1049. Found: 188.1053.

5C. Preparation of a Compound of Formula (8) where R^1 and R^2 taken together with the Carbon to which they are attached represent Piperidine. a Compound of Formula (8)

Lithium diisopropylamide was prepared by the addition of 1.6M *N*-butyl lithium (29.1 ml, 46.6 mmol) in hexanes to a solution diisopropylamine (6.5 ml, 46.6 mmmol) in tetrahydrofuran (150 ml) at 0°C with stirring for 20 minutes at -78°C. Then a solution of neat *N*-(*tert*-butoxycarbonyl)-piperidine-4-carboxylic acid ethyl ester (10 g, 38.9 mmol) was added over 5 minutes, and the resulting solution was stirred an additional 50 minutes. Solid paraformaldehyde (13.5 g, 155.4 mmol) was added in one portion, and the mixture slowly allowed to warm to room temperature over 9 hours. The mixture was diluted with 2M aqueous hydrochloric acid (100 ml), filtered over a pad of celite, washed with diethyl ether (2 x 200 ml). The combined organic layers were washed once with 2M aqueous hydrochloric acid (100 ml), saturated aqueous sodium bicarbonate (100 ml), dried over magnesium sulfate, and concentrated *in vacuo.* Chromatography on silica gel, and eluting with 50% ethyl acetate/hexanes, yielded slightly impure *N*-(*tert*-butoxycarbonyl)-4-(hydroxymethyl)piperidine-4-carboxylic acid ethyl ester (10.57 g, 95%) as a pale yellow oil which was taken immediately into the hydrolysis reaction (LiOH): ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.4 Hz, 3H), 1.40-1.53 (m, 2H), 1.46 (s, 9H), 2.00-2.12 (m, 2H), 3.05-3.16 (m, 2H), 3.65 (s, 2H), 3.70-3.83 (m, 2H), 4.23 (q, J = 7.2 Hz, 2H).

5D. Preparation of a Compound of Formula (9) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran. a Compound of Formula (9a)

Lithium hydroxide monohydrate (16.7 g, 398.5 mmol) was added to a solution of 4-(hydroxymethyl)tetrahydropyran-4-carboxylic acid ethyl ester (25.0 g, 132.8 mmol) in 4.5:1 methanol/water (220 ml). The mixture was heated to reflux for 40 minutes and the methanol removed *in vacuo* by concentration using a bath temperature no higher than 45°C. The aqueous layer was then extracted into diethyl ether (4 x 100 ml) and the combined ether layers washed twice with 2M sodium hydroxide (15 ml). The combined aqueous base layers were cooled to 0°C, acidified to pH 3.0 with 8M aqueous hydrochloric acid, saturated with solid sodium chloride and extracted with ethyl acetate (8 x 250 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo.* The white fluffy powder residue was recrystallized from the minimum amount of methylene chloride/hexanes to afford pure 4-(hydroxymethyl)tetrahydropyran-4-carboxylic acid (17.05 g, 80%).

5E. Alternative Preparation of a Compound of Formula (9) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran

Lithium diisopropylamide was prepared by the addition of 2.45M *N*-butyl lithium (16.5 ml) in hexanes to a solution diisopropylamine (5.80 ml, 41.4 mmmol) in tetrahydrofuran (40 ml) at 0°C with stirring for 20 minutes. Then a solution of tetrahydropyran-4-carboxylic acid (2.5 g, 19.2 mmol) in tetrahydrofuran (10 ml) was added to the solution of lithium diisopropylamide over 15 minutes to form a slurry, followed by hexamethylphosphoramide (2 ml). The resulting solution was stirred for 25 minutes, then immediately warmed to room temperature after a stream of gaseous formaldehyde (prepared by heating 4 g of paraformaldehyde at 175-200°C over 5-10 minutes) was passed through the solution. The slurry was carefully concentrated at ambient temperature, acidified to pH 3 with 8M hydrochloric acid, saturated with solid sodium chloride, and extracted with ethyl acetate (8 x 100 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo.* Chromatography over silica gel (80 g), and eluting with 10% methanol/methylene chloride, yielded 4-(hydroxymethyl)tetrahydropyran-4-carboxylic acid as a white solid (1.80 g, 58%). mp 113.7-115°C; IR (KBr) 3420 (br), 1724 cm^{-1}, ^{1}HNMR (DMSO-d_6) δ 1.43 (ddd, J = 13.5, 11.0,4.4 Hz, 2H), 1.85 (dm, J = 13.4 Hz, 2H), 3.37 (td, J = 11.3, 3.0 Hz, 2H), 3.43 (s, 2H), 3.71 (dt, J = 11.6, 3.9 Hz, 2H), 4.81 (br, s, 1H); 12.24 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 30.42 (t), 46.38 (s), 64.35 (t), 68.15 (t), 69.02 (t), 176.08 (s); HRMS Calcd. for $C_7H_{12}O_4$: 160.0735. Found: 160.0731. Anal. Calcd. for $C_7H_{12}O_3$: C, 52.49; H, 7.55. Found: C, 52.50; H, 7.62.

5F. Preparation of a Compound of Formula (9) where R^1 and R^2 taken together with the Carbon to which they are attached represent Piperidine. a Compound of Formula (9b)

Lithium hydroxide monohydrate (6.95 g, 165.6 mmol) was added to solution of *N*-(*tert*-butoxycarbonyl)-4-(hydroxymethyl)piperidine-4-carboxylic acid ethyl ester (9.52 g, 33.1 mmol) in 2:1 methanol/water (100 ml). The mixture was heated to reflux for 30 minutes, the methanol removed *in vacuo* by concentration using a bath temperature no

higher than 45°C. The aqueous layer was cooled to 0°C, acidified to pH 3.0 using 6M aqueous hydrochloric acid, and extracted with ethyl acetate (4 x 75 ml). The combined organic layers were dried over magnesium sultate, and concentrated *in vacuo*, and recrystallized from dichloromethane/hexanes to afford *N*-(*tert*-butoxycarbonyl)-4-(hydroxymethyl)piperidine-4-carboxylic acid (8.59 g, 100%).

5G. Alternative Preparation of a Compound of Formula (9) where R^1 and R^2 taken together with the Carbon to which they are attached represent Piperidine

Lithium diisopropylamide was prepared by the addition of 2.45M *N*-butyllithium (69 ml, 168.8 mmol) in hexanes to a solution diisopropylamine (24 ml, 171.2 mmmol) in tetrahydrofuran (40 ml) at 0°C with stirring for 20 minutes. Then a solution of *N*-(*tert*-butoxycarbonyl)-piperidine-4-carboxylic acid (18 g, 78.5 mmol) in tetrahydrofuran (35 ml) was added to the solution of lithium diisopropylamide over 15 minutes to form a slurry, followed by hexamethylphosphoramide (2 ml). The resulting solution was stirred for 25 minutes, then stream of gaseous formaldehyde (prepared by heating paraformaldehyde (16.4 g, 189 mmol) at 175-200°C over 5-10 minutes) was passed through the solution, which was allowed to immediately warm to room temperature. The slurry was concentrated at ambient temperature, acidified to pH 4 with 6M hydrochloric acid, saturated with solid sodium chloride, and extracted with ethyl acetate (8 x 100 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 1% methanol/ methylene chloride, afforded *N*-(*tert*-butoxycarbonyl)-4-(hydroxymethyl)piperidine-4-carboxylic acid as a white solid (4 g, 20%). mp 156.6-157.3 °C; ^{1}HNMR (DMSO-d_6) δ 1.25-1.37 (m, 2H), 1.38 (s, 9H), 1.85 (dm, J = 13.7 Hz, 2H), 2.78-2.94 (br m, 2H), 3.41 (s, 1H), 3.70 (dm, J = 12.8 Hz, 2H), 4.87 (br s, 1H), 12.34 (s, 1H); Anal. Calcd. for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.72; H, 8.10; N, 5.53.

5H. Preparation of (10) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran a Compound of Formula (10a)

Trifluoromethanesulfonic anhydride (11.1 ml, 66.2 mmol), followed by triethylamine (17.8 ml, 127.4 mmol) was added to a slurry of 4-(hydroxymethyl)tetrahydropyran-4-carboxylic acid (10.20 g, 63.68 mmol) in anhydrous diethyl ether cooled to 0°C (115 ml). The biphasic solution was stirred for 20 hours, warmed to room temperature, stirred an additional 2 hours. The layers were separated by decantation, and the lower layer diluted with 2% aqueous sodium bicarbonate solution (50 ml) and extracted with methylene chloride (4 x 200 ml). The combined organic extracts were washed with additional 2% aqueous sodium bicarbonate (100 ml), dried over magnesium sulfate, and concentrated *in vacuo* to afford 2,7-dioxa-spiro[3.5]nonane-1-one as a pale yellow oil (10.8 g). IR (KBr) 1821 cm^{-1}; ^{1}HNMR (CD_3Cl_3) δ 1.92 (ddd, J = 13.4, 8.1, 4.0 Hz, 2H), 2.10 (dddd, J = 13.4, 6.1,3.4, 0.8 Hz, 2H), 3.70 (ddd, J = 11.8, 6.3, 3.9 Hz, 2H), 3.92 (ddd, J = 11.8, 7.9, 3.4 Hz, 2H), 4.15 (s, 2H); ^{13}CNMR (CD_3Cl_3) δ 30.78 (t), 55.78 (s), 64,46 (t), 71.50 (t), 173.42 (s), MS(EI) m/e=142. MS(CI) M+ =H m/e=143, M+ +HNH_4 m/e=160.

5I. Preparation of a Compound of Formula (10) where R^1 and R^2 taken together with the Carbon to which they are attached represent Piperidine, a Compound of Formula (10b)

Trifluoromethanesulfonic anhydride (2.60 ml, 15.39 mmol), followed by triethylamine (4.30 ml, 30.78 mmol) was added to a slurry of *N*-(*tert*-butoxycarbonyl)-4-hydroxymethylpiperidine-4-carboxylic acid (3.80 g, 14.65 mmol) in anhydrous diethyl ether (27 ml) cooled to 0°C. The biphasic solution was stirred for 23 hours, warmed to room temperature, stirred an addition 1 hour, and the upper diethyl ether layer separated by decantation. The lower was extracted with additional portions of diethyl ether (2 x 100 ml), and the combined organic extracts washed with aqueous sodium bicarbonate solution (2 x 50 ml), dried over magnesium sulfate, and concentrated *in vacuo* to afford 7-(butoxycarbonyl)-2-oxa-7-azaspiro[3.5]nonan-1-one as a pale yellow oil (2.88 g, 82%). ^{1}HNMR ($CDCl_3$) δ 1.48 (s, 9H), 1.79-1.89 (m, 2H), 2.02-2.10 (m, 2H), 3.48-3.66 (m, 4H), 4.13 (s, 2H).

EXAMPLE 6

Preparation of a Compound of Formula (13)

6A. Preparation of (13) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran, and X is Iodo

Lithium diisopropylamide was prepared by the addition of 2.5M *N*-butyl lithium (5.6 ml, 13.9 mmol) in hexanes to a solution of diisopropylamine (1.95 ml, 13.9 mmmol) in tetrahydrofuran (30 ml) at 0°C with stirring for 20 minutes. Then a solution of tetrahydropyran-4-carboxylic acid ethyl ester (2 g, 12.7 mmol) in tetrahydrofuran (8 ml) was added to the solution of lithium diisopropylamide at a temperature of -78°C over 15 minutes. The resulting solution was stirred an

additional 50 minutes, and diiodomethane (1.14ml, 14.2 mmol) was added. The resulting mixture was stirred an additional 50 minutes, warmed to room temperature over 30 minutes, then recooled to 0°C. The mixture was diluted with 1M aqueous hydrochloric acid (25 ml), extracted with diethyl ether (2 x 100 ml), and washed with additional portions of diethyl ether (2 x 50 ml). The combined organic layers were washed once with 1M aqueous hydrochloric acid (100 ml), saturated aqueous sodium bisulfite (100 ml), saturated aqueous sodium bicarbonate (100 ml), and dried over magnesium sulfate, and concentrated *in vacuo*. The residue was filtered over a plug of silica gel, eluting successively with hexanes and ethyl acetate, removing excess alkylating agent with the hexane wash, to afford pure 4-(iodomethyl)tetrahydropyran-4-carboxylic acid ethyl ester as a pale yellow oil which was taken directly into the next reaction without further purification (3.20 g, 85%). IR (KBr) 1732 cm^{-1}; ^{1}HNMR ($CDCl_3$) 1.31 (q, J = 7.3 Hz, 3H), 1.56 (ddd, J = 14.6, 10.9, 4.5, 2H), 2.17 (ddd, J = 14.6, 5.7, 3.3, 2H), 3.31 (s, 2H), 3.51 (ddd, J = 11.7, 11.1, 2.5 Hz, 2H), 3.51 (td, J = 11.7, 4.3 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 14.33 (q), 15.04 (t), 34.70 (t), 45.26 (s), 61.34 (t), 65.22 (t), 172.89 (s); EIHRMS Calcd. for $C_9H_{15}IO_3$ (M^+): 298.0066. Found: 298.0066. Anal. Calcd. for $C_9H_{15}IO_3$: C, 36.26; H, 5.07. Found: C, 36.56; H, 5.09.

6B. Preparation of (13) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran, and Varying X

Similarly, replacing diiodomethane with dibromomethane or bromochloromethane, the following compounds of Formula (13) were prepared:

4-(bromomethyl)tetrahydropyran-4-carboxylic acid ethyl ester: IR (neat) 1732 cm^{-1}; ^{1}HNMR ($CDCl_3$) 1.30 (q, J = 7.1 Hz, 3H), 1.59 (ddd, J = 14.6, 10.9, 4.5, 2H), 2.17 (dm, J = 14.7, 2H), 3.48 (s, 2H), 3.53 (dt, J = 11.9, 4.5 Hz, 2H), 3.84 (dt, J = 11.9, 4.5 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H); ^{13}CNMR ($CDCl_3$)δ 14.27 (q), 33.17 (t), 40.16 (t), 46.05 (s), 61.29 (t), 64.97 (t), 172.91 (s); CIMS (M^+ + H): 251, (M^+ + NH_4+) 268.

4-(chloromethyl)tetrahydropyran-4-carboxylic acid ethyl ester: IR (neat) 1734 cm^{-1}; ^{1}HNMR ($CDCl_3$) 1.30 (q, J = 7.1 Hz, 3H), 1.59 (ddd, J = 14.6, 10.9, 4.5, 2H), 2.16 (dm, J = 14.7, 2H), 3.53 (dt, J = 11.9, 4.5 Hz, 2H), 3.61 (s, 2H), 3.84 (dt, J = 11.7, 4.3 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 14.24 (q), 32.14 (t), 46.69 (s), 51.40 (t), 61.29 (t), 64.85 (t), 173.01 (s); CIMS (M^+ + H): 207. Anal. Calcd. for $C_9H_{15}ClO_3$: C, 52.31; H, 7.32. Found: C, 52.51; H, 7.30.

6C. Alternative Preparation of a Compound of Formula (13) where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran, and X is *p*-Tosyl

To a solution of tetrahydropyran-4-carboxylic acid ethyl ester (820 mg, 4.356 mmol) in pyridine (10 ml) at 0°C, was added *p*-toluenesulfonyl chloride (997 mg, 5.23 mmol), and the mixture allowed to warm to room temperature over 1 hour period. The mixture was stirred 36 hours and partitioned between methylene chloride (150 ml) and 3N aqueous hydrochloric acid (50 ml). The organic layer was washed with 25 ml of saturated aqueous sodium bicarbonate, dried ($MgSO_4$), concentrated and the residue chromatographed over 45 g of silica gel, eluting with 30% ethyl acetate/hexanes, to afford the tosylate as a white solid (1.03 g, 69%). mp 87.7-88.6 °C; IR (KBr) 1717 cm^{-1}; 1 NMR ($CDCl_3$) δ 1.21 (q, J = 17.1 Hz, 3H), 1.52 (ddd, J = 13.4, 10.6, 4.1 Hz, 2H), 2.00 (dm, J = 13.4 Hz, 2H), 2.46 (s, 3H), 3.49 (ddd, J = 11.7, 10.6, 2.5 Hz, 2H), 3.76 (dt, J = 11.9, 4.1 Hz, 2H), 4.03 (s, 2H), 4.13 (q, J = 7.1 Hz, 2H), 7.35; ^{13}C NMR ($CDCl_3$) δ 14.10 (q), 21.67 (q), 30.43 (t), 44.93 (s), 61.37 (t), 64.43 (t), 74.65 (t), 127.95 (d), 129.89 (d), 132.67 (s), 145.05 (s), 172.57 (s); HRMS Calcd for $C_{16}H_{22}O_6$: 343.1215. Found: 343.1217. Anal. Calcd. for $C_{16}H_{22}O_6$: C, 56.12; H, 6.48. Found: C, 56.22; H, 6.46.

EXAMPLE 7

Preparation of Compounds of Formula Ia

7A. Preparation of Ia where R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached represent Piperidine, and R^5 is Diphenylether, from a Compound of Formula (4)

1. 4-Phenoxythiophenol (7.4 g, 36.3 mmol), 4-carboxymethylene-*N*-CBZ-piperidine (10 g, 36.3 mmol) and piperidine (1.8 ml, 36.3 mmol) were stirred overnight at 100-110°C in a sealed flask. After cooling, the crude reaction mixture was partitioned between ethyl acetate and 1N hydrochloric acid, the organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo* to give a yellow solid. The solid was triturated in 1:1 (v/v) ethyl ether/hexane (500 ml) to give 2-[4-(4-phenoxyphenylthio)-*N*-CBZ-piperidin-4-yl]-acetic acid as a white solid.

2. A solution of 2-[4-(4-phenoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetic acid (150 mg, 0.29 mmole) in dry 1,2 dichloroethane (3 ml) under nitrogen was cooled to -10°C and saturated with hydrochloric acid gas for 15 minutes. The reaction vessel was then sealed and the solution stirred for two days at 25°C. The tube was cooled to -10°C prior to opening to release gaseous hydrochloric acid, and then allowed to warm to 25°C. The solvent was removed *in vacuo* and the product triturated with ethyl acetate to give 2-[4-(4-phenoxyphenylthio)-piperidin-4-yl)]-acetic acid hydrochloride as a white powder. ^{1}HNMR (CD_3OD): 7.93 (d,2H); 7.45 (t,2H); 7.27 (t,1H), 7.14 (t,4H); 3.52 (m,2H); 3.25 (m,2H); 2.70 (s,2H), 2.35 (m,4H).

7B. Preparation of Ia where R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached represent Cyclopentyl, and R^5 is Diphenylether, from a Compound of Formula (4)

A mixture of cyclopentylideneacetic acid (2 mmol) and *p*-(phenoxy)-thiophenol (2 mmol) was heated at 110°C under nitrogen in the presence of piperidine (100 μL) for 24 hours. The residue was dissolved in ethyl acetate and washed with dilute hydrochloric acid. The organic layer was separated, dried and evaporated under reduced pressure to give crude 2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetic acid, which can be used in the next reaction without further purification.

7C. Preparation of Ia where R^1, R^2 and R^3 are Hydrogen, R^4 is Benzyl, and R^5 is 4-Bromophenyl

A mixture of *E*-2-benzylacrylic acid (1 g) and *p*-bromothiophenol (1.12 g) were stirred overnight at 110°C in the presence of piperidine (300 μL). The residue was partitioned between ethyl acetate and dilute hydrochloric acid. The organic layer was separated, dried and evaporated under reduced pressure to give 3-benzyl-3-(4-bromophenylthio)-propionic acid (Iaa), which was used in the next reaction with no further purification.

7D. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl, from a Compound of Formula (10)

2,7-dioxa-spiro[3.5]nonane-1-one (10.8 g), obtained as described in Example 5H, was immediately dissolved in *N,N*-dimethylformamide (95 ml) and slowly added to a solution containing the sodium salt of 4-(4-chlorophenoxy)thiophenol (generated by the addition of sodium hydride powder (2.14 g, 89.2 mmol) to a solution of 4-(4-chlorophenoxy)thiophenol (15.83 g, 66.8 mmol) in *N,N*-dimethylformamide (19 ml) at 0°C and stirring for 30 minutes) over a 10-15 minute period, and then stirred an additional 15 minutes. The resulting slurry was heated to 40°C, stirred for 5 minutes, *tert*-butanol (2 ml) was added, and the mixture cooled to room temperature over 20 minutes. The majority of the *N,N*-dimethylformamide was removed *in vacuo*, the pH adjusted to 9.2, the resultant slurry diluted with 30% diethyl ether-hexanes (120 ml) and filtered. The filter cake was washed with additional portions of ether (3 x 70 ml), acidified to pH 3.5 with 2N aqueous hydrochloric acid, and extracted into methylene chloride (4 x 350 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo*. The solid residue was recrystallized from the minimum amount of methylene chloridehexanes to afford pure 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid as a white crystalline solid (19.50 g). mp 140.6-141.9°C; IR (KBr) 3429 (br), 1732 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.54 (ddd, *J* = 14.2, 10.0, 4.2 Hz, 2H), 1.95 (dm, *J* = 14.2 Hz, 2H), 3.19 (s, 2H), 3.56 (ddd, *J* = 11.8, 10.0, 4.2 Hz, 2H), 3.70 (dt, J = 11.8, 4.2 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.02 (d, J = 8.9 Hz, 2H), 7.02 (d, *J* = 8.9 Hz, 2H), 7.42 (d, *J* = 9.0 Hz, 4H), 12.66 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 33.06 (t), 43.56 (t), 45.03 (s), 64.13 (t), 119.43 (d), 120.11 (d), 110.43 (d), 127.35 (s), 129.80 (d), 131.09 (s), 131.59 (d), 154.90 (s), 155.50 (s), 175.25 (s); HRMS Cald. for $C_{19}H_{19}SO_4Cl$: 378.0693. Found: 378.0685. Anal. Calcd. for $C_{19}H_{19}SO_4Cl.O.25\ H_2O$: C,59.53; H, 513. Found: C, 59.53; H, 5.07.

Similarly, replacing 4-(4-chlorophenoxy)thiophenol with 4-(4-bromophenoxy)thiophenol and 4-(4-fluorophenoxy)thiophenol, the following compounds were prepared:

4-[4-(4-bromophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid: mp 143.7-144.5 °C; IR (KBr) 3434 (br), 1732 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.54 (ddd, *J* = 13.8, 10.1, 4.3 Hz, 2H), 1.94 (dm, *J* = 13.5 Hz, 2H), 3.19 (s, 2H), 3.37 (ddd, *J* = 11.8, 10.1, 2.5 Hz, 2H), 3.70 (dt, *J* = 11.8 Hz, 4.0 Hz, 2H), 6.96 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 12.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 33.04 (t),43.34 (t), 45.00 (s), 64.10 (t), 115.14 (s), 119.59 (d), 120.53 (d), 131.15 (s), 131.51 (d), 132.77 (s), 154.71 (s), 156.06 (s), 175.28 (s); EIMS (M^+): 424. Anal. Calcd. for $C_{19}H_{19}SO_4Br$: C, 53.91; H, 4.52. Found: C, 53.53; H, 4.54;

4-[4-(4-fluorophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid: mp 143.0-143.4 °C; IR (KBr) 3436 (br), 1721 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.54 (ddd, *J* = 13.5, 10.1, 4.0 Hz, 2H), 1.94 (dm, *J* = 13.5 Hz, 2H), 3.17 (s, 2H), 3.38 (td, *J* = 11.8, 2.5 Hz, 2H), 3.70 (dt, *J* = 11.8 Hz, 4.0 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 2H),7.05 (dd, *J* = 9.2. 4.6 Hz, 2H), 7.21 (dd, *J* = 9.1, 8.4 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H), 12.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 33.05 (t), 43.65 (t),

45.49 (s), 64.12 (t), 116.53 (dd, J_{C-F} = 23.2 Hz), 118.71 (d), 120.63 (dd, J_{C-F} = 8.5 Hz), 130.31 (s), 131.69 (d), 152.38 (s), 155.85 (s), 158.29 (d, J_{C-F} = 239.9 Hz), 175.28 (s); EIMS (M^+): 362. Anal. Calcd. for $C_{19}H_{19}SO_4F$: C, 62.97; H, 5.28. Found: C, 62.79; H, 5.26.

7E. Alternative Preparation of Ia where R^1 and R^2 are both Methyl. R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

Sodium hydride powder (0.86 g, 35.8 mmol) was added to a mixture of 4-(4-chlorophenoxy)thiophenol (3.55 g, 15 mmol) in *N,N*-dimethylformamide (12 ml) at 0°C. The mixture was warmed to room temperature over 5 minutes, stirred for an additional 20 minutes, and solid chloropivalic acid (1.64 g, 12.0 mmol) was added in one portion. This mixture was heated to 80°C for 18 hours, cooled to room temperature, and water (1 ml) added. The residue was partitioned between methylene chloride (50 ml) and 2N hydrochloric acid (25 ml). The aqueous layer was separated and washed with additional methylene chloride (2 x 25 ml). The combined organic extracts were dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 5% methanol/methylene chloride, gave slightly impure 3-[4-(4-chlorophenoxy)-phenylthio]-2,2-dimethyl propionic acid (4 g, 99%). This material was recrystallized from the minimum amount of diethyl ether/hexanes to afford analytically pure acid as a white solid (3.20 g, 80%). mp 84.4-84.9°C; IR (KBr) 3433 (br), 1732 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.19 (s, 6H), 3.14 (s, 2H), 6.97 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.9, 2H), 7.40 (d, J = 8.8 Hz, 2H), 12.36 (br s, 1H). EIMS(M+): 378. Anal. Calcd. for $C_{17}H_{17}SO_3Cl$: C, 60.62; H, 5.09. Found: C, 60.31; H, 4.96.

7F. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent *N*-BOC-Piperidine. R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl, from a Compound of Formula (10b)

7-(*tert*-Butoxycarbonyl)-2-oxa-7-azaspiro[3.5]nonan-1-one obtained in Example 5l above, was immediately dissolved in *N,N*-dimethylformamide (4 ml), slowly added to a solution containing the sodium salt of 4-(4-chlorophenoxy)thiophenol (generated by the addition of sodium hydride power (340 mg, 14.17 mmol) to a solution of 4-(4-chlorophenoxy)thiophenol (3.00 g, 12.7 mmol) in *N,N*-dimethylformamide (19 ml), at 0°C and stirred for 30 minutes) over a 10-15 minute period, and was stirred an additional 15 minutes. The resulting slurry was heated to 80°C, stirred for 5 minutes, *tert*-butanol (2 ml) added, and the mixture cooled to room temperature over 20 minutes. The majority of the *N,N*-dimethylformamide was removed *in vacuo*, the pH adjusted to 3.5 using 2M aqueous hydrochloric acid and extracted into ethyl acetate (4 x 150 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo* and the residue chromatographed over silica gel, eluting with 1% to 10% methanol/methylene chloride, to afford the piperidine acid, 4-[4-(4-chlorophenoxy)phenylthiomethyl]-*N*-(*tert*-butoxycarbonyl)-piperidin-4-yl carboxylic acid as a pale yellow oil (5 g, 89%). ^{1}HNMR (OH not observed; $CDCl_3$) δ 1.37 (s, 9H), 1.55 (m_c, 2H), 2.10 (m_c, 2H), 3.05 (m_c, 2H), 3.06 (s, 2H), 3.72 (m_c, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.9 Hz, 2H), 7.21 (d, J = 8.9 Hz, 2H), 7.30 (d, J = 8.7 Hz, 4H).

7G. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, R^5 is 4-(4-Chlorophenoxy)phenyl, from a Compound of Formula Ia where R is Ethyl

To a solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid ethyl ester (70 mg, 0.17 mmol) in ethanol (2 ml) containing two drops of water, was added potassium hydroxide (58.3 mg, 1.04 mmol). The mixture was refluxed for 13 hours, cooled to room temperature, acidified to pH 4, and extracted with ethyl acetate (4 x 50 ml). The combined organic layers were dried over magnesium sulfate, and concentrated to afford 4-[4-(4-chlorophenoxy)-phenylthiomethyl]-tetrahydropyran-4-carboxylic acid (66 mg, 100%), which is spectroscopically identical to that isolated from the prior procedure of Example 7D.

7H. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, R^5 is 4-(4-Bromophenoxy)phenyl, from a Compound of Formula Ia where R is Ethyl

Similarly, following the procedure of Example 7G above, 4-[4-(4-bromophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid and 4-[4-(4-fluorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid were prepared.

7I. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, R^5 is 4-(4-Chlorophenoxy)phenyl, and R is Methyl, from the Corresponding Carboxylic Acid

To a solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid (580 mg, 1.53 mmol) and *N, N*-dimethylformamide catalyst (22 μL) in methylene chloride (15 ml) at 0°C was added oxalyl chloride (0.33 ml, 3.83 mmol) dropwise over 10 minutes. The mixture was warmed to room temperature over 1 hour, the partial slurry stirred an additional 12 hours, and concentrated *in vacuo* until the theoretical mass of the acid chloride was obtained. The residue was suspended in tetrahydrofuran (7.5 ml), and methanol (0.19 ml, 4.59 mmol), followed by triethylamine (0.64 ml, 4.59 mmol) was added. The mixture was heated to reflux for 14 hours, concentrated, and the resulting residue partitioned between methylene chloride (150 ml) and 1M aqueous hydrochloric acid (50 ml). The aqueous layer was back extracted with additional portions of methylene chloride (2 x 30 ml), the combined extracts dried over magnesium sulfate, and concentrated to afford crude 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid methyl ester, which was taken directly into the next reaction without further purification. ^{1}HNMR ($CDCl_3$) δ 1.62 (m_c, 2H), 2.15 (dm, *J* = 13.6 Hz, 2H), 3.13 (s, 2H), 3.47 (td, *J* = 11.9, 2.4 Hz, 2H), 3.59 (s, 3H), 3.81 (dt, *J* = 12.0, 4.1 Hz, 2H), 6.92 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 2H).

7J. Preparation of Ia where R^1 and R^2 taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, R^5 is 4-4-Chlorophenoxy)phenyl, and R is Ethyl, from a Compound of Formula (13)

4-(Iodomethyl)tetrahydropyran-4-carboxylic acid ethyl ester (300 mg, 1 mmol) was added to a solution containing the sodium salt of 4-(4-chlorophenoxy)thiophenol (generated by the addition of sodium hydride powder (36 mg, 1.5 mmol) to a solution of 4-(4-chlorophenoxy)thiophenol (262 mg, 1.1 mmol) in *N,N*-dimethylformamide (2 ml) at 0°C and stirring for 30 minutes). The mixture was warmed to room temperature over 5 minutes, stirred for an additional 20 minutes, cooled to room temperature, and 1M aqueous hydrochloric acid (5 ml) added. The mixture was then partitioned between ethyl acetate (100 ml) and 2M hydrochloric acid (25 ml). The aqueous layer was separated and washed with additional ethyl acetate (2 x 50 ml). The organic extracts were combined, washed with 1M sodium hydroxide (2 x 30 ml), dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 20% ethylacetate/ hexanes, yielded pure 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid ethyl ester (370 mg, 91%), followed by impure 4-[4-(4-chlorophenoxy)-phenylthiomethyl]tetrahydropyran-4-carboxylic acid ethyl ester (40 mg). IR (KBr) 1728 cm^{-1}; ^{1}HNMR ($CDCl_3$) 1.23 (q, *J* = 7.1 Hz, 3H), 1.56 (ddd, *J* = 14.6, 10.9, 4.4, 2H), 1.63 (ddd, *J* = 14.6, 5.7, 3.3, 2H), 3.13 (s, 2H), 3.51 (ddd, *J* = 11.8, 11.1, 2.4 Hz, 2H), 3.80 (dt, *J* = 11.8, 4.1 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 6.91 (d, *J* = 8.9 Hz, 2H), 6.92 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 9.0 Hz, 2H), 7.39 (d, *J* = 8.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.20 (q), 33.72 (t), 45.72 (t), 46.07 (s), 60.92 (t), 65.06 (t), 119.29 (d), 120.20 (d), 128.43 (s), 129.85 (d), 130.57 (s), 133.05 (s), 155.40 (s), 156.21(s), 174.02 (s); EIHRMS Calcd. for $C_{21}H_{23}SO_4Cl$ (M^+): 406.1006. Found: 406.1008. Anal. Calcd. for $C_{21}H_{23}SO_4Cl$: C, 61.98; H, 5.70. Found: C, 61.86; H, 5.68.

7K. Preparation of Ia where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, R^5 is 4-(4-Bromophenoxy)phenyl, and R is Ethyl, from a Compound of Formula (13)

Similarly, replacing 4-(4-chlorophenoxy)thiophenol with 4-(4-bromophenoxy)thiophenol, and following the procedures of Example 7J above, 4-[4-(4-bromophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid ethyl ester was prepared (2.10 g, 93%). IR (KBr) 1728 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.22 (q, *J* = 7.1 Hz, 3H), 1.60 (ddd, *J* = 14.6, 10.9, 4.5, 2H), 2.14 (ddd, *J* = 14.6, 5.7, 3.3, 2H), 3.13 (s, 2H), 3.81 (ddd, *J* = 11.8, 11.1, 2.4 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 9.0 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 14.20 (q), 33.71 (t), 45.69 (t), 46.05 (s), 60.92 (t), 65.05 (t), 116.06 (s), 119.40 (d), 120.59 (d), 130.69 (s), 132.81 (d), 133.03 (s), 156.04 (s), 156.16 (s), 174.01 (s); EIHRMS Calcd. for $C_{21}H_{23}SO_4Br$ (M^+): 450.0500. Found: 450.0505. Anal. Calcd. for $C_{21}H_{23}SO_4Cl$: C, 55.88; H, 5.14. Found: C, 55.52; H, 5.09.

Similar reactions were carried out, starting from compounds of Formula (13) where X is iodo, bromo, and chloro, and moderate to good yields were obtained in all cases.

7L. Preparation of Ia, varying R^1, R^2, R^3, R^4, and R^5

Similarly, optionally replacing 4-carboxymethylene-*N*-CBZ-piperidine with other *N*-protected compounds of Formula (4) and following the procedures of Example 7A (1) and (2) above, or optionally replacing cyclopentylideneacetic acid with other compounds of Formula (4) and following the procedures of Example 7B above, and optionally replacing p-phenoxythiophenol with other compounds of Formula (5), the following compounds of Formula Ia were prepared:

2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl-]-acetic acid;
2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-acetic acid;
2-benzyl-3-(3-methoxyphenylthio)-propionic acid;
2-benzyl-3-(4-methoxyphenylthio)-propionic acid;
3-benzyl-3-(4-methoxyphenylthio)-propionic acid;
3,3-dimethyl-3-[(4-chlorophenoxy)phenylthio]-propionic acid;
2-{4-[4-(4-fluorophenoxy)phenylthio]-piperidin-4-yl}-acetic acid;
2-{4-[4-(4-fluorophenoxy)phenylthio}-*N*-CBZ-piperidin-4-yl}-acetic acid;
3-benzyl-3-[(4-phenylthiophenyl)thio]-propionic acid;
3-benzyl-3-(phenylthio)-propionic acid;
3-benzyl-3-(4-phenoxphenylthio)-propionic acid;
3-benzyl-3-[(4-biphenyl)thio]-propionic acid;
3-benzyl-3-(2-naphthylthio)-propionic acid;
3-benzyl-3-(4-methoxystyrylphenylthio)-propionic acid;
3-cyclopentylmethyl-3-(4-methoxyphenylthio)-propionic acid;
3-cyclopentylmethyl-2-isopropyl-3-(4-methoxyphenylthio)-propionic acid;
3-ethyl-2-methyl-3-(4-methoxyphenylthio)-propionic acid;
3,3-dimethyl-(4-methoxyphenylthio)-propionic acid;
2-[1-(4-methoxyphenylthio)-cyclopent-1-yl]-acetic acid;
2-[4-(4-methoxyphenylthio)-cyclohexanone-4-yl]-acetic acid ethylene ketal;
2-[1-(4-methoxyphenylthio)-(4-methylcyclohex-1-yl]-acetic acid;
2-[1-(4-phenoxyphenylthio)-cyclohex-1-yl]-acetic acid;
2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetic acid;
{4-[4-(4-benzo[b]thiophen-2-yl-phenoxy)phenylthio)-tetrahydropyran-4-yl]-acetic acid;
2-{4-[4-(phenylmethyl)phenylthio]-tetrahydropyran-4-yl}-acetic acid;
2-{4-[4-(4-fluorophenoxy)phenylthio]-tetrahydropyran-4-yl}-acetic acid;
2-{4-[4-(4-chlorophenoxy)phenylthio]-tetrahydropyran-4-yl}-acetic acid: mp 138.5-138.8 °C; ^{1}HNMR ($CDCl_3$, OH not seen) δ 1.73 (d, J = 14.7, 2H), 1.91 (ddd, J = 14.7, 10.1, 4.3 Hz, 2H), 2.58 (s, 2H), 3.76 (dt, J = 11.8, 4.1 Hz, 2H), 4.02 (dt, J = 11.8, 2.6 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.9 Hz, 2H), 7.33 (d, J = 8.9 Hz, 2H), 7.53 (d, J = 8.8 Hz, 4H); FABMS (M^+): 379.2. Anal. Calcd. for $C_{19}H_{19}SO_4Cl$: C, 60.23; H, 5.05. Found: C, 60.39; H, 5.01;
2-{4-[4-(4-chlorophenoxy)phenylthio]-tetrahydropyran-4-yl}-acetic acid;
2-{4-[4-(4-bromophenoxy)phenylthio]-tetrahydropyran-4-yl}-acetic acid;
2-[4-(4-phenoxyphenylthio)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetic acid;
trans-2-(4-methoxyphenylthio)-cyclopentanecarboxylic acid; and
2-(4-methoxyphenylthio)-cyclohexanecarboxylic acid.

7M. Preparation of Ia, varying R^1, R^2, R^3, R^4, and R^5

Similarly, optionally replacing 2,7-dioxa-spiro[3.5]nonane-1-one with other compounds of Formula (10) and following the procedures of Example 7D above, and optionally replacing 4-(4-chlorophenoxy)-thiophenol with other compounds of Formula (5), the following compounds of Formula Ia were prepared:

4-[4-(4-fluorophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid;
4-[4-(4-bromophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid;
3-(4-benzoylphenylthio)-2,2-dimethyl propionic acid;
3-[4-(4-chlorophenoxy)phenylthio]-2,2-dimethyl propionic acid;
4-[(4-phenoxypyrid-4-yl)thiomethyl]tetrahydropyran-4-carboxylic acid: ^{1}HNMR (OH not observed; $CDCl_3$) δ 1.65 (m_c, 2H), 2.16 (dm, J = 14.2 Hz, 2H), 3.20 (s, 2H), 3.57 (tm, J = 11.4 Hz, 2H), 3.84 (dm, J = 12.0 Hz, 2H), 6.87 (d, J =6.2 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 8.9 Hz, 2H), 8.43 (d, J = 6.0 Hz, 2H).

7N. Preparation of Ia, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 7 above, other compounds of Formula Ia are prepared.

EXAMPLE 8

Preparation of Compounds of Formula Iba

8A. Preparation of Iba where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

Oxalyl chloride (37.5 ml, 429.5 mmol) was added dropwise over 10 minutes to a suspension of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid (65.1 g, 171.8 mmol) and *N,N*-dimethylformamide catalyst (2 ml) in methylene chloride (1 litre) at 0°C. The mixture was warmed to room temperature over 1 hour and the resultant partial slurry stirred an additional 20 hours, concentrated under reduced pressure until the theoretical mass of the acid chloride was obtained. This mixture was dissolved in methylene chloride (600 ml), cooled to 0°C, and *N,O*-bis(trimethylsilyl)hydroxylamine (109.1 ml, 510.45 mmol) added dropwise over 10 minutes. The mixture was immediately warmed to room temperature, stirred 3 hours, and recooled to 0°C. Aqueous 2.4M hydrochloric acid solution (400 ml, 960 mmol) was added to the solution, causing precipitation of the hydroxamic acid product within several minutes after the addition. The slurry was stirred an additional 30 minutes and filtered. The filter cake was washed with water (3 x 30 ml) and 50% diethyl ether-hexanes (2 x 25 ml) and dried at 70°C to afford 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) (61.8 g, 92%). mp 146.6-148.0 °C; IR (KBr) 3426 (br), 1636 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.54 (ddd, J = 13.8, 10.2, 4.0 Hz, 2H), 2.00 (dm, J = 13.8 Hz, 2H), 3.16 (s, 2H), 3.39 (m, 2H), 3.66 (dt,J= 11.7,3.8 Hz, 2H), 6.98 (d,J = 8.8Hz, 2H), 7.02 (d,J=9.0Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.9 Hz, 2H), 8.78 (s, 1H), 10.63 (s, 1H); ^{13}CNMR ($CDCl_3$) δ 32.79 (t), 43.60 (s), 43.70 (t), 63.93 (t), 119.56 (d), 120.07 (d), 127.19 (s), 129.85 (d), 131.24 (d), 131.34 (s), 154.62 (s), 155.59 (s), 169.69 (s); FABHRMS Calcd. for $C_{19}H_{21}NSO_4Cl$ (M^+ + H): 394.0880. Found: 378.0872. Anal. Calcd. for $C_{19}H_{20}NSO_4Cl$: C, 57.94; H, 5.12; N, 3.56. Found: C, 57.98; H, 5.04; N, 3.68.

8B. Alternative Preparation of Iba where R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

Oxalyl chloride (37.5 ml, 429.5 mmol) was added dropwise over 10 minutes to a solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid (65.1 g, 171.8 mmol) and *N,N*-dimethylformamide catalyst (2 ml) in methylene chloride (1 litre) at 0°C. The mixture was warmed to room temperature over 1 hour, and the resultant partial slurry stirred an additional 20 hours and concentrated *in vacuo* until the theoretical mass of the acid chloride was obtained. A solution of the acid chloride mixture (650 mg, 1.68 mmol) in methylene chloride (3.4 ml) was added dropwise over 2 minutes to a solution of 50% aqueous hydroxylamine (556 mg) in 2:1 tetrahydrofuran/*tert*-butanol (5.1 ml). The mixture was stirred 1.5 hours and concentrated until approximately 1 ml of aqueous solution was remaining. The slurry was filtered, washed with 1:1 diethyl ether-hexanes (3 X 15 ml) and the solid dried overnite at 70°C in a vacuum oven, to afford 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) (584 mg, 91%). mp 146.6-148.0 °C; IR (KBr) 3426 (br), 1636 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.54 (ddd, J = 13.8, 10.2, 4.0 Hz, 2H), 2.00 (dm, J = 13.8 Hz, 2H), 3.16 (s, 2H), 3.39 (m, 2H), 3.66 (dt, J = 11.7, 3.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.9 Hz, 2H), 8.78 (s, 1H), 10.63 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 32.79 (t), 43.60 (s), 43.70 (t), 63.93 (t), 119.56 (d), 120.07 (d), 127.19 (s), 129.85 (d), 131.24 (d), 131.34 (s), 154.62 (s), 155.59 (s), 169.69 (s); FABHRMS Calcd. for $C_{19}H_{21}NSO_4Cl$ (M^+ + H): 394.0880. Found: 378.0872. Anal. Calcd. for $C_{19}H_{20}NSO_4Cl$: C, 57.94; H, 5.12; N, 3.56. Found: C, 57.98; H, 5.04; N, 3.68.

8C. Preparation of Iba, varying R^1, R^2, R^3, R^4, and R^5

Similarly, replacing 4-[4-(4-chlorophenoxy)phenyl-thiomethyl]-tetrahydropyran-4-carboxylic acid with other compounds of Formula Ia and following the procedures of Example 8A above, the following compounds of Formula Iba were prepared:

4-[4-(4-fluorophenoxy)phenylthiomethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 146.2-146.5 °C; IR (KBr) 3431 (br), 1628 cm^{-1}; ^{1}HNMR ($CDCl_3$; NH and OH not observed) δ 1.35 (ddd, J = 13.8, 10.2, 4.0 Hz, 2H), 1.83 (dm, J = 13.8 Hz, 2H), 2.85 (s, 2H), 3.23 (m, 2H), 3.46 (dt, J = 11.9, 3.9 Hz, 2H), 6.58 (d, J = 8.8 Hz, 2H), 6.57 (d, J = 8.8 Hz, 2H), 6.65-6.78 (m, 4H),7.06 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 32.99 (t), 44.27 (s), 45.49 (t), 64.63 (t), 116.28 (dd, J_{C-F} = 23.2 Hz), 118.64 (d), 120.49 (dd, J_{C-F} = 8.5 Hz), 130.41 (s), 132.49 (d), 152.46 (s), 156.49 (s), 160.29 (d, J_{C-F} = 241.9 Hz), 170.23 (s); FABMS (M^+ + H): 378. Anal. Calcd. for $C_{19}H_{20}NSO_4F$: C, 60.46; H, 5.34; N, 3.71. Found: C, 60.08; H, 5.29; N, 3.65.

4-[4-(4-bromophenoxy)phenylthiomethyl]tetrahydropyran-4-*N*-hydroxycarboxamide: mp 153.1-154.0 °C; IR (KBr) 3434 (br), 1634 cm^{-1}; 1HNMR ($CDCl_3$; NH and OH not observed) δ 1.68 (ddd, J = 14.0, 10.0, 4.0 Hz, 2H), 2.13 (dm, J = 14.0 Hz, 2H), 3.15 (s, 2H), 3.55 (ddd, J = 12.0, 10.2, 2.5 Hz, 2H), 3.76 (dt, J = 12.0 Hz, 4.1 Hz, 2H), 6.87

(d, J = 9.0 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 9.0 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 33.01 (t), 44.32 (s), 45.40 (t), 64.65 (t), 115.95 (s), 119.50 (d), 120.53 (d), 130.67 (s), 132.76 (d), 132.80 (d), 155.92 (s), 156.16 (s), 170.60 (s); FABMS (M^+ + H): 438. Anal. Calcd. for $C_{19}H_{20}NSO_4Br$: C, 52.06; H, 4.60; N, 3.20. Found: C, 51.84; H, 4.52; N, 3.54.

3-(4-benzoylphenylthio)-2,2-dimethyl-N-hydroxypropionamide;
3-[4-(4-chlorophenoxy)phenylthio]-2,2-dimethyl-N-hydroxypropionamide: mp 114.7-115.3 °C; ^{1}HNMR ($CDCl_3$) δ 1.30 (s, 6H), 3.14 (s, 2H), 6.90 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 8.9 Hz, 2H), 7.37 (d, J = 8.8 Hz, 1H); FABHRMS Calcd. for $C_{17}H_{18}NSO_3Cl$ (M^+ + H): 352.0772. Found: 352.0774. Anal. Calcd. for $C_{17}H_{18}NSO_3Cl$: C, 58.03; H, 5.16; N, 3.98. Found: C, 57.85; H, 5.10; N, 4.12.
3,3-dimethyl-3-[(4-chlorophenoxy)phenylthio]-N-hydroxypropionamide;
{4-[4-(4-benzo[b]thiophen-2-yl-phenoxy)phenylthio)-tetrahydropyran-4-yl]-N-hydroxyacetamide;
2-{4-[4-(phenylmethyl)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-chlorophenoxy)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide; and
2-{4-[4-(4-bromophenoxy)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide.

8D. Preparation of Iba, varying R^1, R^2, R^3, R^4, and R^5

Similarly, replacing 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid with other compounds of Formula Ia and following the procedures of Example 8A above, other compounds of Formula Iba are prepared, for example:

4-(4-phenoxyphenylthiomethyl)tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-fluorophenoxy)phenylthiomethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]piperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]-1-methylpiperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]-1-(cyclopropyl-methyl)piperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]-1-acetylpiperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]-1-(3-pyridyl)-piperidine-4-(*N*-hydroxycarboxamide);
4-[4-(4-chlorophenoxy)phenylthiomethyl]-1-(3-pyridoyl)-piperidine-4-(*N*-hydroxycarboxamide);
2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl-]-*N*-hydroxyacetamide;
2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-*N*-hydroxyacetamide;
2-benzyl-3-(3-methoxyphenylthio)-*N*-hydroxypropionamide;
2-benzyl-3-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
3-benzyl-3-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-piperidin-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-*N*-CBZ-piperidin-4-yl}-*N*-hydroxyacetamide;
3-benzyl-3-[(4-phenylthiophenyl)thio]-*N*-hydroxypropionamide;
3-benzyl-3-(phenylthio)-*N*-hydroxypropionamide;
3-benzyl-3-(4-phenoxphenylthio)-*N*-hydroxypropionamide;
3-benzyl-3-[(4-biphenyl)thio]-*N*-hydroxypropionamide;
3-benzyl-3-(2-naphthylthio)-*N*-hydroxypropionamide;
3-benzyl-3-(4-methoxystyrylphenylthio)-*N*-hydroxypropionamide;
3-cyclopentylmethyl-3-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
3-cyclopentylmethyl-2-isopropyl-3-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
3-ethyl-2-methyl-3-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
3,3-dimethyl-(4-methoxyphenylthio)-*N*-hydroxypropionamide;
2-[1-(4-methoxyphenylthio)-cyclopent-1-yl]-*N*-hydroxyacetamide;
2-[4-(4-methoxyphenylthio)-cyclohexanone-4-yl]-*N*-hydroxyacetamide ethylene ketal;
2-[1-(4-methoxyphenylthio)-(4-methylcyclohex-1-yl]-*N*-hydroxyacetamide;
2-[1-(4-phenoxyphenylthio)-cyclohex-1-yl]-*N*-hydroxyacetamide;
2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-[4-(4-phenoxyphenylthio)-tetrahydrothiopyran-1,1-dioxide-4-yl]-*N*-hydroxyacetamide;
trans-2-(4-methoxyphenylthio)-cyclopentanecarboxylic acid; and
2-(4-methoxyphenylthio)-cyclohexanecarboxylic acid.

EXAMPLE 9

Preparation of Compounds of Formula Ib

9A. Preparation of Ib where R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Cyclopentyl, and R^5 is 4-Phenoxyphenyl

The 2-[1-(4-phenoxypheny]thio)-cyclopent-1-yl]-acetic acid obtained in Example 5 was dissolved in methylene chloride (8 ml) and treated with 4-dimethylaminopyridine (180 mg), *O*-(*tert*-butyl)-hydroxylamine hydrochloride (360 mg), triethylamine (540 μL), pyridine (400 μL), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (750 mg). After stirring overnight the reaction mixture was partitioned between ethyl acetate and water, the organic layer separated, and the solvent removed under reduced pressure. Preparative TLC of the residue and elution with 2:1 hexane/ethyl acetate gave *N*-(*tert*-butoxy)-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide (270 mg) as a white foam, which can be used in the next reaction without further purification.

9B. Preparation of Ib where R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Tetrahydropyran, and R^5 is 4-Phenoxyphenyl

O-(*tert*-Butyl)hydroxylamine hydrochloride (9.57 g), 4-methylmorpholine (15.64 ml), hydroxybenzotriazole (6.87 g), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (19.5 g) was added to a solution of 2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetic acid (17.5 g) in methylene chloride (200 ml). After stirring for 3 hours at room temperature, 0.5 M hydrochloric acid (200 ml) was added to the mixture, and the mixture extracted with methylene chloride. The solvent was removed from the combined extracts under reduced pressure. Silica gel chromatography of the residue and elution with 35%-80% ethyl acetate/hexane gave *N*-*tert*-butoxy-2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetamide (15.3 g) as an oil, which can be used in the next reaction without further purification.

9C. Preparation of Ib where R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached are *N*-BOC-Piperidine. and R^5 is 4-(4-Chlorophenoxy)phenyl

4-Methylmorpholine (2.60 ml, 23.68 mmol) was added dropwise to a solution of 2-{4-[4-(4-chlorophenoxy)phenylthiomethyl]-*N*-BOC-piperidin-4-yl}-carboxylic acid obtained in Example 6 (2.83 g, 5.92 mmol), *O*-(*tert*-butyl)hydroxylamine hydrochloride (2.23 g, 17.76 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.27 g, 11.84 mmol) in anhydrous methylene chloride (25 ml) cooled to 0°C. After the resulting mixture was allowed to warm to room temperature over 1 hour and stirred for an additional 12 hours, the mixture was partitioned between diethyl ether/1 N aqueous hydrochloric acid (300 ml). The acid layer was back extracted using diethyl ether (2 x 100 ml), and the combined ether extracts dried over magnesium sulfate and concentrated. Chromatography over silica gel, and eluting with 25% ethyl acetate/hexanes, gave *N*-(*tert*-butoxy)-2-{4-[4-(4-chlorophenoxy)phenylthiomethyl]-*N*-BOC-piperidin-4-yl}-carboxamide (2.88 g, 89%). ^{1}HNMR ($CDCl_3$) δ 1.31 (s, 9H), 1.45 (s, 9H), 1.58 (m_c, 2H), 2.10 (br d, J = 14.2 Hz, 2H), 3.13 (s, 2H), 3.19 (m_c, 2H), 3.73 (m_c, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.9 Hz, 2H), 7.30 (d, J = 8.9 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H), 8.15 (br s, 1H).

9D. Preparation of Ib, varying R^1 , R^2 , R^3 , R^4, and R^5

Similarly, following the procedures of Example 9A above, but replacing 2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetic acid with other compounds of Formula Ia, the following compounds of Formula Ib were prepared:

N-*tert*-butoxy-2-[4-(4-phenoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-*tert*-butoxy-2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-*tert*-butoxy-2-{4-[4-(4-fluorophenoxy)phenylthio]-*N*-CBZ-piperidin-4-yl}-acetamide;
N-*tert*-butoxy-2-{4-[4-(4-fluorophenoxy)phenylthio]-piperidin-4-yl}-acetamide;
N-*tert*-butoxy-2-[4-(4-phenoxyphenylthio)-piperidin-4-yl)]-acetamide;
N-*tert*-butoxy-2-[4-(3-methoxyphenylthio)-piperidin-4-yl)]-acetamide;
N-*tert*-butoxy-2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-acetamide;
N-*tert*-butoxy-2-benzyl-3-(phenylthio)-propionamide;
N-*tert*-butoxy-3-benzyl-3-(phenylthio)-propionamide;
N-*tert*-butoxy-3-benzyl-3-(4-methoxyphenylthio)-propionamide;
N-*tert*-butoxy-3-benzyl-3-[(4-phenylthiophenyl)thio]-propionamide;
N-*tert*-butoxy-3-benzyl-3-(4-phenoxyphenylthio)-propionamide;
N-*tert*-butoxy-3-benzyl-3-[(4-biphenyl)thio]-propionamide;

N-tert-butoxy-3-benzyl-3-(2-naphthylthio)-propionamide;
N-tert-butoxy-3-benzyl-3-(4-methoxystyrylphenylthio)-propionamide;
N-tert-butoxy-3-cyclopentylmethyl-3-(4-methoxyphenylthio)-propionamide;
N-tert-butoxy-3-cyclopentylmethyl-2-isopropyl-3-(4-methoxyphenylthio)-propionamide;
N-tert-butoxy-3-ethyl-2-methyl-3-(4-methoxyphenylthio)-propionamide;
N-tert-butoxy-3,3-dimethyl-(4-methoxyphenylthio)-propionamide;
N-tert-butoxy-2-[1-(4-methoxyphenylthio)-cyclopent-1-yl]-acetamide;
N-tert-butoxy-2-[1-(4-methoxyphenylthio)-(4-methylcyclohex-1-yl]-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylthio)-cyclohexanone-4-yl]-acetamide ethylene ketal;
N-tert-butoxy-2-[1-(4-phenoxyphenylthio)-cyclohex-1-yl]-acetamide;
N-tert-butoxy-2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-tert-butoxy-2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-acetamide.
N-tert-butoxy-2-{4-[4-(4-fluorophenoxy)phenylthio]-tetrahydropyran-4-yl}-acetamide;
N-tert-butoxy-2-{4-[4-(4-chlorophenoxy)phenylthio]-tetrahydropyran-4-yl]-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylthio)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide;
N-tert-butoxy-4-[4-(4-pyridyloxy)phenylthiomethyl]-tetrahydropyran-carboxamide: ^{1}HNMR ($CDCl_3$) δ 1.31 (s, 9H), 1.70 (m_c, 2H), 2.14 (dm, J = 11.8 Hz, 2H), 3.21 (s, 2H), 3.63 (m_c, 2H), 3.82 (m_c, 2H), 6.84 (d, J =6.4 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 8.20 (s, 1H), 8.48 (d, J = 5.8 Hz, 2H).
N-tert-butoxy-4-[4-(5-chloro-2-pyridyloxy)phenylthiomethyl]-tetrahydropyran-carboxamide: mp 100.5-102.7 °C; IR (KBr) 3438 (br), 1657 cm^{-1}; ^{1}HNMR (DMSO-d_6) 1.19 (s, 9H), 1.57 (ddd, J = 13.5, 10.1, 4.0 Hz, 2H), 2.05 (dm, J = 13.5 Hz, 2H), 3.34 (s, 2H), 3.42 (m_c, 2H), 3.65 (dm, J = 11.6 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.95 (dd, J = 8.8, 2.7 Hz, 1H), 8.19 (d, J = 2.7 Hz, 1H), 10.37 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 26.66 (q), 33.03 (t), 43.20 (t), 44.25 (s), 64.10 (t), 80.78 (s), 113.00 (d), 121.88 (d), 124.88 (s), 130.43 (d), 132.67 (s), 139.93 (d), 145.51 (d), 151.89 (s), 161.58 (s), 171.64 (s); FABHRMS Calcd. for $C_{22}H_{28}N_2SO_4Cl$ (M^+ + H): 451.1458. Found: 451.1461. Anal. Calcd. for $C_{22}H_{27}N_2SO_4Cl$: C, 58.59; H, 6.03; N, 6.21. Found: C, 58.70; H, 6.05; N, 6.43.
N-tert-butoxy-3-[4-(5-chloro-2-pyridyloxy)phenylthio]-2,2-dimethyl-*N*-hydroxypropionamide: mp 90.8-91.9°C; IR (KBr) 3438 (br), 1651 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.18 (s, 9H), 1.21 (s, 6H), 3.20 (s, 2H), 7.08 (m_c, 3H), 7.40 (d, J = 8.7 Hz, 2H), 7.93 (dd, J = 8.7, 2.7 Hz, 1H), 8.17 (d, J = 2.7 Hz, 1H), 10.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 24.67 (q), 26.48 (q), 42.54 (s), 44.31 (t), 80.62 (s), 112.95 (d), 121.79 (d), 125.28 (s), 130.32 (d), 133.31 (s), 139.86 (d), 145.48 (d), 151.77 (s), 161.58 (s), 173.77 (s); FABHRMS Calcd. for $C_{20}H_{26}N_2SO_3Cl$ (M^+ + H): 409.1353. Found: 409.1354. Anal. Calcd. for $C_{20}H_{25}N_2SO_3Cl$: C, 58.74; H, 6.16; N, 6.85. Found: C, 58.91; H, 6.13; N, 7.07.
N-tert-butoxy-2-(4-methoxyphenylmercapto)-cyclohexane-carboxamide; and
N-tert-butoxy-*trans*-2-(4-methoxyphenylmercapto)-cyclopentanecarboxamide.

9E. Preparation of Ib, varying R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 9A above, but replacing 2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetic acid with other compounds of Formula Ia, other compounds of Formula Ib are prepared.

EXAMPLE 10

Preparation of Compounds of Formula Id

10A. Preparation of Id where n is 0, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Cyclopentyl, and R^5 is 4-Phenoxyphenyl

The *N-tert*-butoxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide was dissolved in trifluoroacetic acid (6 ml) and allowed to stand for 24 hours. The acid was evaporated off under reduced pressure and the product purified by preparative TLC, eluting with 6.5% methanol/methylene chloride gave *N*-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide (100 mg).

10B. Preparation of Id where n is 0, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 10A above, but replacing *N-tert*-butoxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide with other compounds of Formula Ib, the following compounds of Formula Id where n is 0 are prepared:

N-hydroxy-2-[4-(4-phenoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;

N-hydroxy-2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-*N*-CBZ-piperidin-4-yl}-*N*-hydroxy-acetamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-piperidin-4-yl}-*N*-hydroxy-acetamide;
3-benzyl-*N*-hydroxy-3-(3-methoxyphenylthio)-propionamide;
N-hydroxy-2-[4-(4-phenoxyphenylthio)-piperidin-4-yl)]-acetamide;
N-hydroxy-2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-acetamide;
2-benzyl-*N*-hydroxy-3-(phenylthio)-propionamide;
3-benzyl-*N*-hydroxy-3-(phenylthio)-propionamide;
3-benzyl-*N*-hydroxy-3-(4-methoxyphenylthio)-propionamide;
3-benzyl-*N*-hydroxy-3-[(4-phenylthiophenyl)thio]-propionamide;
3-benzyl-*N*-hydroxy-3-(4-phenoxyphenylthio)-propionamide;
3-benzyl-*N*-hydroxy-3-[(4-biphenyl)thio]-propionamide;
3-benzyl-*N*-hydroxy-3-(2-naphthylthio)-propionamide;
3-benzyl-*N*-hydroxy-3-(4-methoxystyrylphenylthio)-propionamide;
3-cyclopentylmethyl-*N*-hydroxy-3-(4-methoxyphenylthio)-propionamide;
3-cyclopentylmethyl-*N*-hydroxy-2-isopropyl-3-(4-methoxyphenylthio)-propionamide;
3-ethyl-*N*-hydroxy-2-methyl-3-(4-methoxyphenylthio)-propionamide;
3,3-dimethyl-*N*-hydroxy-(4-methoxyphenylthio)-propionamide;
N-hydroxy-2-[1-(4-methoxyphenylthio)-cyclopent-1-yl]-acetamide;
N-hydroxy-2-[1-(4-methoxyphenylthio)-(4-methylcyclohex-1-yl]-acetamide;
N-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclohex-1-yl]-acetamide;
N-hydroxy-2-[4-(4-methoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-hydroxy-2-[4-(4-methoxyphenylthio)-piperidin-4-yl)]-acetamide;
N-hydroxy-2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetamide; 2-{4-[4-(4-chlorophenoxy)-phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxy-acetamide;
2-{4-[4-(4-fluorophenoxy)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxy-acetamide, m.p. 50-55°C; and
N-hydroxy-2-[4-(4-phenoxyphenylthio)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide.

10C. Preparation of Id where n is 0, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 10A above, but replacing *N*-*tert*-butoxy-2-[1-(4-phenoxyphenylthio)cyclopent-1-yl]-acetamide with other compounds of Formula Ib, other compounds of Formula Id where n is 0 are prepared.

EXAMPLE 11

Preparation of Compounds of Formula Id

11A. Preparation of Id where n is 1, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Cyclopentyl, and R^5 is 4-Phenoxyphenyl

A solution of *N*-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide (45 mg) in acetone (4 ml) was treated with sodium periodate (260 mg) in water (2 ml). Over the course of 24 hours, two additional portions of sodium periodate (260 mg) were added. After complete disappearance of starting material the solution was diluted with methylene chloride, filtered, dried, and the solvent evaporated under reduced pressure. Preparative TLC on silica gel and elution with 10% methanol/methylene chloride gave *N*-hydroxy-2-[1-(4-phenoxyphenylsulfinyl)-cyclopent-1-yl]-acetamide (15 mg), ^{1}H NMR (CDCl3) 7.64 (d,2H), 7.44 (t,2H), 7.30-7.05 (m,5H), 2.97 (d,1H), 2.53 (d,1H), 2.15-1.65 (m,8H).

11B. Preparation of Id where n is 1, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Tetrahydropyran-4-yl, and R^5 is 4-(4-Fluorophenoxy)-phenyl

2-{4-[4-(4-Fluorophenoxy)phenylthio]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide (500 mg) was dissolved in methanol (25 ml). OXONE (400 mg) in water (5 ml) was added. After stirring for 30 minutes, the mixture was partitioned between methylene chloride and water. Preparative TLC on silica gel and elution with 10% methanol/methylene chloride gave 2-{4-[4-(4-fluorophenoxy)phenyl-sulfinyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide (402 mg, m.p. 120°C).

11C. Preparation of Id where n is 1, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 11A or 11B above, but replacing *N*-hydroxy-2-[1-(4-phenoxyphe-

nylthio)-cyclopent-1-yl]-acetamide with other compounds of Formula Id where n is 0, other compounds of Formula Id where n is 1 are prepared, for example;

N-hydroxy-2-[4-(4-phenoxyphenylsulfinyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-hydroxy-2-[4-(4-phenoxyphenylsulfinyl)-piperidin-4-yl)]-acetamide;
N-hydroxy-2-[4-(4-methoxyphenylsulfinyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;
2-{4-[4-(4-fluorophenoxy)phenylsulfinyl]-piperidin-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-[4-(4-methoxyphenylsulfinyl)-piperidin-4-yl)]-acetamide;
2-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfinyl)-propionamide;
3-benzyl-*N*-hydroxy-3-(3-methoxyphenylsulfinyl)-propionamide;
3-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfinyl)-propionamide;
3-benzyl-*N*-hydroxy-3-[(4-phenylthiophenyl)sulfinyl]-propionamide;
3-benzyl-*N*-hydroxy-3-(4-phenoxyphenylsulfinyl)-propionamide;
3-benzyl-*N*-hydroxy-3-[(4-biphenyl)sulfinyl]-propionamide;
3-benzyl-*N*-hydroxy-3-(2-naphthylsulfinyl)-propionamide;
3-benzyl-*N*-hydroxy-3-(4-methoxystyrylphenylsulfinyl)-propionamide;
3-cyclopentylmethyl-*N*-hydroxy-3-(4-methoxyphenylsulfinyl)-propionamide;
3-cyclopentylmethyl-*N*-hydroxy-2-isopropyl-3-(4-methoxyphenylsulfinyl)-propionamide;
3-ethyl-*N*-hydroxy-2-methyl-3-(4-methoxyphenylsulfinyl)-propionamide;
3,3-dimethyl-*N*-hydroxy-(4-methoxyphenylsulfinyl)-propionamide;
N-hydroxy-2[1-(4-methoxyphenylsulfinyl)-cyclopent-1-yl]-acetamide;
N-hydroxy-2-[1-(4-methoxyphenylsulfinyl)-(4-methylcyclohex-1-yl]-acetamide;
N-hydroxy-2-[1-(4-phenoxyphenylsulfinyl)-cyclohex-1-yl]-acetamide;
N-hydroxy-2-[4-(4-methoxyphenylsulfinyl)-*N*-CBZ-piperidin-4-yl)]-acetamide; and
N-hydroxy-2-[4-(4-methoxyphenylsulfinyl)-piperidin-4-yl)]-acetamide.
N-hydroxy-2-[4-(4-phenoxyphenylsulfinyl)-tetrahydropyran-4-yl]-acetamide;
4-[4-(4-chlorophenoxy)phenylsulfinylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 141.3-142.1 °C; IR (KBr) 3436 (br), 1649 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.67 (dm, *J* = 13.9 Hz, 1H), 1.79 (dm, *J* = 13.9 Hz, 1H), 1.97 (dm, *J* = 13.9 Hz, 1H), 2.24 (dm, *J* = 13.9 Hz, 1H), 2.97 (d, *J* = 13.7 Hz, 1H), 3.07 (d, *J* = 13.7 Hz, 1H), 3.33-3.54 (m_c, 2H), 3.69 (m_c, 2H), 7.12 (d, *J* = 8.9 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.48 (d, *J* = 8.9 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 8.87 (br s, 1H), 10.76 (s, 1H), ^{13}CNMR (DMSO-d_6) ∂32.43 (t), 33.71 (t), 42.69 (s), 63.65 (t), 67.12 (t), 118.90 (d), 121.07 (d), 126.11 (d), 128.19 (s), 130.07 (d), 139.51 (s), 154.62 (s), 158.72 (s), 169.68 (s); FABHRMS Calcd. for $C_{19}H_{21}NSO_5Cl$ ($M^+ + H$): 410.0829 Found: 426.0825. Anal. Calcd. for $C_{19}H_{20}NSO_5Cl$: C, 55.68; H, 4.92; N, 3.42. Found: C, 55.70; H, 4.93; N, 3.64.
2-{4-[4-(4-chlorophenoxy)-phenylsulfinyl]-tetrahydropyran-4-yl } -*N*-hydroxyacetamide; and
N-hydroxy-2-[4-(4-phenoxyphenylsulfinyl)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide.

EXAMPLE 12

Preparation of Compounds of Formula Id

12A. Preparation of Id where n is 2, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Cyclopentyl, and R^5 is 4-Phenoxyphenyl

A solution of *N*-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide (45 mg) in methanol (4 ml) was treated with a solution of OXONE (260 mg) in water (2 ml). The mixture was stirred for 1 hour, then partitioned between methylene chloride and water. The organic layer was separated, and the solvent removed under reduced pressure. Preparative TLC on silica gel and elution with 10% methanol/methylene chloride gave *N*-hydroxy-2-[1-(4-phenoxyphenylsulfonyl)cyclopent-1-yl]-acetamide (20 mg), m/e = 393 (MNH_4^+, CIMS).

12B. Preparation of Id where n is 2, R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a mechanically stirred suspension of 4-[4-(4-chlorophenoxy)-phenylthiomethyl]tetrahydropyran-4-(N-hydroxycarboxamide) (59.8 g, 151.8 mmol) in 20% tetrahydrofuran-methanol (1570 ml) cooled to 5°C was added dropwise a solution of OXONE (152 g, 247 mmol) in water (1 litre), maintaining an internal temperature of 15-20°C. The mixture was stirred for 5.5 hours, and the mixture then partitioned between 30% ethyl acetate/water (3 litres). The aqueous layer was washed with ethyl acetate (2 x 300 ml), the combined ethyl acetate layers dried over magnesium sulfate, concentrated under reduced pressure, and the residue crystallized from the minimum amount of methylene chloride/hexanes,

to afford analytically pure 4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) as a white powder (54.2 g, 84%). mp 147.7-148.9 °C; IR (KBr) 3429 (br), 1636 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.70 (dm, *J* = 13.9, 2H), 1.96 (dm, *J* = 13.9 Hz, 2H), 3.38-3.48 (m, 2H), 3.58-3.68 (m, 2H), 3.58-3.68 (m, 2H), 3.66 (s, 2H), 7.19 (d, *J* = 8.9 Hz, 2H), 7.19 (d, *J* = 8.9 Hz, 2H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.85 (d, *J* = 8.9 Hz, 2H), 8.68 (d, *J* = 2.0 Hz, 1H), 10.54 (d, *J* = 2.0 Hz, 1H), ^{13}CNMR (DMSO-d_6) δ 32.83 (t), 41.70 (s), 61.02 (t), 63.19 (t), 118.01 (d), 121.71 (d), 128.73 (s), 130.08 (d), 130.19 (d), 135.20 (s), 153.83 (s), 160.86 (s), 168.96 (s); FABHRMS Calcd. for $C_{19}H_{20}NSO_6Cl$: 426.0778. Found: 426.0774. Anal. Calcd. for $C_{19}H_{20}NSO_6Cl$: C, 53.59; H, 4.73; N, 3.29. Found: C, 53.58; H, 4.70; N, 3.40.

12C. Preparation of Id where n is 2, R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 is hydrogen, R^4 is Benzyl, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a solution of 3-benzyl-4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid (316 mg, 0.63 mmol) and *N,N*-dimethylformamide catalyst (10 μL) in methylene chloride (6 ml) at 0°C was added oxalyl chloride (200 μL, 2.20 mmol) dropwise over 10 minutes. The mixture was warmed to room temperature over 1 hour, the partial slurry stirred an additional 8 hours, and concentrated *in vacuo* until the theoretical mass of the acid chloride was obtained. This mixture was dissolved in methylene chloride (8 ml), cooled to 0°C, and a neat solution of *N,O*-bis(trimethylsilyl)hydroxylamine (0.56 g, 3.15 mmol) added dropwise over 5 minutes. The mixture was immediately warmed to room temperature, stirred for 48 hours, and recooled to 0°C. To this solution was added aqueous 1M hydrochloric acid (5 ml, 150 mmol), and the solution stirred for an additional 30 minutes, partitioned between ethyl acetae (150 ml) and brine (50 ml). The organic layer was dried over magnesium sulfate, concentrated *in vacuo*, chromatographed over silica gel, eluted with 4% methanol/methylene chloride) to afford 280 mg (86%) of 3-benzyl-4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarbamide) hydroxamic acid. mp 108-113°C; IR (KBr) 3422 (br), 1653 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.76-1.86 (m, 1H), 2.08-2.27 (m, 2H), 2.34 (dm, *J* = 13.8 Hz, 1H), 2.91 (dd, *J* = 16.5, 7.2 Hz, 1H), 3.17 (dd, *J* = 16.4, 4.0 Hz, 1H), 3.19-3.23 (tm, *J* = 9.0 Hz, 1H), 3.43 (td, *J* = 11.9, 2.4 Hz, 2H), 6.65-6.72 (m, 2H), 6.76 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.98-7.04 (m, 3H), 7.30 (d, *J* = 8.9 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 31.76 (t), 34.23 (t), 47.30 (s), 64.07 (t), 64.66 (t), 72.68 (d), 117.50 (d), 121.64 (d), 126.47 (d), 127.96 (d), 128.53 (d), 130.31 (d), 130.69 (d), 132.91 (s), 137.83 (s), 153.34 (s), 162.12 (s), 171.30 (s); FABMS (M^+ +H): 516; Anal. Calcd. for $C_{26}H_{26}NSO_6Cl$: C, 60.52; H, 5.08; N, 2.71. Found: C, 60.45; H, 5.10; N, 2.55.

12D. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 12C above, but replacing 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) with other compounds of Formula Iba, the following compounds of Formula Id where n is 2 were prepared:

4-[4-(4-fluorophenoxy)phenylsulfonylmethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 153.1-153.9 °C; IR (KBr) 3434 (br), 1636 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.87 (ddd, *J* = 13.6, 8.8, 4.0 Hz, 2H), 2.22 (dm, *J* = 13.6 Hz, 2H), 3.52-3.78 (m, 4H), 7.00-7.16 (m, 6H), 7.84 (d, *J* = 8.9 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 33.12 (t), 42.19 (s), 62.52 (t), 63.96 (t), 116.88 (dd, J_{C-F} = 21.3 Hz), 117.30 (d), 121.97 (dd, J_{C-F} = 8.4 Hz), 130.18 (s), 134.21 (d), 150.66 (d, J_{C-F} = 2.6 Hz), 159.73 (d, J_{C-F} = 243.8 Hz), 162.61 (s), 169.73 (s); FABMS (M^+ + H): 410. Anal. Calcd. for $C_{19}H_{20}NSO_6F$: C, 55.74; H, 4.92; N, 3.42. Found: C, 55.45; H, 4.91; N, 3.38.

4-[4-(4-bromophenoxy)phenylsulfonylmethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 150.1-151.0 °C; IR (KBr) 3432 (br), 1636 cm^{-1}; ^{1}HNMR ($CDCl_3$; NH and OH not observed) ∂ 1.87 (ddd, *J* = 13.6, 8.7, 3.9 Hz, 2H), 2.12 (dm, *J* = 13.6 Hz, 2H), 3.52 (s, 2H), 3.62-3.80 (m, 4H), 6.97 (d, *J* = 8.8 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H); ^{13}CNMR ($CDCl_3$) δ 33.10 (t), 42.16 (s), 62.49 (t), 63.93 (t), 117.66 (s), 117.83 (d), 121.93 (d), 130.20 (d), 133.17 (d), 134.61(s), 154.13 (s), 161.79 (s), 169.53 (s); FABHRMS Calcd. for $C_{19}H_{20}NSO_6Br$ (M^+ + H): 470.0273. Found: 470.0268. Anal. Calcd. for $C_{19}H_{20}NSO_6Br$: C, 48.51; H, 4.28; N, 2.98. Found: C, 48.29; H, 4.02; N, 2.94.

3-(4-benzoylphenylsulfonyl)-2,2-dimethyl-N-hydroxypropionamide;

3-[4-(4-chlorophenoxy)phenylsulfonyl]-2,2-dimethyl-*N*-hydroxypropionamide: mp 154.9-156.1 °C; ^{1}HNMR ($CDCl_3$) δ 1.45 (s, 6H), 3.48 (s, 2H), 7.02 (d, *J* = 8.9 Hz, 2H), 7.04 (d, *J* = 8.9 Hz, 2H), 7.38 (d, *J* = 8.9 Hz, 2H), 7.85 (d, *J* = 8.9 Hz, 2H); FABMS (M^+ +H): 384.0. Anal. Calcd. for $C_{17}H_{18}NSO_5Cl$: C, 53.19; H, 4.73; N, 3.65. Found: C, 52.98; H, 4.69; N, 3.73.

4-(4-phenoxyphenylsulfonylmethyl)-tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 141.8-142.9 °C; IR (KBr) 3432 (br), 1636 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.74 (ddd, *J* = 13.8, 10.0, 3.9 Hz, 2H), 1.98 (dm, *J* = 13.8 Hz, 2H), 3.45 (m_c, 2H), 3.64 (m_c, 2H), 3.65 (s, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 7.26 (d, *J* = 7.5 Hz, 2H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 8.68 (s, 1H), 10.52 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.87 (t), 41.76 (s), 61.19 (t), 63.28 (t), 117.71 (d), 119.99 (d), 124.91 (d), 130.04 (d), 130.34 (d), 134.85 (s), 154.85 (s), 161.39 (s), 168.97 (s); FABHRMS Calcd. for $C_{19}H_{22}NSO_6$ (M^+ + H): 392.1168. Found: 392.1162. Anal. Calcd. for $C_{19}H_{21}NSO_6.0.5H_2O$: C,

56.99; H, 5.54; N, 3.50. Found: C, 57.06; H, 5.35; N, 3.93.

4-[4-(4-thiophen-2-yl)phenoxyphenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 172.2-176.5 °C; IR (KBr) 3428 (br), 1636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.72 (dm, J = 14.5 Hz, 2H), 1.99 (dm, J = 14.5 Hz, 2H), 3.46 (m_c, 2H), 3.65 (m_c, 2H), 3.66 (s, 2H), 7.14 (dd, J = 4.9, 3.6 Hz,1H), 7.19 (d, J = 8.7 Hz, 2H), 7.20 (d, J = 8.9 Hz, 2H), 7.48 (dd, J = 3.6, 1.2 Hz, 1H), 7.52 (dd, J = 4.9, 1.2 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 8.68 (s, 1H), 12.58 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 32.89 (t), 41.78 (s), 61.20 (t), 63.28 (t), 117.88 (d), 120.55 (d), 123.66 (d), 125.56 (d), 127.34 (d), 128.45 (d), 130.07 (d), 130.62 (s), 135.04 (s), 142.45 (s), 154.30 (s), 161.16 (s), 169.03 (s); FABHRMS Calcd. for $C_{23}H_{24}NS_2O_6$ (M^+ + H): 474.1045. Found: 474.1050. Anal. Calcd. for $C_{23}H_{23}NS_2O_6$: C, 58.33; H, 4.90; N, 3.00. Found: C, 58.18; H, 4.84; N, 3.19.

4-[4-(4-thiophen-3-yl)phenoxyphenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 183.5-184.4 °C; IR (KBr) 3432 (br), 1636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.72 (m_c, 2H), 1.98 (m_c, 2H), 3.48 (m_c, 2H), 3.65 (m_c, 4H), 7.18 (m_c, 4H), 7.55 (dd, J = 5.1 Hz, 1H), 7.62 (d, J = 4.9, 3.7 Hz, 2H), 7.80 (d, J = 8.6 Hz, 2H), 7.86 (m_c, 3H), 8.69 (s, 1H), 10.58 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.88 (t), 41.79 (s), 61.19 (t), 63.28 (t), 117.71 (d), 120.42 (d), 120.81 (d), 126.09 (d), 127.10 (d), 127.97 (d), 130.06 (d), 132.10 (s), 134.89 (s), 140.54 (s), 153.86 (s), 168.85 (s); FAB-HRMS Calcd. for $C_{23}H_{24}NS_2O_6$ (M^+ + H): 474.1045. Found: 474.1049. Anal. Calcd. for $C_{23}H_{23}NS_2O_6.0.75H_2O$: C, 56.72; H, 5.07; N, 2.88. Found: C, 56.74; H, 4.78; N, 3.22.

3,3-dimethyl-3-[(4-chlorophenoxy)phenylsulfonyl]-*N*-hydroxypropionamide;

{4-[4-(4-benzo[b]thiophen-2-yl-phenoxy)phenylsulfonyl)-tetrahydropyran-4-yl]-*N*-hydroxyacetamide;

2-{4-[4-(phenylmethyl)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;

2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]tetrahydropyran-4-yl}-*N*-hydroxyacetamide; and

2-{4-[4-(4-bromophenoxy)phenylsulfonyl]tetrahydropyran-4-yl}-*N*-hydroxyacetamide.

12E. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 12A or 12B above, but replacing *N*-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide with other compounds of Formula Id where n is 0, the following compounds of Formula Id where n is 2 are prepared, for example;

4-(4-phenoxyphenylsulfonylmethyl)tetrahydropyran-4-(*N*-hydroxycarboxamide);

4-[4-(4-fluorophenoxy)phenylsulfonylmethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]piperidine-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-methylpiperidine-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-cyclopropylmethylpiperidine-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-acetylpiperidine-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-(3-pyridyl)-piperidine-4-(*N*-hydroxycarboxamide);

4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-(3-pyridoyl)-piperidine-4-(*N*-hydroxycarboxamide);

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;

N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;

2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-*N*-CBZ-piperidin-4-yl}-*N*-hydroxyacetamide;

2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;

N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide;

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide;

2-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide;

3-benzyl-*N*-hydroxy-3-(3-methoxyphenylsulfonyl)-propionamide;

3-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide;

3-benzyl-*N*-hydroxy-3-[(4-phenylthiophenyl)sulfonyl]-propionamide;

3-benzyl-*N*-hydroxy-3-(phenylsulfonyl)-propionamide;

3-benzyl-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide;

3-benzyl-3-[(4-biphenyl)sulfonyl]-*N*-hydroxypropionamide;

3-benzyl-*N*-hydroxy-3-(2-naphthylsulfonyl)-propionamide;

3-benzyl-*N*-hydroxy-3-(4-methoxystyrylphenylsulfonyl)-propionamide;

3-(cyclopentylmethyl)-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide;

3-(cyclopentylmethyl)-*N*-hydroxy-2-isopropyl-3-(4-methoxyphenyl-sulfonyl)-propionamide;

3-ethyl-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-2-methylpropionamide;

3,3-dimethyl-*N*-hydroxy-(4-methoxyphenylsulfonyl)-propionamide;

N-hydroxy-2-[1-(4-methoxyphenylsulfonyl)-cyclopent-1-yl]-acetamide;

N-hydroxy-2-[1-(4-methoxyphenylsulfonyl)-(4-methylcyclohex-1-yl]-acetamide;

N-hydroxy-2-[1-(4-phenoxyphenylsulfonyl)-cyclohex-1-yl]-acetamide;

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide;

2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-N-hydroxyacetamide;

2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide; and

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide.

12F. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 12A above, but replacing *N*-hydroxy-2-[1-(4-phenoxyphenylthio)-cyclopent-1-yl]-acetamide with other compounds of Formula Id where n is 0, other compounds of Formula Id where n is 2 are prepared.

EXAMPLE 13

Preparation of Compounds of Formula I where Y is *tert*-BuONH-

13A. Preparation of Ic where n is 2, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Tetrahydropyran, and R^5 is 4-Phenoxyphenyl

To a cooled solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetamide (14.1 g, 33.9 mmol) in methanol (340 ml) was added a solution of OXONE (33.9 g) in water (170 ml). The reaction mixture was stirred for 5 hours at room temperature, concentrated to half the original volume under reduced pressure, and the residue then partitioned between ethyl acetate and water. The solvent was removed from the ethyl acetate extracts under reduced pressure. The residue chromatographed on silica gel, eluting with 10% methanol/methylene chloride, to give *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide as a white foam.

13B. Preparation of Ic where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached are *N*-BOC-Piperidine, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylthiomethyl)-*N*-BOC-piperidin-4-yl]-carboxamide (4.96 g, 9.03 mmol) in anhydrous methylene chloride (70 ml) cooled to 0°C, was added 60% 3-chloroperoxybenzoic acid (4.96 g). After the resulting mixture was allowed to warm to room temperature over 30 minutes and stirred for 5 minutes, 13.6M aqueous methyl sulfide (1 ml, 13.62 mmol) was added in one portion. The mixture was stirred 10 minutes, partitioned with saturated aqueous sodium bicarbonate (2 x 50 ml), dried over magnesium sulfate, and concentrated *in vacuo*. Chromatography over silica gel, and eluting with 25% ethyl acetate/hexanes, gave *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-*N*-BOC-piperidin-4-yl]-carboxamide as a white foam (4.70 g, 90%). ^{1}HNMR ($CDCl_3$) δ 1.31 (s, 9H), 1.46 (s, 9H), 1.59 (m_c, 2H), 2.18 (m_c, 2H), 3.42 (m_c, 2H), 3.45 (s, 2H), 3.62 (m_c, 2H), 7.01 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 8.44 (br s, 1H).

13C. Preparation of Ic where n is 2 and Y is *tert*-BuONH-, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 13B above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylthiomethyl)-*N*-BOC-piperidin-4-yl]-carboxamide with other compounds of Formula Ib, the following compound of Formula Ic where n is 2 and Y is *tert*-BuONH- was prepared:

N-tert-butoxy-4-[4-(4-pyridyloxy)phenylsulfonylmethyl]-tetrahydropyran-carboxamide: IR (KBr) 3434, 1684 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.33 (s, 9H), 2.01 (m_c, 2H), 2.24 (m_c, 2H), 3.55 (s, 2H), 3.79 (m_c, 4H), 6.93 (d, J =6.3 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 8.8 Hz, 2H), 8.38 (s, 1H), 8.57 (d, J = 6.3 Hz, 2H); FABHRMS Calcd. for $C_{22}H_{28}N_2SO_6$ (M^+ + H) 449.1746. Found: 449.1757.

N-tert-butoxy-4-[4-(5-chloro-2-pyridyloxy)phenylsulfonylmethyl]-tetrahydropyran-carboxamide: mp (broad) 100.8-135.8 °C; IR (KBr) 3436 (br), 1684 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.20 (s, 9H), 1.72 (m_c, 2H), 2.03 (m_c, 2H), 3.48 (m_c, 2H), 3.67 (m_c, 2H), 3.76 (s, 2H), 7.23 (dd, J = 8.8, 0.5 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 8.03 (dd, J = 8.8, 2.7 Hz, 1H), 8.25 (dd, J = 2.7, 0.5 Hz, 1H), 8.30 (s, 1H), 10.32 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 26.66 (q), 33.09 (t), 42.37 (s), 61.03 (t), 63.36 (t), 80.64 (s), 113.89 (d), 121.38 (d), 126.33 (s), 129.53 (d), 137.00 (s), 140.34 (d), 145.74 (d), 157.87 (s), 160.66 (s), 171.25 (s); FABHRMS Calcd. for $C_{22}H_{28}N_2SO_6Cl$ (M^+ + H): 483.1357. Found: 483.1354. Anal. Calcd. for $C_{22}H_{27}N_2SO_6Cl$: C, 54.71; H, 5.63; N, 5.80. Found: C, 54.46; H, 5.60; N, 5.98.

N-tert-butoxy-3-[4-(5-chloro-2-pyridyloxy)phenylsulfonyl]-2,2-dimethyl-propionamide: mp (broad) 64.5-70.5 °C; ^{1}HNMR (DMSO-d_6) δ 1.19 (s, 9H), 1.29 (s, 6H), 3.65 (s, 2H), 7.24 (d, J = 8.7 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 8.04 (dd, J = 8.8, 2.7 Hz, 1H), 8.26 (d, J = 2.7 Hz, 1H), 10.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.01 (q), 26.47 (q), 40.74 (s), 63.03 (t), 80.79 (s), 113.91 (d), 121.38 (d), 126.32 (s), 129.35 (d), 130.66 (s), 140.36

(d), 145.75 (d), 157.72 (s), 160.68 (s), 173.14 (s); FABHRMS Calcd. for $C_{20}H_{26}N_2SO_5Cl$ (M^+ + H): 441.1251. Found: 441.1248. Anal. Calcd. for $C_{20}H_{25}N_2SO_5Cl$: C, 54.48; H, 5.71; N, 6.35. Found: C, 54.37; H, 5.69; N, 6.57.

13D. Preparation of Ic where n is 2 and Y is *tert*-BuONH-, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 13A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylthio)-tetrahydropyran-4-yl]-acetamide with other compounds of Formula Ib, the following compounds of Formula Ic where n is 2 and Y is *tert*-BuONH-were prepared;

N-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-tert-butoxy-2-[4-(4-methoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide;
N-tert-butoxy-2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-piperidin-4-yl}-acetamide;
N-tert-butoxy-2-[4-(4-methoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide;
2-benzyl-*N-tert*-butoxy-3-(4-methoxyphenylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-(3-methoxyphenylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-(4-methoxyphenylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-[(4-phenylthiophenyl)sulfonyl]-propionamide;
3-benzyl-*N-tert*-butoxy-3-(phenylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-[(4-biphenyl)sulfonyl]-propionamide;
3-benzyl-*N-tert*-butoxy-3-(2-naphthylsulfonyl)-propionamide;
3-benzyl-*N-tert*-butoxy-3-(4-methoxystyrylphenylsulfonyl)-propionamide;
N-tert-butoxy-3-(cyclopentylmethyl)-3-(4-methoxyphenylsulfonyl)-propionamide;
N-tert-butoxy-3-(cyclopentylmethyl)-2-isopropyl-3-(4-methoxyphenylsulfonyl)-propionamide;
N-tert-butoxy-3-ethyl-2-methyl-3-(4-methoxyphenylsulfonyl)-propionamide;
N-tert-butoxy-3,3-dimethyl-(4-methoxyphenylsulfonyl)-propionamide;
N-tert-butoxy-2-[1-(4-methoxyphenylsulfonyl)-cyclopent-1-yl]-acetamide;
N-tert-butoxy-2-[1-(4-methoxyphenylsulfonyl)-(4-methylcyclohex-1-yl]-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-cyclohexanone-4-yl]-acetamide ethylene ketal;
N-tert-butoxy-2-[1-(4-phenoxyphenylsulfonyl)-cyclohex-1-yl]-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide;
N-tert-butoxy-2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-acetamide;
N-tert-butoxy-2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-acetamide;
N-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide;
N-tert-butoxy-2-(4-methoxyphenylsulfonyl)-cyclohexanecarboxamide; and
N-tert-butoxy-*trans*-2-(4-methoxyphenylsulfonyl)-cyclopentanecarboxamide.

13E. Preparation of Ic where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 13A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylthio)-*N*-CBZ-piperidin-4-yl)]-acetamide with other compounds of Formula Ib, other compounds of Formula Ic where n is 2 and Y is *tert*-BuONH- are prepared.

EXAMPLE 14

Preparation of Compounds of Formula Ic where Y is *tert*-BuONH-

14A. Preparation of Ic where n is 2, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Piperidine and R^5 is 4-Phenoxyphenyl

To a solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide (1.2 g, 2.1 mmol) in ethanol (21 ml) was added 10% palladium on carbon (1 g) and ammonium formate (6.7 g), and the mixture refluxed for 1 hour. The mixture was filtered through Celite, the filter cake washed with ethanol (150 ml) followed by 10% methanol in methylene chloride (150 ml). Solvent was removed from the filtrate under reduced pressure and the residue was dissolved in hot ethyl acetate. Filtration, concentration of the filtrate, followed by silica gel chromatography and elution with 10% methanol/methylene chloride gave *N*-tert-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide as a colorless oil.

14B. Preparation of Ic where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 14A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide with other *N*-CBZ protected compounds of Formula I, other compounds of Formula I where n is 2 and Y is *tert*-BuONH- are prepared.

EXAMPLE 15

Preparation of Compounds of Formula Id where Y is HONH-

15A. Preparation of Id where n is 2, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Piperidine, and R^5 is 4-Phenoxyphenyl

A solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperid-4-yl)]-acetamide (27 mg, 0.05 mmol) in dichloroethane (2 ml) was cooled to -20°C, and saturated with hydrochloric acid gas for 30 minutes. The reaction vessel was then sealed and the solution stirred for two days at 25°C. Solvent was removed from the reaction mixture under reduced pressure, and the residue dissolved in 50% methanol in methylene chloride. Addition of hexane precipitated *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide, m/e = 391 (MH^+, FAB).

15B. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 15A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide with other compounds of Formula Ic where Y is *tert*-BuONH-, the following compounds of Formula Id where n is 2 and Y is HONH- were prepared:

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide, m/e = 525 (MH^+);
N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-*N*-CBZ-piperidin-4-yl)]-acetamide, m/e = 463 (MH^+, FAB);
2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide, m.p. 196-197°C;
2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide, m.p. 200-201°C;
2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide: mp 135.7-136.1 °C; ^{1}HNMR ($CDCl_3$) δ 1.60 (m_c, 2H), 1.83 (m_c, 2H), 3.00 (s, 2H), 3.66 (m_c, 2H), 3.88 (m_c, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.9 Hz, 2H), 7.79 (d, *J* = 8.9 Hz, 2H), 7.25 (s, 1H), 9.49 (s, 1H); FABHRMS Calcd. for $C_{19}H_{20}NSO_6Cl$ (M^+ + H): 426.0778. Found: 426.0775. Anal. Calcd. for $C_{19}H_{20}NSO_6Cl$: C, 53.59; H, 4.73; N, 3.29. Found: C, 53.30; H, 4.67; N, 3.35.
2-[4-(4-cyclohexyloxyphenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide: m.p. 77-78°C;
N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide, m/e = 329 (MH^+);
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide, m/e = 391 (MH^+);
2-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide, m/e = 350.2 (MH^+);
3-benzyl-*N*-hydroxy-3-(3-methoxyphenylsulfonyl)-propionamide, m/e = 350.2 (MH^+);
3-benzyl-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide, m/e = 350.2 (MH^+);
3-benzyl-*N*-hydroxy-3-[(4-phenylthiophenyl)sulfonyl]-propionamide, m/e = 427 (MH^+);
3-benzyl-*N*-hydroxy-3-(phenylsulfonyl)-propionamide, m/e = 320 (MH^+);
3-benzyl-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide, m/e = 412.2 (MH^+);
3-benzyl-*N*-hydroxy-3-[(4-biphenyl)sulfonyl]-propionamide; m/e = 395 (MH^+);
3-benzyl-*N*-hydroxy-3-(2-naphthylsulfonyl)-propionamide, m/e = 370.1 (MH^+);
3-benzyl-*N*-hydroxy-3-[(4-methoxystyrylphenylsulfonyl]-propionamide, m/e = 452.2 (MH^+);
3-(cyclopentylmethyl)-*N*-hydroxy-3-(4-methoxyphenylsulfonyl)-propionamide, m/e = 342 (MH^+);
3-(cyclopentylmethyl)-*N*-hydroxy-2-isopropyl-3-(4-methoxyphenylsulfonyl)-propionamide;
3-ethyl-*N*-hydroxy-2-methyl-3-(4-methoxyphenylsulfonyl)-propionamide, m/e = 301 (MH^+);
3,3-dimethyl-3-(4-methoxyphenylsulfonyl)-*N*-hydroxypropionamide, elemental analysis: C_1H_1N;
N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-cyclopent-1-yl]-acetamide, m/e = 313 (MH^+);
N-hydroxy-2-[4-(4-methoxyphenylsulfonyl)-(4-methylcyclohex-1-yl]-acetamide, m/e = 341 (MH^+);
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)cyclohex-1-yl]-acetamide, m/e = 389 (MH^+);
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide, m.p. 88.5-90°C, m/e = 391 (MH^+);
2-{ 4-[4-(4-chlorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide;
2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide, m.p. 91-95°C;
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)tetrahydrothiopyran-1,1-dioxide-4-yl]-acetamide, m/e = 440.1 (MH^+);
N-hydroxy-*trans*-2-(4-methoxyphenylsulfonyl)-cyclopentanecarboxamide, m/e = 313 (MH^+);
N-hydroxy-*trans*-2-(4-methoxyphenylsulfonyl)-cyclohexanecarboxamide, m/e = 327 (MH^+); and

2-benzyl-*N*-hydroxy-*trans*-2-(4-methoxyphenylsulfonyl)-cyclopentane-carboxamide, m/e = 390 (MH^+, FABMS).

15C. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 15A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide with other compounds of Formula Ic where Y is *tert*-BuONH-, other compounds of Formula Id where n is 2 and Y is HONH-are prepared, for example:

2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-*N*-CBZ-piperidin-4-yl}-*N*-hydroxyacetamide;
2-{1-methyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide;
N-hydroxy-2-{1-methyl-4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-acetamide; and
2-{4-[4-(4-bromophenoxy)-phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide.

15D. Preparation of Id where n is 2, R^1 and R^2 are Hydrogen, R^3 and R^4 when taken together with the Carbon to which they are attached are Cyclohexanone, and R^5 is 4-Phenoxyphenyl

Following the procedure outlined in Example 15A, *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-cyclohexanone-4-yl]-acetamide ethylene ketal (400 mg) was prepared from the corresponding *N-tert*-butoxy precursor. The above product was dissolved in a 1:1 mixture of acetone and 1M hydrochloric acid (40 ml) and stirred at room temperature for 18 hours. The reaction was concentrated under reduced pressure and extracted with ethyl acetate. Silica gel chromatography using 10% methanol/methylene chloride gave 2-[4-(4-phenoxyphenylsulfonyl)cyclohexanone-4-yl]-*N*-hydroxyacetamide as a white solid: m.p. 106°C (dec), m/e = 404 (MH^+, FABMS).

15E. Preparation of Id where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached are Piperidine, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a sealed tube containing the free base *N-tert*-butoxy-2-{4-[4-(4-phenoxy)phenylsulfonylmethyl]-piperidin-4-yl}-carboxamide (780 mg, 1.62 mmol) in 1,2-dichloroethane (35 ml) at -30°C, was bubbled in gaseous hydrochloric acid until the saturation point was reached. The reaction vessel was then sealed and the solution stirred for two days. After the vessel was recooled to -30°C and opened, a stream of nitrogen gas bubbled through the solution, which was then warmed to room temperature. The mixture was concentrated to afford 2-{4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-piperidin-4-yl}-*N*-hydroxycarboxamide (747 mg, 100%). mp 166.7-176.2°C; ^{1}HNMR (CD_3OD) δ 2.39 (m_c, 2H), 3.12 (m_c, 2H), 3.36 (m_c, 2H), 3.63 (s, 2H), 7.12 (d, *J* = 8.9 Hz, 2H), 7.15 (d, *J* = 8.9 Hz, 2H), 7.44 (d, *J* = 9.0 Hz, 2H), 7.89 (d, *J* = 8.9 Hz, 2H); FABMS (M^+ +H): 425.0; Anal. Calcd. for $C_{19}H_{21}N_2SO_5Cl.HCl.1.5\,H_2O$: C, 46.73; H, 4.33; N, 5.74. Found: C, 46.83; H, 4.66; N, 5.71.

15F. Preparation of Id where n is 2, varying R^1, R^2, R^3, R^4, and R^5

Similarly, following the procedures of Example 15E above, but replacing *N-tert*-butoxy-2-{4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-piperidin-4-yl)}-carboxamide with other compounds of Formula Ic where Y is *tert*-BuONH-, other compounds of Formula Id where n is 2 and Y is HONH- were prepared, for example:

2-{4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-1-(cyclopropylmethyl)piperidin-4-yl}-*N*-hydroxycarboxamide hydrochloride (1.30 g, 84%). mp 120.5-124.0 °C; IR (KBr) 3429 (br), 1582 cm^{-1}; ^{1}HNMR (CD_3OD) δ 0.40-0.50 (m, 2H), 0.73-0.81 (m, 2H), 1.12 (m_c, 1H), 2.18 (m_c, 2H), 2.41 (d, *J* = 14.8 Hz, 2H), 2.63 (d, *J* = 14.3 Hz, 2H), 3.03 (m_c, 2H), 3.10 (m_c, 2H), 3.60 (m_c, 3H), 7.13 (m_c, 4H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 8.8 Hz, 2H); FABMS (M^+ +H): 479.1. Anal. Calcd. for $C_{23}H_{27}N_2SO_5Cl.HCl.H_2O$: C, 51.77; H, 5.09; N, 5.25. Found: C, 51.90; H, 5.53; N, 5.26.

2-{4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-*N*-hydroxy-1-nicotinoylmethylpiperidin-4-yl}-carboxamide hydrochloride (590 mg, 89%). mp 160.5 °C (effervescence); IR (KBr) 3426 (br), 1638 cm^{-1}; ^{1}HNMR (CD_3OD) δ 1.97 (m_c, 2H), 2.25 (m_c, 2H), 3.55 (m_c, 4H), 3.64 (s, 2H), 7.10 (d, *J* = 8.9 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.6 Hz, 2H), 8.12 (m_c, 1H), 8.61 (d, *J* = 7.9 Hz, 2H), 8.92 (d, *J* = 5.5 Hz, 2H), 8.98 (br s, 1H); FABMS (M^+ +H): 530.0. Anal. Calcd. for $C_{25}H_{29}N_3SO_6Cl.HCl.0.5H_2O$: C, 51.38; H, 4.14; N, 7.19. Found: C, 51.80; H, 4.46; N, 7.25.

2-{4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-*N*-hydroxy-1-methansulfonylpiperidin-4-yl}-carboxamide hydrochloride (682 mg, 69%). mp 107.3-112.3 °C; ^{1}HNMR ($CDCl_3$) δ 1.95 (m_c, 2H), 2.40 (m_c, 2H), 2.79 (s, 3H), 3.12 (m_c, 2H), 3.42 (s, 2H), 3.51 (m_c, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 7.07 (d, *J* = 8.9 Hz, 2H), 7.39 (d, *J* = 8.9 Hz, 2H), 7.83 (d, *J* = 8.9 Hz, 2H); FABMS (M^+ +H): 503.2. Anal. Calcd. for $C_{20}H_{23}N_2S_2O_7Cl$: C, 47.76; H, 4.61; N, 5.57. Found: C, 47.32; H, 4.56; N, 5.52.

4-[4-(4-pyridyloxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) hydrochloride: mp 188-

197°C; IR (KBr) 3431, 1638 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.73 (m_c, 2H), 2.01 (dm, J =14.7 Hz, 2H), 3.43 (m_c, 2H), 3.65 (m_c, 2H), 3.78 (s, 2H), 7.56 (m_c, 4H), 8.02 (d, J = 8.7 Hz, 2H), 8.82 (d, J = 6.6 Hz, 2H), 10.64 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 33.01 (t), 39.78 (t), 61.13 (s), 63.26 (t), 114.48 (d), 121.81 (d), 130.87 (d), 138.41 (s), 144.92 (d), 156.14 (s), 168.4 (s), 168.8 (s); Anal. Calcd. for $C_{18}H_{21}N_2SO_6Cl.HCl.0.6\ H_2O$: C, 49.17; H, 5.09; N, 6.37. Found: C, 49.16; H, 5.03; N, 6.27.

4-[4-(5-chloro-2-pyridyloxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide): mp 141.9-142.7°C; IR (KBr) 3432, 1636 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.73 (m_c, 2H), 2.01 (dm, J = 14.7 Hz, 2H), 3.33 (s, 2H), 3.46 (m_c, 2H), 3.64 (m_c, 2H), 7.23 (dd, J = 8.7, 0.4 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.7, 2.7 Hz, 2H), 8.26 (dd, J = 2.7, 0.4 Hz, 1H), 8.69 (s, 1H), 10.62 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 32.89 (t), 41.81 (s), 60.96 (t), 63.26 (t), 113.88 (d), 121.32 (d), 126.31 (s), 129.58 (d), 136.93 (s), 140.33 (s), 145.74 (d), 157.82 (s), 160.69 (s), 169.02 (s); FABHRMS Calcd. for $C_{18}H_{19}N_2SO_6Cl$ (M^+ + H): 427.0731. Found: 427.0726. Anal. Calcd. for $C_{18}H_{19}N_2SO_6C1.0.5H_2O$: C, 49.49; H, 4.61; N, 6.41. Found: C, 49.54; H, 4.35; N, 6.47.

3-[4-(5-chloro-2-pyridyloxy)phenylsulfonyl]-2,2-dimethyl-*N*-hydroxypropionamide: mp 115.8-116.6 °C; IR (KBr) 3412 (br), 1644 cm^{-1}; ^{1}HNMR (CD_3OD) δ 1.38 (s, 6H), 3.58 (s, 2H), 7.13 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 8.8 Hz, 2H), 7.89 (dd, J = 8.7, 2.7 Hz, 2H), 7.95 (d, J = 8.8 Hz, 1H), 8.15 (d, J = 2.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 25.55 (q), 41.76 (s), 65.06 (t), 114.91 (d), 122.35 (d), 128.40 (s), 130.98 (d), 138.21 (s), 141.44 (d), 146.88 (d), 159.89 (s), 162.32 (s), 174.51 (s); FABHRMS Calcd. for $C_{16}H_{18}N_2SO_5Cl$ (M^+ + H): 385.0625. Found: 383.0625. Anal. Calcd. for $C_{16}H_{17}N_2SO_5Cl$: C, 49.94; H, 4.48; N, 7.28. Found: C, 49.58; H, 4.42; N, 7.30.

15G. Preparation of Id where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached are 1-Picolylpiperidine. and R^5 is 4-(4-Chlorophenoxy)-phenyl

A solution containing *N-tert*-butoxy-2-{4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-1-picolylpiperidin-4-yl}-carboxamide (324 mg, 0.566 mmol) in trifluoroacetic acid (5 ml) was heated to 30°C for 1.5 hours, cooled to room temperature, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 ml), washed with saturated sodium bicarbonate (2 x 30 ml), dried over magnesium sulfate, and concentrated *in vacuo*. Chromatography over silica gel, eluting with 6% methanol/methylene chloride, yielded 2-{4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-1-picolylpiperidin-4-yl}-*N*- hydroxycarboxamide hydrochloride: mp 222.5-223.9°C; IR (KBr) 3436 (br), 1645 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 2.15 (m_c, 3H), 2.40 (m_c, 2H), 3.32 (m_c, 2H), 3.57 (m_c, 2H), 3.97 (m_c, 2H), 4.44 (m_c, 2H), 4.51 (m_c, 2H), 7.19 (m_c, 4H), 7.50 (d, J = 8.8 Hz, 2H), 7.87 (m_c, 3H), 8.49 (m_c, 1H), 8.85 (m_c, 1H), 8.99 (br s, 1H); FABMS (M^+ +H): 516.1. Anal. Calcd. for $C_{29}H_{34}N_3SO_5Cl.2HCl.0.5\ H_2O$: C, 50.22; H, 4.89; N, 7.03. Found: C, 50.17; H, 4.65; N, 7.00.

EXAMPLE 16

Preparation of Compounds of Formula Ih

16A. Preparation of Ie where R^1 , R^2 and R^3 are Hydrogen, and R^4 is Benzyl

To a cooled solution of 3-benzyl-3-(4-bromophenylthio)-propionic acid in methanol (50 ml) was added a solution of OXONE (8 g) in water (50 ml). The reaction mixture was stirred for 2 hours at room temperature, and then partitioned between methylene chloride and water. The solvent was removed from the organic layer under reduced pressure, to give 3-benzyl-3-(4-bromophenylsulfonyl)-propionic acid, as a crystalline solid.

16B. Preparation of If where R^1 , R^2 and R^3 are Hydrogen, and R^4 is Benzyl

1. A solution of 3-(4-bromophenyl)sulfonyl-4-benzylpropionic acid (200 mg, 0.52 mmol), phenylboronic acid (127 mg, 1.04 mmol), and tetrakis(triphenylphospine)palladium(0) (24 mg, 0.021 mmol) in a 1:1 mixture of ethanol and benzene (5 ml) was heated to reflux temperature with stirring. A solution of 2M sodium carbonate (1 ml) was added to the reaction mixture, and stirring continued at reflux for approximately 2 hours. The mixture was cooled and then partitioned between ethyl acetate and water. The solvent layer was washed with brine, dried over magnesium sulfate, filtered, and solvent removed under reduced pressure. The residue was chromatographed, eluting with 7% methanol/methylene chloride, to yield 3-(4-biphenyl)-sulfonyl-4-benzylpropionic acid. ^{1}HNMR ($CDCl_3$): 7.75 ppm (m, 14H); 3.42 ppm (dd, 1H); 2.82 ppm (dd, 1H); 2.77 ppm (dd, 1H); 2.51 ppm (dd, 1H).

16C. Preparation of Ih where R^1, R^2, and R^3 are Hydrogen and R^4 is Benzyl

The 3-(4-biphenyl)sulfonyl-4-benzylpropionic acid, prepared as shown above, was then converted to 3-(4-biphenyl)sulfonyl-4-benzyl-*N*-hydroxypropionamide, m.p. 65°C (shrinks with decomposition) as described in Examples 10A.

16D. Preparation of Ifb where R^1 and R^2 Together with the Carbon to which they are attached represent Tetrahydropyran-4-yl, R^3 and R^4 are Hydrogen, R^5 is 4-(Thiophen-2-yl)phenoxyphenyl

1. To a mechanically stirred suspension of 4-[4-(4-bromophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid (5.50 g, 13.0 mmol) in 20% tetrahydrofuran/methanol (135 ml) cooled to 15°C, was added a solution of OXONE (13.0 g, 21.2 mmol) in water (86 ml) dropwise, maintaining an internal temperature of 15-20°C. The mixture was stirred for 12 hours and dissolved in 40% ethyl acetate/water (1200 ml). The layers were partitioned, and the water layer back extracted using ethyl acetate (2 x 300 ml). The combined ethyl acetate layers were dried ($MgSO_4$), concentrated, and the residue crystallized from the minimum amount of methylene chloride/hexanes to afford 4-[4-(4-bromophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid as a white powder, which was used without further purification (5.00 g, 84%).

2. To a solution of 4-[4-(4-bromophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid (1.10 g, 2.42 mmol) of in *N, N*-dimethylformamide (15 ml) was added tetrakis(triphenylphosphine)-palladium(0) (108 mg), 2-thiophene boronic acid (857 mg, 6.70 mmol), followed by 2M aqueous sodium carbonate (2.7 ml, 5.4 mmol). The reaction was heated to reflux for 10 hours, cooled to room temperature, and the mixture partitioned between methylene chloride (100 ml) and 1N aqueous hydrochloric acid (20 ml). The aqueous layer was back extracted with methylene chloride (100 ml), and the combined organic layers dried ($MgSO_4$), the residue chromatographed over 100 g of silica gel (eluted with methylene chloride to 10% methanol/methylene chloride), and the resulting foam crystallized from the minimum amount of methylene chloride/hexanes to afford 4-[4-(4-(thiophen-2-yl)phenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid (1.04 g, 94%). mp 181.2-193.3°C; IR (KBr) 3432 (br), 1718.9 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.67 (ddd, J = 13.8, 9.4, 4.0 Hz, 2H), 1.95 (dm, J = 13.8 Hz, 2H), 3.47 (m_c, 2H), 3.67 (m_c, 2H), 3.68 (s, 2H), 7.14 (dd, J = 4.9, 3.6 Hz, 1H), 7.20 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.9 Hz, 2H), 7.50 (dd, J = 3.6, 1.2 Hz, 1H), 7.54 (dd, J = 4.9, 1.2 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 12.80 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 32.92 (t), 42.25 (s), 61.73 (t), 63.26 (t), 117.82 (d), 123.75 (d), 125.66 (d), 127.39 (d), 128.50 (d), 130.08 (d), 130.74 (s), 134.90 (s), 142.42 (s), 154.13 (s), 161.33 (s), 174.39 (s); FABHRMS Calcd. for $C_{23}H_{24}S_2O_6$ (M^+ + H): 459.0936. Found: 459.0936. Anal. Calcd. for $C_{23}H_{23}S_2O_6$: C, 60.24; H, 4.83. Found: C, 60.57; H, 4.90.

16E. Preparation of Ifb where R^1 and R^2 Together with the Carbon to which they are attached represent Tetrahydropyran-4-yl, R^3 and R^4 are Hydrogen, R^5 is 4-(Thiophen-3-yl)phenoxyphenyl

Similarly, following the above procedure, other compounds of Formula Ifb, were prepared, for example replacing 2-thiophene boronic acid with 3-thiophene boronic acid, 4-[4-(4-(thiophen-3-yl)phenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid was prepared: mp 206.6-212.4 °C; IR (KBr) 3430 (br), 1719 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.67 (m_c, 2H), 1.95 (m_c, 2H), 3.47 (m_c, 2H), 3.66 (m_c, 2H), 3.67 (s, 2H), 7.20 (m_c, 4H), 7.56 (dd, J = 5.0, 1.4 Hz, 1H), 7.64 (d, J = 5.0, 2.9 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.87 (m_c, 2H), 7.96 (s, 1H), 12.77 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 32.92 (t), 40.38 (s), 61.19 (t), 63.26 (t), 117.66 (d), 120.54 (d), 120.87 (d), 126.04 (d), 127.07 (d), 127.96 (d), 130.02 (d), 132.00 (s), 134.66 (s), 140.45 (s), 160.80 (s), 174.32 (s); FABHRMS Calcd. for $C_{23}H_{23}S_2O_6$ (M^+ + H): 459.0936. Found: 459.0934. Anal. Calcd. for $C_{23}H_{22}S_2O_6.0.5H_2O$: C, 59.08; H, 4.96. Found: C, 58.82; H, 4.69.

16F. Catalytic Reduction of 4-[4-(4-bromophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid

A solution of 660 mg (1.45 mmol) of 4-[4-(4-bromophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid in 80% ethanol/tetrahydropyran (40 ml) was hydrogenated at atmospheric pressure for 14 hours using palladium on carbon catalyst, filtered over a celite pad washing with methylene chloride and concentrated *in vacuo* to afford 4-[4-phenoxyphenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid as a light orange solid (546 mg, 100%), which was taken directly into the next reaction without further purification: mp 162.5-165.3°C; IR (KBr) 3431 (br), 1727 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 1.67 (ddd, J = 14.1, 10.0, 4.0 Hz, 2H), 1.95 (dm, J = 14.1 Hz, 2H), 3.47 (m_c, 2H), 3.65 (m_c, 2H), 3.66 (s, 2H), 7.15 (d, J = 8.8 Hz, 2H), 7.27 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.5 Hz, 2H), 7.86 (d, J = 7.9 Hz, 2H), 12.74 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.88 (t), 42.26 (s), 61.75 (t), 63.26 (t), 117.64 (d), 120.11 (d), 125.03 (d), 130.04 (d), 130.39 (s), 134.69 (s), 154.69 (s), 161.53 (s), 174.39 (s); FABHRMS Calcd for $C_{19}H_{21}SO_6$ (M^+ + H): 377.1059. Found: 378.1064. Anal. Calcd. for $C_{19}H_{20}SO_6.0.75H_2O$: C, 58.52; H, 5.56. Found: C, 58.54; H, 5.19.

EXAMPLE 17

Preparation of Compounds of Formula Ij

17A. Preparation of Ii where R^1, R^2 and R^3 are Hydrogen, and R^4 is Benzyl

Thiophenol (80 mg) was stirred for 45 min with potassium hydride (40 mg) in *N,N*-dimethylformamide (1 ml) to produce a homogeneous solution of potassium thiophenolate. To this mixture was added 3-benzyl-3-(4-bromophenylsulfonyl)-propionic acid (100 mg) dissolved in *N,N*-dimethylformamide (1 ml) at room temperature. After stirring for 16 hours at 75°C the mixture was partitioned between aqueous citric acid and water, giving a product which was purified by preparative TLC to afford 3-benzyl-3-(4-phenylthiophenylsulfonyl)-propionic acid (30 mg).

17B. Preparation of Ij where R^1, R^2 and R^3 are Hydrogen, and R^4 is Benzyl

The 3-benzyl-3-(4-phenylthiophenylsulfonyl)-propionic acid, prepared as shown above, was then converted to 3-benzyl-3-(4-phenylthiophenylsulfonyl)-*N*-hydroxypropionamide as descibed in Example 10A.

EXAMPLE 18

Preparation of Compounds of Formula Ik

18A. Preparation of Ik where R^1, R^2 and R^3 are Hydrogen, and R^4 is Benzyl

A mixture of 3-benzyl-3-(4-bromophenylsulfonyl)-propionic acid (250 mg), *p*-methoxystyrene (0.1 ml), diisopropylethylemine (0.25 ml), palladium acetate (5 mg) and tri(o-methylphenyl)phosphine (16 mg) was stirred overnight at 80°C. The reaction mixture was dissolved in methylene chloride and washed with aqueous citric acid. Solvent was removed from the methylene chloride solution, and the residue chromatographed on silica gel (preparative TLC, eluting with 10% methanol/methylene chloride), to afford 3-benzyl-3-(4-styrylphenylsulfonyl)-propionic acid (21 mg).

18B. Preparation of Ik where R^1, R^2 and R^3 are Hydrogen, and R^4 is Benzyl

The 3-benzyl-3-(4-styrylphenylsulfonyl)-propionic acid, prepared as shown above, was then converted to 3-benzyl-3-(4-styrylphenylsulfonyl)-*N*-hydroxypropionamide, LSIMS m/e=452.2 $(M+H)^+$, as descibed in Example 10A.

EXAMPLE 19

Preparation of Compounds of Formula Il

Preparation of Il where n is 2, R^1 and R^2 together with the Carbon to which they are attached are Piperidine, R^2 and R^3 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

Trifluoroacetic acid (4 ml) was added to a solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-*N*-BOC-piperidin-4-yl]-carboxamide (2 g, 3.64 mmol) dissolved in methylene chloride (4 ml). The reaction mixture was stirred for 1.3 hours and concentrated *in vacuo*. The crude salt residue was dissolved in ethyl acetate (150 ml), washed with saturated aqueous sodium bicarbonate (2 x 50 ml), dried over magnesium sulfate, concentrated *in vacuo*, to afford the free base, *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-piperidin-4-yl]-carboxamide (1.57 g, 90%). ^{1}HNMR ($CDCl_3$) δ 1.28 (s, 9H), 2.23 (m_c, 2H), 2.56 (m_c, 2H), 3.30 (m_c, 2H), 3.44 (m_c, 2H), 3.53 (m_c, 2H), 7.00 (d, J = 8.9 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 8.25 (br s, 1H), 8.48 (br s, 1H).

EXAMPLE 20

Preparation of Compounds of Formula Im

20A. Preparation of Im where n is 2, R is Ethoxycarbonylmethyl, R^1 and R^2 are Hydrogen, and R^5 is 4-Phenoxyphenyl

A solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide (750 mg) in *N, N*-dimethylformamide (10 ml) was treated with ethyl bromoacetate (0.2 ml) and potassium carbonate (600 mg). The mixture was stirred overnight at room temperature, and then partitioned between ethyl acetate and water. After drying, solvent was removed from the organic layer under reduced pressure to yield *N-tert*-butoxy-2- [4-(4-phenoxyphenyl-sulfonyl)-1-

(ethoxycarbonylmethyl)piperidin-4-yl]-acetamide, which was used in the next step without further purification.

20B. Preparation of Im where n is 2, R is Isopropyl, R^1 and R^2 are Hydrogen, and R^5 is 4-Phenoxyphenyl

To a solution of *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide (500 mg) in acetone (20 ml) was added 10% palladium on carbon (100 mg), and the mixture stirred under hydrogen for three days. The catalyst was filtered off, and solvent removed from the filtrate under reduced pressure. The residue was chromatographed on silica gel, eluting with 10% methanol/methylene chloride, to give *N-t*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(isopropyl)piperidin-4-yl)]-acetamide (300 mg).

20C. Preparation of Im where n is 2, varying R

Similarly, following the procedures of Example 20A above, but replacing ethyl bromoacetate with 3-picolyl chloride, *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(3-picolyl)piperidin-4-yl]-acetamide was prepared.

Similarly, following the procedures of Example 20A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)piperidin-4-yl)]-acetamide with *N-tert*-butoxy-2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-acetamide, and replacing ethyl bromoacetate with cyclopropylmethyl bromide, *N-tert*-butoxy-2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-1-(cyclopropylmethyl)-piperidin-4-yl}-acetamide was prepared.

Similarly, *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(acetamidocarbonylmethyl)piperidin-4-yl]-acetamide was prepared.

20D. Preparation of Im where n is 2, varying R

Similarly, following the procedures of Example 20A above, but optionally replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperid-4-yl)]-acetamide with other compounds of Formula Iy, and optionally replacing ethyl bromoacetate with other compounds of formula RX, where R is lower alkyl, cycloalkylalkyl, acyl, alkoxycarbonylalkyl, picoline, $-SO_2R^a$, where R^a is lower alkyl or $-NR^bR^c$, where R^b and R^c are independently hydrogen or lower alkyl; and the like, and X is chloro, bromo or iodo, other compounds of Formula Im were prepared:

N-tert-butoxy-2-[1-ethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide;
N-tert-butoxy-2-[1-methyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide, m.p. 152-155°C;
N-tert-butoxy-2-[1-(2-methylpropyl)-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide;
N-tert-butoxy-2-[1-cyclopropylmethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide;
N-tert-butoxy-2-[1-cyclopropylmethyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl]-acetamide; and
N-tert-butoxy-2-[1-acetyl-4-[4-(4-fluorophenoxy)phenylsulfonyl]-piperidin-4-yl]-acetamide.

20E. Preparation of Ic where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached is 1-CyclopropylmethylPiperidine, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a solution of the free base *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-piperidin-4-yl]-carboxamide (1.28 g, 2.66 mmol) dissolved in *N,N*-dimethylformamide (17 ml), was added cyclopropylmethyl bromide (0.26 ml, 2.66 mmol), followed by potassium carbonate (1.84 g, 13.3 mmol). After the reaction mixture was stirred for 20 hours, water was added (100 ml), and the aqueous solution extracted with ethyl acetate (3 x 100 ml). The combined organic extracts were washed with brine (2 x 50 ml), dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 25% ethyl acetate/hexanes, gave *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-1-(cyclopropyl)piperidin-4-yl]-carboxamide (1.30 g, 92%). ^{1}HNMR ($CDCl_3$) δ 0.10 (ddd, *J* = 5.6, 4.7, 4.6 Hz, 2H), 0.53 (ddd, *J* =8.7, 4.7, 4.5 Hz, 2H), 0.85 (m_c, 1H), 1.31 (s, 3H), 1.64 (m_c, 2H), 2.06 (m_c, 2H), 2.24 (m_c, 2H), 2.28 (d, *J* = 6.5 Hz, 2H), 2.67 (m_c, 4H), 3.50 (m_c, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H), 8.33 (br s, 2H); FABMS (M^+ +H): 535.2.

20F. Preparation of Ic where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached is 1-(3-Picolyl)piperidine, and R^5 is 4-(4-Chlorophenoxy)-phenyl

Similarly, following the procedures of Example 20E above, but replacing cyclopropylmethyl bromide with 1.25 equivalents of 3-picolyol chloride hydrochloride, *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-1-(3-picolyl)piperidin-4-yl]-carboxamide was prepared: mp 83.3-93.8°C; IR (KBr) 3436, 1661 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.31 (s, 9H), 2.00 (m_c, 2H), 2.24 (m_c, 2H), 2.55 (m_c, 4H), 3.48 (s, 2H), 3.53 (s, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 7.04 (d, *J* = 8.9 Hz, 2H), 7.25 (dd, *J* = 7.6, 4.6 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.64 (brd, *J* = 7.8 Hz, 2H), 7.85 (d, *J* = 8.9 Hz, 2H), 8.36 (br s, 1H), 8.52 (m, 2H); FABMS (M^+ +H): 572.0. Anal. Calcd. for $C_{29}H_{34}N_3SO_5Cl.0.5\ H_2O$: C, 59.03; H, 5.81; N, 7.12. Found: C,

59.37; H, 6.15; N, 7.98.

20G. Preparation of Ic where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached is 1-(Nicotinoyl)Piperidine, and R^5 is 4-(4-Chlorophenoxy)-phenyl

To a solution of the free base *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-piperidin-4-yl]-carboxamide (491 mg, 1.02 mmol) and *N,N*-diisopropylethylamine (444 mg, 2.55 mmol) in methylene chloride (2 ml) cooled to 0°C, was added nicotinyl chloride hydrochloride (219 mg, 1.27 mmol) in one portion. After the reaction mixture was stirred for 3 hours, water (30 ml) was added, and the aqueous solution extracted with ethyl acetate (2 x 60 ml). The combined organic extracts were washed with brine (2 x 50 ml), dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 6% methanol/methylene chloride, afforded *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-1-(nicotinoyl)piperidin-4-yl]-carboxamide (233 mg, 39%). ^{1}HNMR ($CDCl_3$) δ 1.33 (s, 9H), 1.95 (m_c, 2H), 2.35 (m_c, 2H), 3.45 (m_c, 2H), 3.49 (s, 2H), 3.55 (m_c, 4H), 7.01 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.41 (m_c, 2H), 7.79 (m_c, 2H), 7.83 (d, J = 8.8 Hz, 2H), 8.69 (br s, 1H), 8.52 (m_c, 2H).

20H. Preparation of Ic where n is 2, R^3 and R^4 are Hydrogen, R^1 and R^2 when taken together with the Carbon to which they are attached is 1-(Methanesulfonyl)Piperidine, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a solution of the free base *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-piperidin-4-yl]-carboxamide (1.57 g, 3.26 mmol) in 67% methylene chloride/pyridine (16.5 ml) cooled to -78°C, was added a solution of methanesulfonyl chloride (0.51 ml, 6.53 mmol) in methylene chloride (2 ml). After the reaction mixture was stirred for 4 hours, 3N aqueous hydrochloric acid (25 ml) was added, and the aqueous solution extracted with ethyl acetate (2 x 60 ml). The combined organic extracts were washed with brine (2 x 50 ml), dried over magnesium sulfate, concentrated *in vacuo*. Chromatography over silica gel, and eluting with 45% ethyl acetate/hexanes, afforded *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonylmethyl)-1-(methanesulfonyl)piperidin-4-yl]-carboxamide (1.16 g, 64%). ^{1}HNMR ($CDCl_3$) δ 1.33 (s, 9H), 2.05 (m_c, 2H), 2.37 (m_c, 2H), 2.79 (s, 3H), 3.23 (m_c, 2H), 3.43 (s, 2H), 3.47 (m_c, 2H), 7.01 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.85 (d, J = 8.9 Hz, 2H); FABMS (M^+ +H): 559.1.

EXAMPLE 21

Preparation of Compounds of Formula In

21A. Preparation of In where n is 2, R is Ethoxycarbonylmethyl, R^1 and R^2 are Hydrogen, and R^5 is 4-Phenoxyphenyl

The product from Example 20A, *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(ethoxycarbonylmethyl)piperidin-4-yl]-acetamide, was dissolved in dichloroethane (10 ml), cooled to 0°C, and saturated with hydrochloric acid gas. The reaction vessel was then sealed and the solution stirred for two days at 25°C. Solvent was removed from the reaction mixture under reduced pressure, and the residue purified by preparative TLC, eluting with 10% methanol/ methylene chloride, to give *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(ethoxycarbonylmethyl)piperidin-4-yl]-acetamide (420 mg), m/e = 477.1 (MH^+, FABMS).

21B. Preparation of In where n is 2, R is Isopropyl, R^1 and R^2 are Hydrogen, and R^5 is 4-Phenoxyphenyl

The product from Example 20B, *N-t*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(isopropyl)piperidin-4-yl)]acetamide, was reacted with hydrochloric acid gas as described above, to yield *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(isopropyl)piperidin-4-yl)]-acetamide (155 mg), m.p. 128°C, m/e = 432 (MH^+,EIMS).

21C. Preparation of In where n is 2, varying R

Similarly, following the procedures of Example 21A above, but replacing ethyl bromoacetate with 3-picolyl chloride, *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-1-(3-picolyl)piperidin-4-yl]-acetamide was prepared, m.p. 185-192°C (dec).

Similarly, following the procedures of Example 19A above, but replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide with *N-tert*-butoxy-2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-acetamide, and replacing ethyl bromoacetate with cyclopropylmethyl bromide, *N*-hydroxy-2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-1-cyclopropylmethylpiperidin-4-yl}-acetamide was prepared, m.p. 104-105°C.

Similarly, *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-1-acetamidocarbonylmethylpiperidin-4-yl]-acetamide was prepared.

21D. Preparation of In where n is 2, varying R

Similarly, following the procedures of Example 21A above, but optionally replacing *N-tert*-butoxy-2-[4-(4-phenoxyphenylsulfonyl)-piperid-4-yl)]-acetamide with other compounds of Formula Iy, and optionally replacing ethyl bromoacetate with other compounds of formula RX, where R is lower alkyl, cycloalkylalkyl, acyl, alkoxycarbonylalkyl, picoline, $-SO_2R^a$, where R^a is lower alkyl or $-NR^bR^c$, where R^b and R^c are independently hydrogen or lower alkyl; and the like, and X is chloro, bromo or iodo, other compounds of Formula In were prepared:

2-[1-ethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-*N*-hydroxyacetamide, m.p. 182-183°C;
N-hydroxy-2-[1-methyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide, m.p. 152-155°C;
N-hydroxy-2-[1-(2-methylpropyl)-4-(4-phenoxyphenylsulfonyl)-piperid-4-yl]-acetamide, m.p. 226-227°C;
2-[1-cyclopropylmethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide, m.p. 210-211°C;
2-[1-cyclopropylmethyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]-piperidin-4-yl]-*N*-hydroxyacetamide, m.p. 110-112°C; and
2-[1-acetyl-4-[4-(4-fluorophenoxy)phenylsulfonyl]-piperidin-4-yl]-*N*-hydroxyacetamide, m/e = 450 (MH^+).

EXAMPLE 22

Preparation of Compounds of Formula Iab

Preparation of Iab where R^5 is 4-phenoxyphenyl

4-Phenoxythiophenol (4.8 g) was stirred for 45 min with potassium hydride (0.98 g) in *N, N*-dimethylformamide (100 ml) to produce a homogeneous solution of potassium 4-phenoxythiophenolate. The lactone, (S)-3-carbobenzyloxyamino-2-oxetanone (5.3 g) (Arnold, L.D. *et al., J. Am. Chem. Soc.,* **107**, 7105 (1985)), dissolved in *N, N*-dimethylformamide (50 ml) was then added at room temperature. After stirring for 30 minutes the mixture was poured into water and extracted with ethyl acetate. The combined extracts were dried over magnesium sulfate, and solvent removed under reduced pressure to give (*R*)-2-(benzyloxycarbonylamino)-3-(4-phenoxyphenylthio)-propionic acid (9.2 g). It can be used directly in the next step.

EXAMPLE 23

Preparation of Compounds of Formula Io

Preparation of Io where R^5 is 4-phenoxyphenyl

The above-prepared (*R*)-2-(benzyloxycarbonylamino)-3-(4-phenoxyphenylthio)-propionic acid was dissolved in methylene chloride (175 ml), cooled to 0°C, and treated with *O-(tert*-butyl)hydroxylamine hydrochloride (7.7 g), 4-methylmorpholine (9.4 ml), 1-hydroxybenzotriazole (2.8 g), and *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide (7.9 g). The mixture was allowed to warm to room temperature, stirred for 1.5 hours, then partitioned between methylene chloride and water. Solvent was removed from the organic phase under reduced pressure, and the residue purified by flash chromatography on silica gel, eluting with 0 to 50% ethyl acetate/hexane, to provide (*R*)-2-(benzyloxycarbonylamino)-*N-tert*-butoxy-3-(4-phenoxyphenylthio)-propionamide (7.4 g) as a white foam.

EXAMPLE 24

Preparation of Compounds of Formula Ip

Preparation of Ip where n is 2 and R^5 is 4-phenoxyphenyl

(*R*)-*N-tert*-butoxy-2-(benzyloxycarbonylamino)-3-(4-phenoxyphenylthio)-propionamide (1.5 mmol) was dissolved in methanol (140 ml), and a solution of OXONE (15 g) in water (50 ml) was added with vigorous stirring. The oxidation is usually complete within 2 hours. The mixture is then partitioned between methylene chloride and water. Solvent was removed from the dried organic phase under reduced pressure, to afford (*R*)-2-(benzyloxycarbonylamino)-*N-tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (8.3 g) in near-quantitative yield.

EXAMPLE 25

Preparation of Compounds of Formula Iq

Preparation of Iq where n is 2, R^1 is Hydrogen, R^2 is $-NR^6R^7$, in which R^6 is Hydrogen and R^7 is Benzyloxycarbonylamino, and R^5 is 4-phenoxyphenyl

A solution of (*R*)-2-(benzyloxycarbonylamino)-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (1.2 g) obtained from Example 16 in methylene chloride (5 ml) was diluted with trifluoroacetic acid (30 ml). The solution was allowed to stand overnight, and solvent was removed under reduced pressure. This residue was chromatographed on silica gel, eluting with 10% methanol/methylene chloride to give (*R*)-2-(benzyloxycarbonylamino)-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide (400 mg), m.p. 195-202°C.

EXAMPLE 26

Preparation of Compounds of Formula Ir

Preparation of Ir where n is 2 and R^5 is 4-phenoxyphenyl

(*R*)-2-(benzyloxycarbonylamino)-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (6.0 g) obtained from Example 17 was dissolved in ethanol (100 ml) and hydrogenated at 1 atmosphere in the presence of 10% palladium on carbon (6 g) for a period of 18 hours. The catalyst was filtered off and the solvent removed from the filtrate under reduced pressure to give (*R*)-2-amino-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide as a glass.

EXAMPLE 27

Preparation of Compounds of Formula Is

Preparation of Is where n is 2, R^1 is Hydrogen, R^2 is $-NR^6R^7$, in which R^6 and R^7 are both Hydrogen, and R^5 is 4-phenoxyphenyl

Similarly as in Example 25, (*R*)-2-amino-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (6.0 g) was dissolved in 1,2-dichloroethane (5 ml) and cooled to -20°C and bubbled for 20 minutes with hydrochloric acid gas in a pressure tube. The flask was then sealed and the mixture stirred overnight. The tube was cooled, vented, and allowed to warm. The solution was rinsed with methanol, the solvent removed from the filtrate under reduced pressure, triturated with 1:1 hexane/ethyl acetate (4 ml). The residue was filtered and dried to give (*R*)-2-amino-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide hydrochloride, m.p. 178-180°C (dec).

EXAMPLE 28

Preparation of Compounds of Formula It

Preparation of It where n is 2, R^1 is Hydrogen, R^2 is $-NR^6R^7$, in which R^6 is Hydrogen and R^7 is CBZ-(*S*)-Valinamido, and R^5 is 4-phenoxyphenyl

To a solution of (*R*)-2-amino-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (1.9 g) in methylene chloride (30 ml) was added CBZ-(*S*)-valine (1.6 g), 1-hydroxybenzotriazole (0.9 g), triethylamine (1 ml), and *N'*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide (1.3 g). After stirring overnight at room temperature, the solution was partitioned between methylene chloride and water, and after the organic layer was dried over magnesium sulfate, solvent was removed under reduced pressure to give (*R*)-*N*-*tert*-butoxy-2-(CBZ-valinamido)-3-(4-phenoxyphenylsulfonyl)-propionamide, which was used without further purification.

EXAMPLE 29

Preparation of Compounds of Formula Iu

Preparation of Iu where n is 2, R^1 is Hydrogen, R^2 is $-NR^6R^7$, in which R^6 is Hydrogen and R^7 is (*S*)-Valinamido, and R^5 is 4-phenoxyphenyl

A solution of (*R*)-*N*-*tert*-butoxy-2-(CBZ-valinamido)-3-(4-phenoxyphenylsulfonyl)-propionamide (prepared above) in a mixture of methanol (300 ml) and ethanol (100 ml) was stirred under hydrogen at 1 atmosphere with palladium on carbon catalyst (10% Pd, 4 g) for 3 hours. The mixture was filtered, and the filtrate evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with 0-3% methanol in methylene chloride, to give (*R*)-*N*-*tert*-butoxy-2-valinamido-3-(4-phenoxyphenylsulfonyl)-propionamide (1.6 g).

EXAMPLE 30

Preparation of Compounds of Formula Iv

Preparation of Iv where n is 2, R^1 is Hydrogen, R^2 is $-NR^6R^7$, in which R^6 is Hydrogen and R^7 is (*S*)-Valinamido, and R^5 is 4-phenoxyphenyl

A solution of (*R*)-*N*-*tert*-butoxy-2-valinamido-3-(4-phenoxyphenylsulfonyl)-propionamide (1.6 g) in 1,2-dichloroethane (50 ml) was cooled to -20°C and bubbled for 15-20 minutes with hydrochloric acid gas in a pressure tube. The flask was then sealed and the mixture stirred for 24 hours. After cooling the tube was cautiously vented and its contents evaporated to yield a gum, which upon trituration with ethyl acetate gave a crude product as a white powder. This product was stirred overnight with 10% methanol/methylene chloride (20 ml) and filtered to remove impurities. This was repeated three times to give (*R*)-*N*-hydroxy-2-valinamido-3-(4-phenoxyphenylsulfonyl)-propionamide hydrochloride (760 mg), m.p. 214-217°C.

EXAMPLE 31

Preparation of Compounds of Formula Iw

Preparation of Iw where n is 2, Y is hydroxy or lower alkoxy, R^1 and R^2 when taken together with the carbon to which they are attached are Tetrahydropyan-4-yl, R^3 is hydrogen, and R^4 is Benzyl, and R^5 is 4-(4-Chlorophenoxy)phenyl

1. To a solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-carboxylic acid methyl ester in 20% tetrahydrofuran-methanol (9.5 ml) was added dropwise a solution of OXONE (1.53 g, 2.49 mmol) in water (8 ml) while maintaining an internal temperature of 15-20°C. The mixture was stirred 2 hours and the mixture dissolved in 40% ethyl acetate/water (200 ml). The layers were partitioned, and the water layer back extracted using ethyl acetate (2 x 50 ml). The combined organic layers were dried over magnesium sulfate, concentrated, and the residue purified by preparative chromatography (20 x 40-1000 um plates), eluting with 50% ethyl acetate/hexanes) to afford 4-[4-(4-chlorophenoxy)phenyl-sulfonylmethyl]-tetrahydropyran-4-carboxylic acid methyl ester (460 mg, 71%). ^{1}HNMR ($CDCl_3$) δ 1.71-1.82 (m, 2H), 2.23 (dm, *J* = 13.6 Hz, 2H), 3.47 (s, 2H), 3.58-3.67 (m, 2H), 3.59 (s, 3H), 3.73-3.81 (m, 2H), 6.97-7.10 (m, 4H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.84 (d, *J* = 8.7 Hz, 2H).

2. Lithium diisopropylamide was prepared by the addition of 2.5M *N*-butyl lithium (610 μL, 1.53 mmol) in hexanes to a solution of diisopropylamine (200 μL, 1.53 mmol) in tetrahydrofuran (3 ml) at 0°C and stirring for 20 minutes. Then a solution of 4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid methyl ester (540 mg, 1.27 mmol) in tetrahydrofuran (1 ml) was added to the solution of lithium diisopropylamide at -78°C, and stirred for an additional 60 minutes. Benzyl bromide (181 μL, 1.53 mmol) of was added to the mixture, stirred for an 50 minutes, warmed to room temperature over 30 minutes, and stirred for an additional 3 hours. The mixture was then diluted with 0.1M aqueous hydrochloric acid (25 ml) and extracted with methylene chloride (2 x 50 ml). The combined organic layers were dried over magnesium sulfate, concentrated *in vacuo*, chromatographed over silica gel, eluted with 20% ethyl acetate/hexanes, to afford 3-benzyl-4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid methyl ester (440 mg, 67%). IR (KBr) 1736 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.78 (dm, *J* = 13.5 Hz, 1H), 2.02-2.17 (m, 2H), 2.39 (dm, *J* = 13.5 Hz, 1H), 3.19-3.23 (m, 2H), 3.37-3.45 (td, *J* = 11.9, 2.4 Hz, 2H), 3.77-3.85 (m, 1H), 3.84 (s, 3H), 3.88-3.98 (m, 2H), 4.07-4.17 (m, 2H), 6.83-6.90 (m, 4H), 6.94 (d, *J* = 8.7 Hz, 2H), 7.08-7.15 (m, 3H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H); FABMS (M^+ +H): 515.

EXAMPLE 32

Preparation of Compounds of Formula Ix

Preparation of Ix where n is 2, Y is hydroxy, R^1 and R^2 when taken together with the carbon to which they are attached are Tetrahydropyan-4-yl, R^3 is hydrogen, and R^4 is Benzyl, and R^5 is 4-(4-Chlorophenoxy)phenyl

To a solution of 3-benzyl-4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid methyl ester (410 mg, 0.80 mmol) in *N,N*-dimethylformamide (4 ml) was added lithium iodide (1.06 g, 7.96 mmol), followed by sodium cyanide (78 mg, 1.59 mmol). The mixture was heated to 120°C for 8 hours, cooled to room temperature, the *N,N*-dimethylformamide solvent removed by heating under reduced pressure, and the residue partitioned between ethyl acetate (150 ml) and saturated aqueous sodium bisulfite (50 ml). The ethyl acetate layer was dried over magnesium sulfate, concentrated *in vacuo*, purified by preparative chromatography (20 x 40-1000 um plates), eluted with 8% methanol/methylene chloride) to afford 317 mg (80%) of 3-benzyl-4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-carboxylic acid ^{1}HNMR (*N,N*-dimethylformamide contaminant, $CDCl_3$) δ 1.74 (dm, J = 13.5 Hz, 1H), 2.05-2.18 (m, 2H), 2.42 (dm, J = 13.5 Hz, 1H), 3.22-3.26 (m, 2H), 3.48-3.58 (m, 2H), 3.78-4.18 (m, 5H), 6.83-6.88 (m, 4H), 6.93 (d, J = 8.5 Hz, 2H), 7.08-7.13 (m, 3H), 7.36 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H); CIMS (NH_3, $M^+ + NH_4+$): 518.

EXAMPLE 33

Preparation of Compounds of Formula I

Preparation of I where n is 2, R^2 is -NR^6R^7, in which R^6 and R^7 are both Methyl, and R^5 is 4-phenoxyphenyl

To a solution of (*R*)-2-amino-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (1.6 g) in *N,N*-dimethylformamide (5 ml) was added potassium carbonate (0.5 g) and methyl iodide (550 µl). After stirring for 2.5 hours, the mixture was partitioned between ethyl acetate and water, and after the organic layer was dried over magnesium sulfate, solvent was removed under reduced pressure. The residue was chromatographed on silica gel, eluting with 50% ethyl acetate/hexane to give (*R*)-*N*-*tert*-butoxy-2-dimethylamino-3-(4-phenoxyphenylsulfonyl)-propionamide (0.6 g).

This compound, (*R*)-*N*-*tert*-butoxy-2-dimethylamino-3-(4-phenoxyphenylsulfonyl)-propionamide, was dissolved in 1,2-dichloroethane (50 ml), cooled to -30°C and bubbled for 15-20 minutes with hydrochloric acid gas in a pressure tube. The flask was then sealed and the mixture stirred overnight. After cooling the tube was cautiously vented and its contents evaporated, to yield a gum, which upon trituration with 2:1 hexane/ethyl acetate gave a white powder, (*R*)-2-dimethylamino-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide hydrochloride (0.43 g), m.p. 65-70°C.

EXAMPLE 34

Preparation of Compounds of Formula I

Preparation of I where n is 2, R^2 is -NR^6R^7, in which R^6 is Hydrogen and R^7 is Dimethylaminosulfonyl, and R^5 is 4-phenoxyphenyl

To a solution of (*R*)-2-amino-*N*-*tert*-butoxy-3-(4-phenoxyphenylsulfonyl)-propionamide (1.5 g) in methylene chloride (20 ml) and pyridine (1.2 ml) was added dimethylsulfamoyl chloride (1 ml), and the mixture stirred overnight at room temperature. The mixture was partitioned between methylene chloride and water, and after the organic layer was dried over magnesium sulfate, solvent was removed under reduced pressure. The residue was chromatographed on silica gel, eluting with 0-45% ethyl acetate/hexane, to give (*R*)-*N*-*tert*-butoxy-2-dimethylaminosulfonamido-3-(4-phenoxyphenylsulfonyl)-propionamide (1.6 g).

This compound, (*R*)-*N*-*tert*-butoxy-2-dimethylaminosulfonamido-3-(4-phenoxyphenylsulfonyl)-propionamide, was dissolved in trifluoroacetic acid (30 ml) and the mixture stirred overnight at room temperature. The trifluoroacetic acid was removed under reduced pressure, and the residue chromatographed on silica gel, eluting with 10% methanol/methylene chloride, to give (*R*)-2-dimethylaminosulfonamido-3-(4-phenoxyphenylsulfonyl)-*N*-hydroxypropionamide hydrochloride (550 mg). ^{1}H NMR (d6-DMSO) 7.90 (d,2H), 7.47 (d,2H), 7.25 (t,1H), 7.13 (m,4H), 3.95 (m,1H), 3.55 (m,2H), 2.6 (s,6H).

EXAMPLE 35

Example of Preparation of Compounds of Formula I on a Large Scale

Preparation of I where n is 2, R^1 and R^2 when taken together with the Carbon to which they are attached represent Tetrahydropyran, R^3 and R^4 are Hydrogen, and R^5 is 4-(4-Chlorophenoxy)phenyl

1. Preparation of a Compound of Formula (7a)

To a mixture of *N,N*-dimethylformamide (56 Kg) and diethyl malonate (22 Kg) was added a 21% solution of sodium ethoxide in ethanol (45 Kg), followed by 2-chloroethyl ether (19 Kg). The mixture was heated to 85°C, causing ethanol to distil from the mixture. The temperature was raised to 120°C until all the ethanol formed was removed (3 hours), and then the mixture was allowed to cool to 25°C. The mixture was then rewarmed to 120°C and a further 45 Kg of a 21% solution of sodium ethoxide in ethanol added at such a rate as to cause the ethanol formed to distil off. When the distillation was complete, the mixture was cooled to 100°C, and after it was determined that the reaction was complete then cooled to 25°C. The mixture was partitioned between toluene (80 Kg) and water (216 Kg) and solvent removed from the organic layer by distillation. The product was used in the next step with no further purification.

2. Preparation of a Compound of Formula (8a) where R^1 and R^2 when taken together with the Carbon Atom to which they are attached represent Tetrahydropyran

A solution of diethyl tetrahydro-4H-pyran-4,4-dicarboxylate, the compound of Formula (7a), (12 Kg) in toluene (104 Kg) was cooled to between -30°C to -35°C, and diisobutylaluminum hydride (69 Kg) was added at such a rate so as to maintain a reaction temperature of -25°C. After the addition was complete, the temperature was raised to 15°C over 3 hours, and the reaction stirred until all starting material was consumed. The mixture was then recooled to -15°C and allowed to stand overnight. The product was partitioned between ethyl acetate (54 Kg), ethanol (48 Kg), and saturated sodium sulfate solution (60 litres), and the mixture stirred overnight at 25°C. The precipitated salts were filtered off, washed with tetrahydrofuran, and the filtrate washed with brine and separated. The organic layer was dried over magnesium sulfate and solvent removed under reduced pressure, to give ethyl 4-hydroxymethyltetrahydropyran-4-carboxylate (3.8 Kg), the compound of Formula (8a).

3. Preparation of a Compound of Formula (9a) where R^1 and R^2 when taken together with the Carbon Atom to which they are attached represent Tetrahydropyran

To a solution of lithium hydroxide monohydrate (4.46 Kg) in methanol (44 litres) and water (11 Kg) was added ethyl 4-hydroxymethyl-tetrahydropyran-4-carboxylate (8.0 Kg). The mixture was refluxed for 30 minutes, then solvent removed under reduced pressure. The mixture was cooled to 20°C, methyl *tert*-butyl ether (14.8 Kg) added, stirred for 10 minutes, and allowed to settle. The top organic layer was separated. This was repeated twice more, then the remaining mixture cooled to -10°C, and a solution of 31% hydrochloric acid (13 Kg) in water (3 Kg) added, maintaining the temperature below 5°C. The mixture was extracted several times with tetrahydrofuran, and the combined organic phases dried over magnesium sulfate. Approximately 90% of the tetrahydrofuran was removed, and the remaining solution added to a mixture of hexane (64.5 Kg) and methyl *tert*-butylether (23.7 Kg) with stirring. The precipitated solid material was filtered off and dried under reduced pressure at 60°C, to give 4-hydroxymethyl-tetrahydropyran-4-carboxylic acid (3.7 Kg), the compound of Formula (9a).

4. Preparation of a Compound of Formula Ia where R^1 and R^2 when taken together with the Carbon Atom to which they are attached represent Tetrahydropyran

To a mixture of 4-hydroxymethyl-tetrahydropyran-4-carboxylic acid (3.84 Kg), 4-dimethylaminopyridine (0.6 Kg) in dichloromethane (32 litres) was added triethylamine (4.88 Kg). The mixture was cooled to -20°C, and a solution of benzenesulfonyl chloride (4.66 Kg) in dichloromethane (5 litres) was added over a period of 35 minutes, maintaining the temperature below -10°C. The mixture was stirred at -10°C for 30 minutes, then 3N hydrochloric acid (10 litres) and water (10 litres) were added with stirring, then the layers allowed to separate. The organic layer was separated, the aqueous layer washed with dichloromethane (16 litres), the combined organics washed with aqueous 5% sodium bicarbonate solution (12 litres), then with water (12 litres), and solvent removed under reduced pressure, to give 2,7-dioxaspiro[3,5]nonane-1-one, a compound of Formula (10a)

To a mixture of 60% sodium hydride (0.92 Kg) in tetrahydrofran (26 litres) at 0°C was added a solution of 4-(4-chlorophenoxy)thiophenol (4.37 Kg) in tetrahydrofuran (15 litres), maintaining the temperature below 10°C. The mixture was allowed to warm to room temperature for 30 minutes, then recooled to 0°C. The concentrated solution of 2,7-dioxas-

piro[3,5]nonane-1-one obtained above was then added slowly to this mixture, maintaining the temperature below 10°C. The mixture was allowed to warm to room temperature, and stirred for 30 minutes. The mixture was then treated with 3N hydrochloric acid (16 litres) and dichloromethane (30 litres). The organic layer was separated and the aqueous layer extracted twice with dichloromethane (20 litres). The combined organics were washed with water (20 litres), filtered, and 100 litres of solvent removed under atmospheric pressure. To the remaining reaction product was added acetonitrile (60 litres) and after a further 60 litres of solvent were removed by distillation, acetonitrile (40 litres) was added and the total volume of the remainder reduced to 30 litres by distillation. This mixture was then heated to mild reflux (80°C), and then slowly cooled to 0°C. The product was filtered off, washed with hexane, and dried to about 60°C under reduced pressure, to yield 4-[4-(4-chlorophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid (5.61 Kg).

5. Preparation of a Compound of Formula Iba where R^1 and R^2 when taken together with the Carbon Atom to which they are attached represent Tetrahydropyran

A solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]tetrahydropyran-4-carboxylic acid (5.5 Kg) and *N,N*-dimethylformamide (27 ml) in dichloromethane (27.5 litres) was cooled to 5°C, and oxalyl chloride (1.4 litres) added slowly with stirring. After addition was complete, the mixture was allowed to warm to room temperature and stirred for 2 hours, thus forming a compound of Formula (12). The solution was then recooled to 10°C, and a mixture of 50% aqueous hydroxylamine (5.4 litres), *tert*-butanol (12.1 litres) and tetrahydrofuran (30.5 litres) was added slowly, maintaining the temperature below 21°C. The mixture was then allowed to warm to room temperature until the reaction was complete. The solvent was then evaporated under reduced pressure until 90% had been removed, at which point acetonitrile (42.5 litres) was added and the remaining dichloromethane removed by distillation under reduced pressure. The remaining solution was heated under reflux, and water (126 Kg) added at such a rate so as to maintain reflux. The solution was then cooled to 5°C for 12 hours, and the solid thus obtained filtered off. This product was washed with water and dried under vacuum at 50°C to yield 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) (5.06 Kg), a compound of Formula Iba.

6. Preparation of a Compound of Formula Id where R^1 and R^2 when taken together with the Carbon Atom to which they are attached represent Tetrahydropyran

To a solution of 4-[4-(4-chlorophenoxy)phenylthiomethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) (5.06 Kg) in tetrahydrofuran (28 litres) and methanol (112 litres) at 15°C was added a solution of OXONE (14.23 Kg) in water (72 litres) with stirring, ensuring that the temperature did not exceed 16°C. After the addition was complete, the temperature was raised to 20°C and the mixture stirred for 3 hours, then poured into a cold mixture (5°C) of toluene (60 litres) and ethyl acetate (98 litres) with stirring. The resultant mixture was filtered, the organic and aqueous layers thus obtained separated, and the aqueous layer washed with a mixture of ethyl acetate (25 litres) and toluene (10 litres). This wash was repeated twice more. The combined extracts and organic layer was washed twice with water (25 litres), and solvent removed under reduced pressure to a volume of 30 litres. The solution was cooled to 5°C, and the solid filtered off, washed with ethyl acetate/water and dried under vacuum at 50°C, to yield 4-[4-(4-chlorophenoxy)phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide) (4.3 Kg).

7. Similarly other Compounds of Formula I may be prepared.

EXAMPLE 36

This example illustrates the preparation of representative pharmaceutical compositions for oral administration containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g.*, *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide:

A.	
Ingredients	% wt./wt.
Compound of Formula I	20.0%
Lactose	79.5%
Magnesium stearate	0.5%

The above ingredients are mixed and dispensed into hard-shell gelatin capsules containing 100 mg each, one capsule would approximate a total daily dosage.

B.	
Ingredients% wt./wt.	
Compound of Formula I	20.0%
Magnesium stearate	0.9%
Starch	8.6%
Lactose	79.6%
PVP (polyvinylpyrrolidine)	0.9%

The above ingredients with the exception of the magnesium stearate are combined and granulated using water as a granulating liquid. The formulation is then dried, mixed with the magnesium stearate and formed into tablets with an appropriate tablet machine.

C.	
Ingredients	
Compound of Formula I	0.1 g
Propylene glycol	20.0 g
Polyethylene glycol 400	20.0 g
Polysorbate 80	1.0 g
Water	q.s. 100 ml

The compound of Formula I is dissolved in propylene glycol, polyethylene glycol 400 and polysorbate 80. A sufficient quantity of water is then added with stirring to provide 100 ml of the solution which is filtered and bottled.

D.	
Ingredients	% wt./wt.
Compound of Formula I	20.0%
Peanut Oil	78.0%
Span 60	2.0%

The above ingredients are melted, mixed and filled into soft elastic capsules.

EXAMPLE 37

This example illustrates the preparation of a representative pharmaceutical formulation for parenteral administration containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g.*, *N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide:

Ingredients	
Compound of Formula I	0.02 g
Propylene glycol	20.0 g
Polyethylene glycol 400	20.0 g
Polysorbate 80	1.0 g
0.9% Saline solution	q.s. 100 ml

The compound of Formula I is dissolved in propylene glycol, polyethylene glycol 400 and polysorbate 80. A sufficient quantity of 0.9% saline solution is then added with stirring to provide 100 ml of the I.V. solution which is filtered through a 0.2 μ membrane filter and packaged under sterile conditions.

EXAMPLE 38

This example illustrates the preparation of a representative pharmaceutical composition in suppository form containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g., N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide:

Ingredients	% wt./wt.
Compound of Formula I	1.0%
Polyethylene glycol 1000	74.5%
Polyethylene glycol 4000	24.5%

The ingredients are melted together and mixed on a steam bath, and poured into molds containing 2.5 g total weight.

EXAMPLE 39

This example illustrates the preparation of a representative pharmaceutical formulation for insufflation containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g., N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide

Ingredients	% wt./wt.
Micronized compound of Formula I	1.0%
Micronized lactose	99.0%

The ingredients are milled, mixed, and packaged in an insufflator equipped with a dosing pump.

EXAMPLE 40

This example illustrates the preparation of a representative pharmaceutical formulation in nebulized form containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g., N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide:

Ingredients	% wt./wt.
Compound of Formula I	0.005%
Water	89.995%
Ethanol	10.000%

The compound of Formula I is dissolved in ethanol and blended with water. The formulation is then packaged in a nebulizer equipped with a dosing pump.

EXAMPLE 41

This example illustrates the preparation of a representative pharmaceutical formulation in aerosol form containing a compound of Formula I, or a pharmaceutically acceptable salt thereof, *e.g., N*-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl)]-acetamide:

Ingredients	% wt./wt.
Compound of Formula I	0.10%
Propellant 11/12	98.90%
Oleic acid	1.00%

The compound of Formula I is dispersed in oleic acid and the propellants. The resulting mixture is then poured into an aerosol container fitted with a metering valve.

EXAMPLE 42

In Vitro Assay

42A. Isolation of MMPs for Assays

The catalytic domain of human collagenase-1 was expressed as a fusion protein with ubiquitin in *E. Coli* (Gehring, E.R. *et at., J. Biol. Chem.*, **270**, 22507, (1995)). After purification of the fusion protein, the fibroblast collagenase-1 catalytic domain was released by treatment with 1mM of aminophenylmercuric acetate (APMA) for 1 hour at 37°C and purified by zinc chelate chromatography.

Human collagenase-2 and gelatinase B were isolated in active form from buffy coats (Mookhtiar, K.A. *et at., Biochemistry,* **29**, 10620, (1990)).

The propeptide and catalytic domain portion of human collagenase-3 was expressed in *E. Coli* as an *N*-terminal fusion protein with ubiquitin. After purification, the catalytic domain was obtained by treatment with 1 mM APMA for 1 hour at 37°C, and purified by zinc chelate chromatography.

Rat collagenase-3 was purified in active form from the culture media of uterine smooth muscle cells (Roswit, W.T. *et al., Arch. Biochem. Biophys.*, **225**, 285-295 (1983)).

The catalytic and fibronectin-like portion of human progelatinase A was expressed as a fusion protein with ubiquitin in *E. Coli.* Assays were carried out on autolytically activated material. Rat progelatinase A was purified from the culture media of interleukin-1 stimulated keratinocytes and activated by treatment with 1 mM APMA for 1 hour at 37°C, and subsequently dialyzed to remove excess APMA.

Human prostromelysin-1 was purified from the culture medium of synovial fibroblasts by affinity chromatography using an immobilized monoclonal antibody. The zymogen was activated by treatment with trypsin (1.5 μg/ml) for 1 hour at 23°C to give a mixture of 45 and 28 kD species. The catalytic domain of human stromelysin was prepared by expression and purification of prostromelysin-1 from *E. Coli* and activated with 1 mM APMA for 1 hour at 37°C, followed by dialysis. Rat prostromelysin-1 was expressed in Chinese Hampster Ovary cells and purified from the culture media. It was activated by 1 mM APMA for 1 hour at 37°C, followed by dialysis.

Human promatrilysin was expressed and purified from Chinese Hampster Ovary cells (Barnett, *J. et al., Prot.*

Expres. Pur., **5**, 27, (1994)). The zymogen was activated by treatment with 1 mM APMA for 1 hour at 37°C, and purified by zinc chelate chromatography.

Compounds of Formula I exhibited the ability to inhibit the collagenases when tested in this assay.

42B. In Vitro Assay Procedure

Assays were performed in assay buffer (50 mM Tricine pH 7.5, 200 mM sodium chloride, 10 mM calcium chloride, 0.005% Brij-35) containing 2.5% methyl sulfoxide (DMSO) once the substrate and inhibitor were diluted into it. Stock solutions of inhibitors were prepared in 100% DMSO. Stock solutions of the substrate were prepared in 100% DMSO at a concentration of 2 mM.

The assay method was based on the hydrolysis of MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Bachem, Inc.) at 37°C (Knight, C.G. *et al., FEBS,* **296**, 263-266 (1992)). The fluorescence changes were monitored with a Perkin-Elmer LS-50B fluorimeter using an excitation wavelength of 328 nm and an emission wavelength of 393 nm. The substrate concentration used in the assays was 10 μmole. The inhibitor was diluted into the assays from a solution in 100% DMSO, and controls substituted an equal volume of DMSO so that the final DMSO concentration from inhibitor and substrate dilutions in all assays was 2.5%. The inhibition results are expressed as the inhibitor concentration that produced 50% inhibition (IC_{50}) of the activity in the control (non-inhibited) reaction.

EXAMPLE 43

In Vitro Assay

This assay determines the ability of the compounds of Formula I to inhibit the degradation of the collagen matrix (as judged by release of hydroxyproline), and proteoglycan (as judged by the release of ^{35}S-labelled glycosaminoglycans) from cartilage explants.

Small cartilage explants (3 mm diameter) were prepared from freshly sacrificed bovine knee joints and labeled with $^{35}SO_4$. ^{35}S-labelled glycosaminoglycans (GAG's) and collagen fragments are released into the culture medium in response to the addition of rhIL-1-alpha, which induces the expression of chondrocyte matrix metalloproteases (MMP's), including stromelysin and collagenase. The percent inhibition of hydroxyproline and GAG's released was corrected for spontaneous release in the absence of rhIL-1-alpha.

Compounds of Formula I, when tested in this assay, displayed the ability to inhibit the release of both collagen fragments and ^{35}S-labelled GAG's from cartilage explants.

EXAMPLE 44

In Vivo Assay

The cartilage plug implantation assay measures the destruction of the collagen matrix of a cartilage plug implanted in a rat (Bishop, J. *et al., J. Pharm. Tox. Methods,* **30**, 19, (1993)).

Previously frozen bovine nasal cartilage plugs weighing approximately 20 mg were embedded in polyvinyl sponges impregnated with *Mycobacterium tuberculosis* and implanted subcutaneously in female Lewis rats. Dosing was begun 9 days after implantation and the plugs were harvested about one week later. The plugs were weighed, hydrolyzed, and the hydroxyproline content measured. Efficaciousness was determined by the comparison of the compound-treated groups with vehicle treated controls.

The compounds of Formula I exhibited the ability to inhibit the degradation of the cartilage plugs in this assay.

EXAMPLE 45

In Vivo Assay Procedure

45A. Determination of TNF Production Following LPS Stimulation

Female Balb/c mice, 6-8 weeks old (Jackson Labs or Harlan) were used. For each treatment group, 6-8 mice were used. Mice were injected I.P. with LPS (Sigma, 13129, 10-20 μg/mouse) after treatment with a compound of Formula I. The compound of Formula I or vehicle was administered subcutaneously (S.C.) once, 30-60 minutes prior to LPS challenge. Control animals received CMC vehicle alone or CMC + 2-5% DMSO. Animals were bled 1.5 hours after LPS injection under anesthesia with metofane from the retro-orbital plexus, using a Pasteur pipette. Blood was collected in a microtainer serum separator tube (Becton Dickinson #5960). The sera were separated and either tested the next day or they were kept at -20°C until ready to test for TNF-α.

45B. ELISA Assay for Murine TNF-α

The Endogen (EM-TNFA kit) mouse tumor necrosis factor alpha (mTNF-α) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse TNF-α (ordering code: EM-TNFA; Endogen, 30 Commerce Way, Woburn, MA 01801-1059, USA). Standards (lyophilized recombinant *E. coli*-derived mouse TNF-a) or serum samples (50 µl each) were added in duplicate to each well of the precoated anti-mTNF-α plate. Biotinylated antibody (50 µl) was added, the plates were incubated for 2-3 hours at room temperature. The wells were washed five times with wash buffer and 100 µl of diluted strepavidin HRP were added to each well and then were incubated at room temperature for 30 minutes. After washing (5X), 100 µl premixed TMB substrate solution were added to each well and plates were developed at room temperature in the dark for 30 minutes. The reaction was stopped by adding 100 µl of the stop solution. Absorbance at 450-575 nm was measured in a plate reader (ThermoMax, Molecular Devices). Results are calculated at pg/ml TNF-α by comparison to the standard curve, using Immunofit Beckman software. They are expressed as mean pg/ml of TNF-α, and as percentage of inhibition compared to controls (animals injected with LPS alone), considered 100% of TNF-α: production.

The compounds of Formula I, when tested in this assay, exhibited the ability to inhibit TNF-α production.

EXAMPLE 46

TNF Conjugate Immunoassay

Human Monomac 6 cells were cultured at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum to a density of 1 X 10^5 cells/mL. All subsequent incubations were performed at 37°C. 230 µl of these cells were placed in each well of a 96-well tissue culture plate and the cells incubated for 15 minutes. 10 µl of desired concentration of compounds of Formula I in the above mentioned medium were added to the appropriate wells and incubated for an additional 15 minutes. To each well was added 10 µl of an LPS/PMA mixture which brings the final concentration of LPS to 10 ng/mL and the final PMA concentration to 30 ng/mL. The cells were then incubated for 2 hours after which the plate was centrifuged and the medium removed and analyzed for TNF content. The analysis was performed using an R & D Systems TNF Quantikine Immunoassay and following the manufacturer's protocol (R & D. Systems, 614 Mckinley Place N.E., Minneapolis, MN 55413, USA; Catalog No. DTA50). The IC_{50} was calculated from the percent inhibition of TNF released into the medium.

The compounds of Formula I, when tested in this assay, exhibited the ability to inhibit TNF production.

EXAMPLE 47

TNFR Shedding Immunoassay

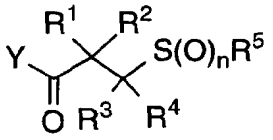
Human Monomac 6 cells are cultured to a density of 1 X 10^6 cells/mL at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum. All subsequent incubations are performed at 37°C. 230 µl of these cells are placed in each well of a 96-well tissue culture plate and the cells are incubated for 15 minutes. 10 µl of desired concentration of compounds of Formula I in the above mentioned medium are added to the appropriate wells and incubated for an additional 15 minutes. To each well is added 10 µl of PMA at a final concentration of 30 ng/mL. The cells are then incubated for 16 hours after which the plate is centrifuged and the medium is removed and analyzed for TNF receptor content. The analysis is performed using the R & D Systems TNF receptor Quantikine Immunoassay following the manufacturer's protocol. Measurements of each TNF receptor (receptor I and receptor II) are performed in this way. The IC_{50} is calculated from the percent inhibition of TNF released into the medium.

The compounds of Formula I, when tested in this assay, exhibited the ability to selectively inhibit TNF production.

While the present invention has been described with respect to specific embodiments thereof, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto.

Claims

1. A compound of the formula:



I

wherein:

n is	0, 1 or 2;
Y is	hydroxy or XONH-, where X is hydrogen or lower alkyl;
R^1	is hydrogen or lower alkyl;
R^2	is hydrogen, lower alkyl, heteroalkyl, aryl, aralkyl, arylheteroalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaralkyl, heteroarylheteroalkyl, heterocyclo, heterocylo-lower alkyl, heterocyclo-lower heteroalkyl or $-NR^6R^7$, wherein:

R^6 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl, aryl, heteroaryl and heteroaralkyl;
R^7 is hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, $-C(O)R^8$, $-C(O)NR^8R^9$, $-SO_2NR^8R^9$, $-SO_2R^{10}$, aryloxycarbonyl, or alkoxycarbonyl; or
R^6 and R^7 together with the nitrogen atom to which they are attached represent a heterocyclo group; wherein

R^8 and R^9 are independently hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl or heteroalkyl; and
R^{10} is lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heteroalkyl or heterocyclo; or

R^1 and R^2	together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group;
R^3 is	hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heteroalkyl or lower alkoxy;
R^4 is	hydrogen, lower alkyl, cycloalkyl or cycloalkylalkyl; or
R^2 and R^3	together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or
R^3 and R^4	together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and
R^5 is	lower alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

or a pharmaceutically acceptable salt or ester thereof.

2. The compound of Claim 1, wherein R^2 is $-NR^6R^7$.

3. The compound of Claim 1, wherein n is 2 and Y is XONH- in which X is hydrogen.

4. The compound of Claim 3, wherein R^1 is hydrogen and R^5 is aryl or heteroaryl.

5. The compound of Claim 4, wherein R^2 is hydrogen and R^3 is aralkyl and R^4 is hydrogen.

6. The compound of Claim 5, wherein R^3 is benzyl and R^5 is optionally substituted phenyl or naphthyl.

7. The compound of Claim 6, wherein R^5 is phenyl, 4-methoxyphenyl, 1-(4-methoxyphenyl)-2-phenylethene, phenylthiophenyl, phenoxyphenyl, or biphenyl.

8. The compound of Claim 7, wherein R^5 is 4-phenylthiophenyl, 4-phenoxyphenyl, or 4-biphenyl.

9. The compound of Claim 4, wherein R^3 and R^4 together with the carbon to which they are attached form a cycloalkyl group.

10. The compound of Claim 9, wherein R^5 is 4-methoxyphenyl or 4-phenoxyphenyl and the cycloalkyl group is cyclopentyl, cyclohexyl, or 4-methylcyclohexyl.

11. The compound of Claim 4, wherein R^3 and R^4 together with the carbon to which they are attached form a heterocyclo group.

12. The compound of Claim 11, wherein the heterocyclo group is optionally substituted piperidine or tetrahydropyranyl.

13. The compound of Claim 12, wherein the heterocyclo group is piperidin-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

14. The compound of Claim 12, wherein the heterocyclo group is 1-methylpiperidin-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

15. The compound of Claim 12, wherein the heterocyclo group is 1-(cyclopropylmethyl)piperidin-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

16. The compound of Claim 12, wherein the heterocyclo group is tetrahydropyran-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

17. The compound of Claim 3, wherein R^2 and R^3 together with the carbons to which they are attached form a cycloalkyl group and R^5 is aryl.

18. The compound of Claim 17, wherein the cycloalkyl group is cyclopentyl or cyclohexyl, R^4 is hydrogen, and R^5 is 4-methoxyphenyl.

19. The compound of Claim 3, wherein R^2 is $-NR^6R^7$, R^1, R^3 and R^4 are hydrogen, and R^5 is aryl.

20. The compound of Claim 19, wherein R^5 is 4-phenoxyphenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

21. The compound of Claim 3, wherein R^1 and R^2 together with the carbon to which they are attached form a heterocyclo group.

22. The compound of Claim 21, wherein R^3 and R^4 are both hydrogen and the heterocyclo group is optionally substituted piperidine or tetrahydropyranyl.

23. The compound of Claim 22, wherein the heterocyclo group is piperidin-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

24. The compound of Claim 22, wherein the heterocyclo group is tetrahydropyran-4-yl and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, 4-(4-fluorophenoxy)phenyl, 4-(thiophen-2-yl)phenoxyphenyl, 4-(thiophen-3-yl)phenoxyphenyl, 4-(2-pyridyloxy)phenyl, 4-(5-chloro-2-pyridyloxy)phenyl.

25. The compound of Claim 3, wherein R^1 and R^2 are both alkyl, R^3 and R^4 are hydrogen, and R^5 is 4-phenoxyphenyl, 4-(4-bromophenoxy)phenyl, 4-(4-chlorophenoxy)phenyl, or 4-(4-fluorophenoxy)phenyl.

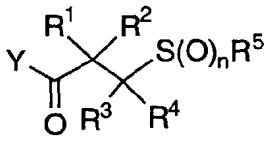
26. A compound of the group comprising

N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-tetrahydropyran-4-yl]-acetamide,
2-{4-[4-(4-chlorophenoxy)phenylsulfonyl]tetrahydropyran-4-yl}-*N*-hydroxyacetamide,
2-{4-[4-(4-fluorophenoxy)phenylsulfonyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide,
N-hydroxy-2-[4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide,
2-{4-[4-(4-chlorophenoxy)-phenylsulfonyl]piperidin-4-yl}-*N*-hydroxyacetamide,
2-{4-[4-(4-fluorophenoxy)-phenylsulfonyl]-piperidin-4-yl}-*N*-hydroxyacetamide,
N-hydroxy-2-[1-methyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-acetamide,
2-[1-cyclopropylmethyl-4-(4-phenoxyphenylsulfonyl)-piperidin-4-yl]-*N*-hydroxyacetamide,
2-{ 1-cyclopropylmethyl-4-[4-(4-chlorophenoxy)-phenylsulfonyl]piperidin-4-yl}-*N*-hydroxyacetamide,
2-{1-cyclopropylmethyl-4-[4-(4-fluorophenoxy)-phenylsulfonyl]piperidin-4-yl}-*N*-hydroxyacetamide
2-{4-[4-(4-fluorophenoxy)-phenylsulflnyl]-tetrahydropyran-4-yl}-*N*-hydroxyacetamide,
(*R*)-2-(CBZ-valinamido)-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide,
(*R*)-*N*-hydroxy-2-valinamido-3-(4-phenoxyphenylsulfonyl)propionamide,

(*R*)-2-dimethylamino-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)propionamide,
(*R*)-2-dimethylaminosulfonamido-*N*-hydroxy-3-(4-phenoxyphenylsulfonyl)-propionamide,
2-{4-[-(4-fluorophenoxy)-phenylthio]-tetrahydropyran-4-yl}-N-hydroxyacetamide,
4-[4-(4-chlorophenoxy)-phenylsulfonylmethyl]-tetrahydropyran-4-(*N*-hydroxycarboxamide),
4-[4-(4-thiophen-2-yl)phenoxyphenyl-sulfonylmethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide),
3-[4-(4-chlorophenoxy)-phenylsulfonyl]-2,2-dimethyl-N-hydroxypropionamide,
4-[4-(4-(thiophen-3-yl)-phenoxy)phenylsulfonylmethyl]tetrahydropyran-4-(*N*-hydroxycarboxamide)

and pharmaceutically acceptable salts thereof.

27. A process for preparing a compound of the Formula:

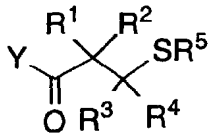


I

wherein:

n	is 1 or 2;
Y	is hydroxy or XONH-, where X is hydrogen or lower alkyl;
R^1	is hydrogen or lower alkyl;
R^2	is hydrogen, lower alkyl, aralkyl, cycloalkyl, cycloalkylalkyl, or heterocyclo; or
R^1 and R^2	together with the carbon atom to which they are attached represent a cycloalkyl or heterocyclo group;
R^3	is hydrogen, lower alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, heteroaralkyl, or lower alkoxy;
R^4	is hydrogen or lower alkyl; or
R^2 and R^3	together with the carbons to which they are attached represent a cycloalkyl or heterocyclo group; or
R^3 and R^4	together with the carbon to which they are attached represent a cycloalkyl or heterocyclo group; and
R^5	is lower alkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

comprising contacting a compound of the Formula:



wherein R^1, R^2, R^3, R^4 and R^5 are as defined before,
with an oxidizing agent.

28. A pharmaceutical composition comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of a compound according to any one of claims 1-26.

29. Compounds according to any one of claims 1-26 for use as a therapeutically active substance.

30. Compounds according to any one of claims 1-16 for use in the treatment of a disease-state which is alleviated by treatment with a matrix metalloprotease inhibitor, especially wherein the disease state is rheumatoid arthritis, osteoarthritis, osteoporosis, periodontal disease, aberrant angiogenesis, multiple sclerosis, tumor metastasis, or corneal ulceration.

31. Compounds according to any one of claims 1-26 for use in the treatment of a disease state which is mediated by tumor necrosis factor, especially wherein the disease state is inflammation, hemorrhage, graft versus host reaction or an autoimmune disease.

32. The use of a compound according to any one of claims 1-26 in the treatment of of a disease-state which is alleviated by treatment with a matrix metalloprotease inhibitor, especially wherein the disease state is rheumatoid arthritis, osteoarthritis, osteoporosis, periodontal disease, aberrant angiogenesis, multiple sclerosis, tumor metastasis, or corneal ulceration.

33. The use of a compound according to any one of claims 1-26 in the treatment of a disease state which is mediated by tumor necrosis factor, especially wherein the disease state is inflammation, hemorrhage, graft versus host reaction or an autoimmune disease.

34. The use of a compound according to any one of claims 1-26 in the preparation of a medicament for the treatment of a disease-state which is alleviated by treatment with a matrix metalloprotease inhibitor, especially wherein the disease state is rheumatoid arthritis, osteoarthritis, osteoporosis, periodontal disease, aberrant angiogenesis, multiple sclerosis, tumor metastasis, or corneal ulceration or wherein the disease-state is mediated by tumor necrosis factor, especially wherein the disease state is inflammation, hemorrhage, graft versus host reaction or an autoimmune disease.

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 96 11 9780

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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X	WO 90 05719 A (BRITISH BIO TECHNOLOGY) 31 May 1990 * examples1-3,8-18,20-24 * ---	1,28,29	
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TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07D
A61K
C07C
C25D

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
BERLIN	10 April 1997	Frelon, D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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E : earlier patent document, but published on, or after the filing date
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Application Number EP 96 11 9780

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EPO FORM 1503 03.82 (P04C10)

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PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 96 11 9780

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Application Number EP 96 11 9780

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
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Y	EP 0 606 046 A (CIBA GEIGY AG) 13 July 1994 * the whole document *	1,28,29	
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Application Number EP 96 11 9780

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Y,D	J. ENZYME INHIBITION, vol. 2, 1987, pages 1-22, XP000197047 W.H. JOHNSON ET AL.: * compound (V), page 7; table III, page 9 * -----	1,28,29	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C10)

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EP 96119780 - C -

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extend that is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely:
Claims searched incompletely: all
Claims not searched:

Reason for the limitation of the search:

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of all claims.
Reason:

The huge number of theoretically conceivable compounds resulting from the combinations of all the substituent definitions claimed in claim 1 prevents the search from being carried out comprehensively. Additionally such an uncertainty on the claimed scope may introduce contradictions and render unity questionable. Guided by the description, the search has been limited to the scope (IPC sub-divisions) which is illustrated by the compounds explicitely mentioned in the application. It is noted nevertheless that many individual compounds fall within the searched scope and therefore it is not possible to cite all of the documents found which are prejudicial to the novelty of the claimed invention. The documents cited as X-documents in the present search report are only a selection thereof.

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(54) Title: INTERLEUKIN CONVERTING ENZYME AND APOPTOSIS

(57) Abstract

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of ICE and ICE-like proteins for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death.

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Interleukin Converting Enzyme and Apoptosis

FIELD OF THE INVENTION

The present invention is to the discovery of a new method to block excessive or inappropriate apoptosis in a mammal.

BACKGROUND

It has been recognized for over a century that there are different forms of cell death. One form of cell death, necrosis, is usually the result of severe trauma and is a process that involves loss of membrane integrity and uncontrolled release of cellular contents, often giving rise to inflammatory responses. In contrast, apoptosis is a more physiological process that occurs in a controlled manner and is generally non-inflammatory in nature. For this reason apoptosis is often referred to as programmed cell death. The name itself (apoptosis: Greek for "dropping off", for example leaves from trees) implies a cell death that is part of a normal physiological process (Kerr et al., Br. J. Cancer, 26: 239-257 (1972)).

Apoptosis appears to be a carefully controlled series of cellular events which ultimately leads to death of the cell. This process for elimination of unwanted cells is active and requires expenditure of cellular energy. The morphological characteristics of apoptosis include cell shrinkage and loss of cell-cell contact, condensation of nuclear chromatin followed by fragmentation, the appearance of membrane ruffling, membrane blebbing and apoptotic bodies. At the end of the process, neighboring cells and macrophages phagocytose the fragments from the apoptotic cell. The process can be very fast, occurring in as little as a few hours (Bright et al., Biosci. Rep., 14: 67-82 (1994)).

The best defined biochemical event of apoptosis involves the orderly destruction of nuclear DNA. Signals for apoptosis promote the activation of specific calcium- and magnesium-dependent endonucleoases that cleave the double stranded DNA at linker regions between nucleosomes. This results in production of DNA fragments that are multiples of 180-200 base pair fragments (Bergamaschi et al., Haematologica, 79: 86-93 (1994); Stewart, JNCI, 86: 1286-1296 (1994)). When examined by agarose gel electrophoresis, these multiple fragments form a ladder pattern that is characteristic for most cells undergoing apoptosis.

There are numerous stimuli that can signal cells to initiate or promote cellular apoptosis, and these can be different in different cells. These stimuli can include glucocorticoids, TNFa, growth factor deprivation, some viral proteins, radiation and anticancer drugs. Some of these stimuli can induce their signals through a variety of cell surface receptors, such as the TNF / nerve growth factor family of receptors, which include CD40 and Fas/Apo-1 (Bright et al., supra). Given this diversity in stimuli that cause apoptosis it has been difficult to map out the signal transduction pathways and molecular factors involved in apoptosis. However, there is evidence for specific molecules being involved in apoptosis.

The best evidence for specific molecules that are essential for apoptosis comes from the study of the nematode C. elegans. In this system, genes that appear to be required for induction of apoptosis are Ced-3 and Ced-4. These genes must function in the dying cells and, if either gene is inactivated by mutation, cell death fails to occur (Yuan et al., Devel. Biol., 138: 33-41 (1990)). In mammals, genes that have been linked with induction of apoptosis include the proto-oncogene c-myc and the tumor suppresser gene p53 (Bright et al., supra; Symonds et al., Cell, 78: 703-711 (1994)).

In this critical determination of whether or not to undergo apoptosis, it is not surprising that these are genes that program for proteins that inhibit apoptosis. An example in C. elegans is Ced-9. When it is abnormally activated, cells survive that would normally die and, conversely, when Ced-9 is inactivated cells die that would normally live (Stewart, B.W., supra). A mammalian counterpart is bcl-2, which had been identified as a cancer-causing oncogene. This gene inhibits apoptosis when its product is overexpressed in a variety of mammalian cells, rendering them less sensitive to radiation, cytotoxic drugs and apoptotic signals such as c-myc (Bright et al., supra). Some viral proteins have taken advantage of this ability of specific proteins to block apoptosis by producing homologous viral proteins with analogous functions. An example of such a situation is a protein produced by the Epstein Barr virus that is similar to bcl-2, which prevents cell death and thus enhances viral production (Wells et al., J. Reprod. Fertil., 101: 385-391 (1994)). In contrast, some proteins may bind to and inhibit the function of bcl-2 protein, an example being the protein bax (Stewart, B.W., supra). The overall picture that has developed is that entry into apoptosis is regulated by a careful balancing act between specific gene products that promote or inhibit apoptosis (Barinaga, Science, 263: 754-756 (1994).

Apoptosis is an important part of normal physiology. The two most often cited examples of this are fetal development and immune cell development. In development of the fetal nervous system, over half of the neurons that exist in the early fetus are lost by apoptosis during development to form the mature brain (Bergamaschi et al., Haematologica, 79: 86-93 (1994)). In the production of immune competent T cells (and

to a lesser extent evidence exists for B cells), a selection process occurs that eliminates cells that recognize and react against self. This selection process is thought to occur in an apoptotic manner within areas of immune cell maturation (Williams, G. T., J. Pathol., 173: 1-4 (1994); Krammer et al., Curr. Opin. Immunol., 6: 279-289 (1994)).

Dysregulation of apoptosis can play an important role in disease states, and diseases can be caused by both excessive or too little apoptosis occurring. An example of diseases associated with too little apoptosis would be certain cancers. There is a follicular B-cell lymphoma associated with an aberrant expression of functional bcl-2 and an inhibition of apoptosis in that cell (Bergamaschi et al., supra). There are numerous reports that associate deletion or mutation of p53 with the inhibition of apoptosis and the production of cancerous cells (Kerr et al., Cancer, 73: 2013-2026 (1994); Ashwell et al., Immunol. Today, 15: 147-151, (1994)). In contrast, one example of excessive or inappropriate apoptosis is the loss of neuronal cells that occurs in Alzheimer disease, possible induced by b-amyloid peptides (Barr et al., BioTechnology, 12: 487-493 (1994)). Other examples include excessive apoptosis of $CD4^+$ T cells that occurs in HIV infection, of cardiac myocytes during infarction / reperfusion and of neuronal cells during ischemia (Bergamaschi et al., supra); Barr et al., supra).

Some pharmacological agents attempt to counteract the lack of apoptosis that is observed in cancers. Examples include topoisomerase II inhibitors, such as the epipodophyllotoxins, and antimetabolites, such as ara-c, which have been reported to enhance apoptosis in cancer cells (Ashwell et al., supra). In many cases with these anti-cancer drugs, the exact mechanism for the induction of apoptosis remains to be elucidated.

In the last few years, evidence has built that ICE and proteins homologous to ICE play a key role in apoptosis. This area of research has been spurred by the observation of homology between the protein coded by Ced-3, a gene known to be critical for C. Elegans apoptosis, and ICE. These two proteins share 29% amino acid identity, and complete identity in the 5 amino acid portion thought to be responsible for protease activity (QACRG) (Yuan et al., Cell, 75: 641-652 (1993)). Additional homologies are observed between ICE and the product of the nedd-2 gene in mice, a gene suspected of involvement in apoptosis in the developing brain (Kumar et al., Genes Dev., 8: 1613-1626 (1994)) and Ich-1 and CPP32 (ICE and Ced-3 homolog-1), human counterparts of nedd-2 isolated from human brain cDNA libraries (Wang et al., Cell, 78: 739-750 (1994); Fernandes-Alnemiri et al., J. Biol. Chem., 269: 30761-30764 (1994)).

Further proof for the role of these proteins in apoptosis comes from transfection studies. Over expression of murine ICE caused fibroblasts to undergo programmed cell

death in a transient transfection assay (Miura et al., Cell, 75: 653-660 (1993)). Cell death could be prevented by point mutations in the transfected gene in the region of greatest homology between ICE and Ced-3. As very strong support for the role of ICE in apoptosis, the authors showed that ICE transfection-induced apoptosis could be antagonized by overexpression of bcl-2, the mammalian oncogene that can prevent programmed cell death (Miura et al., supra). Additional experiments were performed using the crmA gene. This gene of the cowpox virus encodes a serpin protein, a family of proteins that are inhibitors of proteases (Ray et al., Cell, 69: 597-604 (1992)). Specifically, the protein of crmA has been shown to inhibit processing of pro-interleukin -1b by ICE. (Gagliardini et al. Science, 263: 826-828 (1994)) showed that microinjection of the crmA gene into dorsal root ganglion neurons prevented cell death induced by nerve growth factor deprivation. This result shows that ICE is involved in neuronal cell apoptosis. A more direct demonstration of ICE involvement comes from experiments in which ICE transfection is coupled with the co-expression of crmA, demonstrating a crmA-induced suppression of the ICE-induced apoptosis response (Miura et al., supra; Wang et al., supra).

In addition to ICE, researchers have examined the ability of ICE-like genes to promote apoptosis. (Kumar et al. supra) demonstrated that over expression of nedd-2 in fibroblasts and neuroblastoma cells resulted in cell death by apoptosis and that this apoptosis could also be suppressed by expression of the bcl-2 gene. Most recently, Wang et al., (Wang et al. , supra) examined the over expression of Ich-1 in a number of mammalian cells. Expression resulted in cell apoptosis, which could be antagonized by bcl-2 co-expression. Mutation of a cysteine residue, contained within the QACRG motif and presumed to be critical for protease function, to serine abolished apoptotic activity.

Further evidence for a role of a cysteine protease in apoptosis comes from a recent report by Lazebnik et al. (Nature, 371: 346-347 (1994)). These authors have used a cell-free system to mimic and study apoptosis. In their system there is a protease activity that cleaves the enzyme poly(ADP-ribose) polymerase at a site identical to a cleavage site in pre-interleukin-1b. However, this yet to be isolated protease and ICE appear to be different and to act on different substrate proteins. Blockade of protease activity in the system, using non-selective cysteine protease inhibitors, resulted in inhibition of apoptosis.

Taken together, the above evidence provides striking involvement of ICE and ICE-like proteins in the induction of apoptosis in mammalian cells. Brain interleukin-1 has been reported to be elevated in Alzheimer disease and Down syndrome (Griffin et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7611-7615 (1989)). There are also reports that interleukin-1 can increase the mRNA and production of b-amyloid protein, a major

component of senile plaques in Alzheimer disease as well as in brains of people with Down syndrome and with aging (Forloni et al., Mol. Brain Res., 16: 128-134 (1992); Buxbaum et al., Proc. Natl. Acad. Sci. U. S. A., 89: 10075-10078 (1992); Goldgaber et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7606-7610 (1989)). These reports can be viewed as additional evidence for the involvement of ICE in these diseases and the need for use of a novel therapeutic agent and therapy thereby.

To date, no useful therapeutic strategies have blocked excessive or inappropriate apoptosis. In one patent application, EPO 0 533 226 a novel peptide structure is diclosed which is said to be useful for determining the activity of ICE, and therefore useful in the diagnoses and monitoring of IL-1 mediated diseases. Therefore, a need exists to find better therapeutic agents which have non-toxic pharmacological and toxicological profiles for use in mammals. These compounds should block excessive or inappropriate apoptosis cells, and hence provide treatment for diseases and conditions in which this condition appears.

SUMMARY OF THE INVENTION

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of ICE and ICE-like proteins for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death.

Another aspect of the present invention is to a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

Another aspect of the present invention is to a method for the treatment of diseases or disorders associated with excessive IL-1b convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

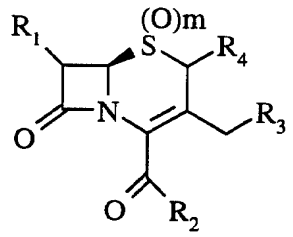
Another aspect of the present invention is to a method of preventing, or reducing apoptosis (i.e.blocking excess or inappropriate apoptosis) in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention is to a method of blocking or decresing the production of IL-1b and/or TNF, in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention may contain one or more asymmetric carbon atoms, in particular positions 6 and 7, and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention. Preferably the compound has a 6R, 7S configuration.

Preferably the compounds of Formula (I) are represented by the structure:



(I)

wherein

R_1 is hydrogen, an optionally substituted alkoxy, or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof.

Suitably, for compounds of Formula (I), R_1 is hydrogen, an optionally substituted C_{1-4} alkoxy or halogen. When R_1 is alkoxy, the carbon chain may be optionally substituted, one or more times, suitably one to three times, independently by hydroxy, halogen, alkoxy, C(O)H, $C(O)_2R_c$, or $C(O)CH_3$ moieties; wherein R_c is hydrogen, C_{1-6} alkyl, aryl, or arylC_{1-4}alkyl. Preferably R_1 is methoxy.

Suitably, for compounds of Formula (I), R_2 is OR_a; wherein R_a is C_{1-4}alkyl, or an optionally substituted arylC_{1-4}alkyl, preferably benzyl. It is recognized that the alkyl group in the arylalkyl moiety may be branched or straightsuch as a methylene or substituted methylene group , i.e., $-CH(CH_3)$ - aryl.

When R_a is an optionally substituted arylC_{1-4}alkyl, the aryl ring may be substituted one or more times independently by hydroxy, halogen, alkyl or alkoxy. When R_a is an alkyl, it is preferably methyl or t-butyl.

Suitably, for compounds of Formula (I), m is 1 or 2. Preferably m is 2.

Suitably, for compounds of Formula (I), R_3 is hydrogen, -OC(O)R_5, S(O)n-R_6, or bromo; provided that when R_3 is hydrogen, then R_4 is other than hydrogen. When R_3 is -OC(O)R_5, the R_5 group is suitably C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; preferably R_5 is C_{1-6} alkyl, more preferably methyl.

When R_3 is S(O)n R_6, R_6 is suitably an optionally substituted aryl, or an optionally substituted aryl heteroaryl; and n is 0, or an integer having a value of 1 or 2. When R_6 is heteroaryl, as defined below, it is preferably a triazole, oxadiazole, or tetrazole moiety. When R_6 is aryl, as also defined below, it is preferably a phenyl; the n value is preferably 1 or 2. When R_6 is a heteroaryl, n is preferably 0. The heteroaryl or aryl ring may be optionally substituted one or more times independently by hydroxy, halogen, alkyl or alkoxy, preferably alkyl, more preferably methyl.

Compounds exemplified by Formula (I) include, but are not limited to:
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Compounds of Formula (I) for use in the methods of the present invention include those noted above and:
tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

The term "excessive IL-1b convertase activity" is used herein to mean an excessive expression of the protein, or activation of the enzyme.

The term "C_{1-6} alkyl" or "alkyl" is used herein to mean both straight and branched chain radicals of 1 to 6 carbon atoms, unless the chain length is otherwise specified, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like.

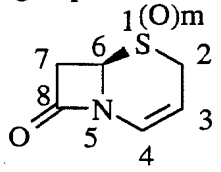
The term "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") is used herein to mean a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, oxazole, oxadiazole, tetrazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole.

The term "aryl" (on its own or in any combination, such as "aryloxy", or "arylalkyl") is used herein to mean a phenyl and naphthyl ring.

The term "cycloalkyl" is used herein to mean cyclic radicals, preferably of 3 to 7 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.

The term"halo" or "halogens", is used herein to include, unless otherwise specified, chloro, fluoro, bromo and iodo.

For purposes herein the "core" group for Formula (I) is numbered as follows:



The present invention is to the inhibition of ICE and ICE-like proteases by compounds of Formula (I). What is meant by the term "ICE-like proteases" are fragment, homologs, analogs and derivatives of the polypeptides Interleukin-1 b converting enzyme (or convertase). These analogs are structurally related to the ICE family. They generally encode a protein (s) which exhibits high homology to the human ICE over the entire sequence. Preferably, the pentapeptide QACRG is conserved. The ICE like proteases , which may include many natural allelic variants (such as substitutions, deletion or addition of nucleotides) does not substantially alter the function of the encoded polypeptide. That is they retain essentially the same biological function or activity as the ICE protease, although it is recognized that the biological function may be enhanced or reduced activity. The suitable activity is not IL-1b convertase activity, but the ability to induce apoptosis or involved in programmed cell death in some manner. Suitable ICE like proteases encompasses within this invention are those described in PCT US94/07127 filed 23 June 1994, Attorney Docket No.: 325800-184; and in USSN 08/334,251, filed 1 November 1994, Attorney Docket No.: 325800-249 whose disclosures are incorporated herein by reference in their entirety.

The term "blocking or inhibiting, or decreasing the production of IL-1b and/or TNF" as used herein refers to:

a) a decrease of excessive levels, or a down regulation, of the cytokine in a human to normal or sub-normal levels by inhibition of the *in vivo* release of the cytokine; or

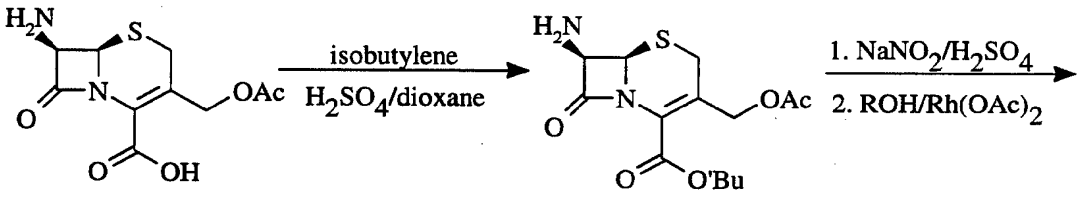
b) a down regulation, at the genomic level, of excessive *in vivo* levels of the cytokine (IL-1 or TNF) in a human to normal or sub-normal levels; or

c) a down regulation, by inhibition of the direct synthesis of the cytokine (IL-1, or TNF) as a postranslational event; or

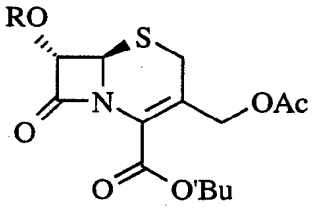
d) a down regulation, at the translational level, of excessive *in vivo* levels of the cytokine (IL-1, or TNF) in a human to normal or sub-normal levels.

The blocking or inhibiting, or decreasing the production of IL-1b and/or TNF is a discovery that the compounds of Formula (I) are inhibitors of the cytokines, IL-1 and TNF is based upon the effects of the compounds of Formulas (I) on the production of the IL-1 and TNF in *in vitro* and *in vivo* assays which are well known and recognized in the art, some of which are described herein.

Compound of the present invention may be synthesized by methods well known in the art, such as those described by the procedures of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513 whose disclosure is incorporated herein by reference. Alternatively, compounds of Formula (I) may be made in accordance with the schemes illustrated below.



I II



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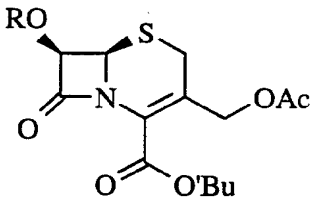

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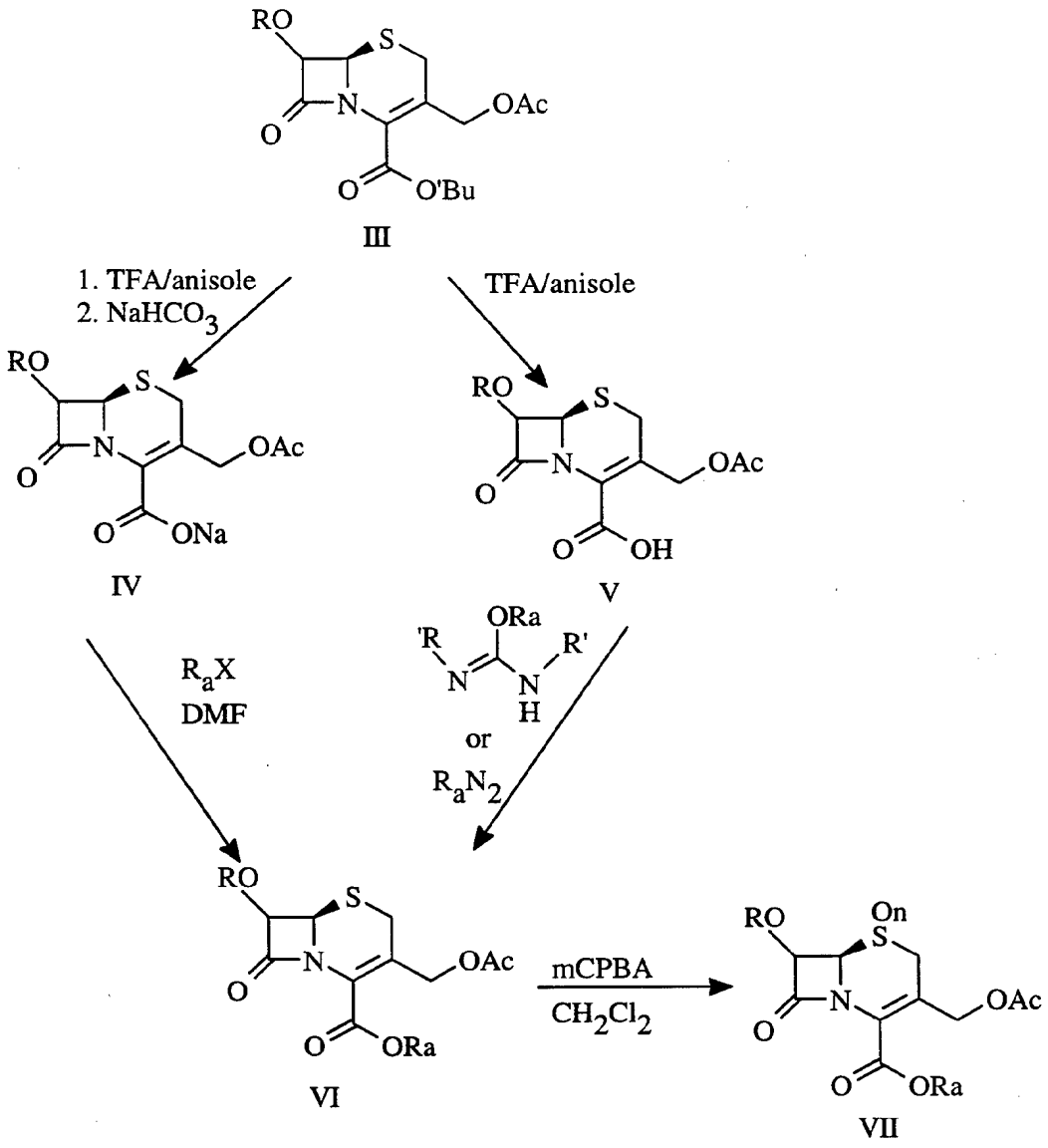
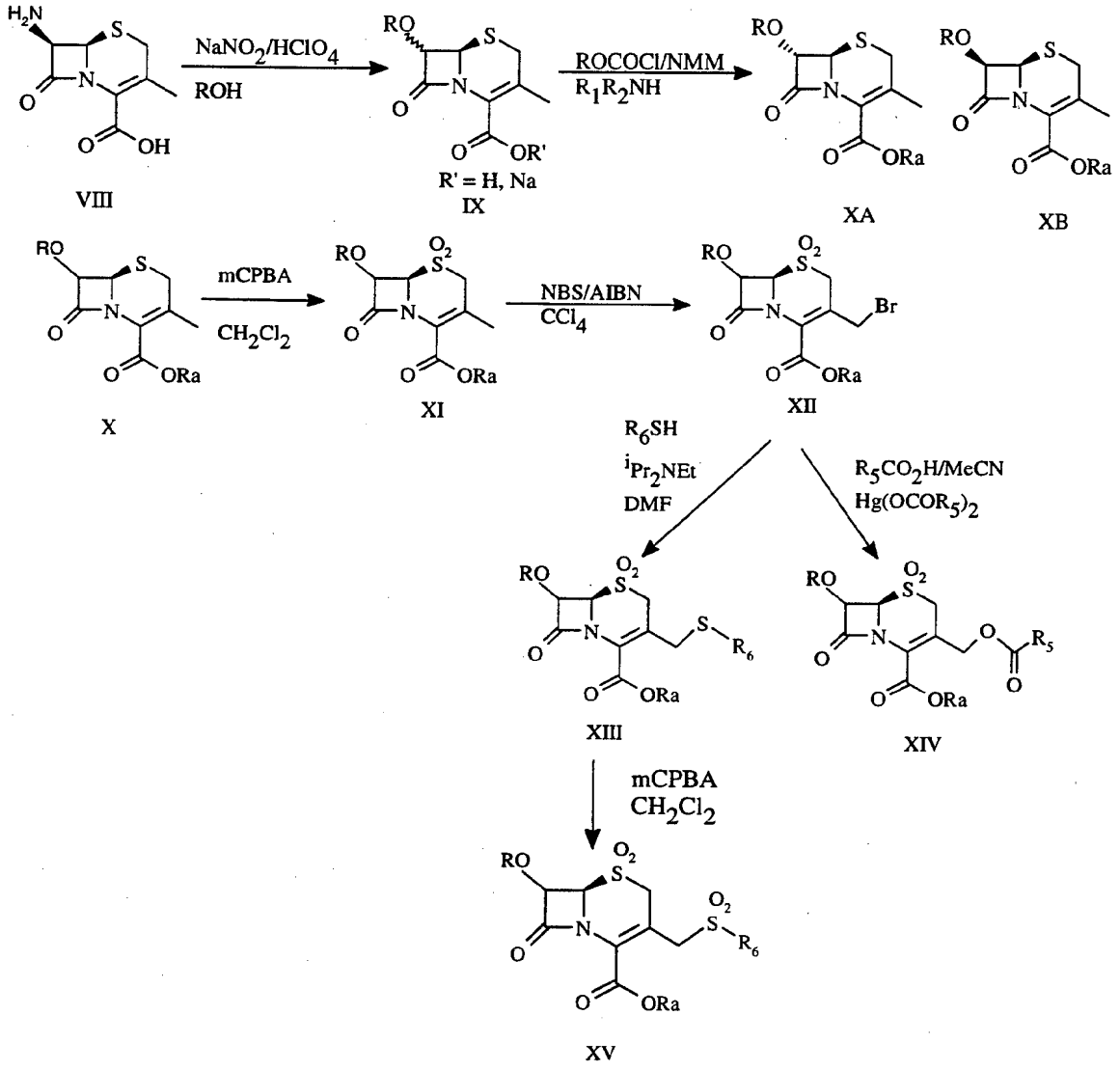


Scheme 1

The t-Butyl ester, 2-Scheme I, is synthesized by treating commercially available 7-aminocephalosporonic acid (1-Scheme I) with isobutylene and sulfuric acid in dioxane. Following the procedure of Doherty et al. (*J. Med. Chem.* 1990, **33**, 2513-2521, which is incorporated herein by reference), 7-alkoxy substituted 3a-Scheme I and 3b-Scheme I are produced as a separable mixture. Deprotection of 3-Scheme I with trifluoroacetic acid/anisole at 0°C gives the free acid 5-Scheme I or the sodium salt 4-Scheme I upon titration with aqueous sodium bicarbonate. Benzyl halide alkylations of IV in DMF give esters 6-Scheme I. Treatment of 5-Scheme I with diazo derivatives (Braun et al. *J. Am. Chem. Soc.* 1958, **80**, 359-363,which is incorporated herein by reference) or with alkoxyisoureas (Schmidt et al. *Justus Liebigs Ann. Chem.* 1965, **685**,

161-166,which is incorporated herein by reference) yields various alkylester derivatives (6-Scheme I). Finally, sulfone or sulfoxides 7-Scheme I are obtained by m-chloroperoxybenzoic acid or oxone oxidation of 6-Scheme I.



VIII IX XA XB X XI XII XIII XIV XV

Scheme 2

Alkoxy derivative 9-Scheme 2 is obtained in one step from 8-Scheme 2 by treatment with $NaNO_2$ and the alcohol in perchloric acid (Alpegiani et al. US 5,254,680, which is incorporated herein by reference). Ester 10-Scheme I is formed by esterification of 9-Scheme 2 by procedures described for 6-Scheme 1; m-chloroperoxybenzoic acid or oxone oxidation of 10-Scheme I yields 11-Scheme 2. The following derivatives can be synthesized according to procedures outlined by Alpegiani et al. *J. Med. Chem.* 1994, **37**, 4003-4019, which is incorporated herein by reference:

exposure of 11-Scheme 2 to N-bromosuccinimide under radical conditions gives the 3-bromomethyl derivative 12-Scheme 2; 13-Scheme 2 and 14-Scheme 2 are accessible through displacement of the bromide by aromatic thiols and mercuric acetate derivatives. Sulfones 15-Scheme 2 are obtained by oxidation of their corresponding thioethers (13-Scheme 2).

SYNTHETIC CHEMISTRY

Without further elaboration, it is believed that one skilled in the art can, using the preceding descriptions, utilize the present invention to its fullest extent. The following examples further illustrate the synthesis of compounds of this invention. The following examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

Temperatures are recorded in degrees centigrade unless otherwise noted.

Example 1

tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

The title compound was prepared according to the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513.

Example 2

tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513, the title compound is isolated as a minor component of the final mixture.

Example 3

3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate

To tert-Butyl (6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate (prepared by the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513) (1.0 g, 2.9 mmol) and anisole (3.2 mL, 29 mmol) was added trifluoroacetic acid (16 mL) at 0_C under Ar. The solution was stirred for 30 min, and concentrated *in vacuo*.

The residue was dissolved in methylene chloride (50 mL), washed with water, washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to an oil. The residue was dissolved in ethyl acetate (30 mL), and water (30 mL) was added. A solution of saturated sodium bicarbonate was dropped in until the aqueous layer reached pH 7. The aqueous layer was separated, and the procedure was repeated with

another 30 mL of water. The aqueous layers were combined and freeze-dried to afford a yellow solid (870 mg).

To the sodium salt (187 mg) in dimethylformamide (6 mL) was added 3,4-dichlorobenzyl chloride (168 uL) under Ar and the solution was stirred for 22 h. To the solution was added ether, the mixture was washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The oil was purified by flash chromatography (silica gel, 25-45% ethyl acetate/hexanes) to yield a 3:2 mixture of the title compound and the Æ^2 regioisomer (85 mg, 30% overall yield). ^{1}H NMR(400 MHz, $CDCl_3$) d 7.1-7.6 (m, 3H), 6.46, 4.5-5.3 (m, 6H), 3.3-3.7 (m, 5H), 3.55 (m, 3H).

b) 3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

To the ester (83 mg, 186 umol) of Example 2(a) in methylene chloride (3 mL) was added 85% m-chloroperoxybenzoic acid (114 mg, 558 umol) and the solution was stirred for 4 h. To the solution was added 20% sodium metabisulfite, followed by saturated sodium bicarbonate and the mixture was extracted with methylene chloride. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 40-50% ethyl acetate/hexanes) to yield the title compound (75 mg, 84%). MS(ES$^+$) m/e 478 $[M+H]^+$.

Example 4

tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide

a) tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate

Following the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513, except substituting ethylene glycol for methanol, the title compound was prepared. ^{1}H NMR(400 MHz, $CDCl_3$) d 4.93 (d, J=13.7 Hz, 1H), 4.73 (d, J=13.7 Hz, 1H), 4.70 (s, 1H), 4.61 (s, 1H), 3.81 (br s, 4H), 3.58 (d, J=18.4 Hz, 1H), 2.07 (s, 3H), 1.54 (s, 9H).

b) tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethyloxy)-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3(b), except substituting the title compound of Example 4(a) for the ester of Example 3(a), the title compound was prepared. MS(ES$^-$) m/e 404 $[M-H]^-$.

Example 5

Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) (6R,7S)-3-Acetoxymethyl-7-methoxy-3-cephem-4-carboxylic acid

The intermediate sodium salt (85 mg) from Example 3(a) was purified by flash chromatography (0.5% acetic acid/10% methanol/methylene chloride) to yield the free acid (50 mg). ^{1}H NMR(400 MHz, 2:1 $CDCl_3/CD_3OD$) d 4.87 (d, J=12.6 Hz, 1H), 4.73 (d, J=12.6 Hz, 1H), 4.61 (s, 1H), 4.40 (d, J=1.7 Hz, 1H), 3.49 (d, J=17.8 Hz, 1H), 3.44 (s, 3H), 3.17 (d, J=17.8 Hz, 1H), 2.09 (s, 3H).

b) Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate

To the acid of Example 5(a) (48 mg, 167 umol) in tetrahydrofuran (3 mL) was added a 0.1 M ethereal solution of diazomethane (10 mL) at 0°C. The solution was stirred for 15 min, and quenched with an excess of acetic acid. The mixture was diluted with methylene chloride, washed with saturated sodium bicarbonate, concentrated in vacuo and purified by flash chromatography (silica gel, 15-25% ethyl acetate/hexanes) to yield the title compound (25 mg, 52%). ^{1}H NMR(400 MHz, $CDCl_3$) d 4.97 (d, J=13.2 Hz, 1H), 4.76 (d, J=13.2 Hz, 1H), 4.69 (s, 1H), 4.51 (s, 1H), 3.89 (s, 3H), 3.58 (d, J=18.3 Hz, 1H), 3.55 (s, 3H), 3.32 (d, J=18.3 Hz, 1H), 2.07 (s, 3H).

c) Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3(b), except substituting the title compound of Example 5(b) for the ester of Example 3(a), the title compound was prepared. MS(ES^-) m/e 332 $[M-H]^-$.

Example 6

Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting benzyl bromide for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^+) m/e 410 $[M+H]^+$.

Example 7

3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 70% 3,4-dimethylbenzyl chloride (30% 2,3-dimethylbenzyl chloride) for 3,4-dichlorobenzyl chloride, the title compound was prepared as a 1:1 mixture with 2,3-dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide. MS(ES^+) m/e 438 $[M+H]^+$.

Example 8

4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 4-nitrobenzyl bromide for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^-) m/e 453 $[M-H]^-$.

Example 9

3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide

Following the procedure of Example 3,.except one equivalent of m-chloroperoxybenzoic acid is used. MS(ES^-) m/e 460 $[M-H]^-$.

Example 10

3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide for tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide, the title compound was prepared. MS(ES^-) m/e 478 $[M-H]^-$.

Example 11

4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 4-iodobenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^+) m/e 536 $[M+H]^+$.

Example 12

3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 3-iodobenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^-) m/e 534 $[M-H]^-$.

Example 13

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 3-iodo-4-methylbenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^+) m/e 550 $[M+H]^+$.

Example 14

3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting the title compound of Example 4(a) for tert-butyl (6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate. MS(ES^-) m/e 506 $[M-H]^-$.

Example 15

3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxyl-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 16, except substituting n-butanol for ethylene glycol, the title compound was prepared. MS(ES^-) m/e 518 $[M-H]^-$.

Example 16

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 16, except substituting ethanol for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^-) m/e 490 $[M-H]^-$.

Example 17

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate

To a solution of (6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylic acid (1 g) in ethyl acetate (30 mL) is added water (30 mL). A solution of saturated sodium bicarbonate is dropped in until a pH of 7 in the aqueous layer is obtained. The aqueous layer is separated and lyophylized to afford sodium-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (670 mg).

To sodium-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (312 mg) in dimethylformamide (2 mL) is added 3,4-dichlorobenzyl chloride (500 uL), and the solution was stirred for 24 h. To the solution was added water and the solution was extracted with ether. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 15-25% ethyl acetate/hexanes) to yield 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (148 mg). ^1H NMR(250 MHz, $CDCl_3$) d 7.2-7.6 (m, 3H), 5.23 (s, 2H), 4.67 (s, 1H), 4.50 (s, 1H), 3.4-3.6 (m, 4H), 3.19 (d, J=18.4 Hz, 1H), 2.10 (s, 3H).

b) 3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3b, except substituting 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate for the ester of example 2a, the sulfone was prepared. ^1H NMR(250 MHz, $CDCl_3$) d 7.2-7.6 (m, 3H), 5.21 (s, 2H), 5.13 (s, 1H), 4.62 (s, 1H), 3.88 (d, J=18.4 Hz, 1H), 3.66 (d, J=18.4 Hz, 1H), 3.56 (s, 3H), 2.10 (s, 3H).

c) 3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide.

To the sulfone of Example 17b (91 mg) in carbon tetrchloride (4 mL) is added AIBN (5 mg) and N-bromosuccinimide (44 mg), and solution was reluxed under argon for 3 h. the reaction mixture was cooled, saturated sodium bicarbonate was added, and the mixture was extracted with methylene chloride. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 15-35% ethyl acetate/hexanes) to yield the title compound (45 mg). MS(ES^-) m/e 496 $[M-H]^-$.

Example 18

3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl-(6R,7S)-3-phenylthiomethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

To the bromide of Example 17 (110 mg) in dimethylformamide (15 mL) at 0°C was added thiophenol (25 uL) and N,N-diisopropyl-N-ethylamine (42 uL). The solution was stirred until the disappearance of starting material. Water was added, and the solution was extracted with ether. The organic extract concentrated *in vacuo*, and the residue was purified by flash chromatography (silica gel, ethyl acetate/hexanes) to yield the title compound. MS(ES$^+$) m/e 528 [M+H]$^+$.

b) 3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3b, except substituting 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate for the ester of example 2a, the sulfone was prepared. MS(ES$^+$) m/e 560 [M+H]$^+$.

Example 19

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting 5-methyl-(1,3,4-oxadiazol)-2-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 534 [M+H]$^+$.

Example 20

3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting (1-methyltetrazole)-5-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 534 [M+H]$^+$.

Example 21

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting (1,2,3-triazole)-4-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 519 [M+H]$^+$.

BIOLOGICAL ASSESSMENTS:

Assay I - DNA Ladder:

The present invention utilizes a model that measures apoptosis, by measuring the production of DNA ladders visualized on agarose gels. The observation of DNA ladders has been a hallmark of the apoptosis response for many years. The model used in our studies is the production of apoptosis in human monocytic HL-60 cells by the anti-cancer ether lipid 1-O-octadecyl-2-O-methyl-*sn*-3-phosphocholine (ET-18-OCH_3) and tumor necrosis factor a (TNF). The production of DNA ladders by ET-18-OCH_3 was recently reported (Mollinedo et al. Biochem. Biophys. Res. Commun., 192: 603-609 (1993)) and confirmed in house. The general method is to treat HL-60 cells with 6 μM ET-18-OCH_3 or 10 units of TNF for 24 hours, followed by extraction of small molecular weight DNA and removal of protein and RNA. The DNA is separated on a agarose gel and visualized with ethidium bromide staining. An internal standard is added to the cells just prior to extraction and preparation of DNA. Drugs are provided to cells 10 minutes prior to the apoptotic insult. This method provides a qualitative assessment of the ability of compounds to inhibit apoptosis.

Cell Conditions

- HL-60 cells (American Type Cell Culture) were grown and kept at log phase in RPMI 1640 w/L-glutamine and 10 % heat inactivated Fetal Bovine Serum (RPMI complete).
- On the day of the experiment, the desired number of cells (for example, 5 x 10^6 cells/treatment group) were resuspend in RPMI complete to give a final cell concentration of approximately 0.5 x 10^6 cells/ml. For each treatment group, 10 mls of cell suspension were placed in a culture flask. Cells were incubated for 2 hours at 37°C.

Exposures:

- For typical expose to ET-18-OCH_3, a 100 mM ET-18-OCH_3 stock solution in $CHCl_3$ was prepared, then diluted in RPMI complete to 600 μM. Then 100 μl of 600 μM ET-18-OCH_3 was added into 10 ml treatment group yielding a final concentration of 6 μM. The cell suspensions are then incubated overnight (18 hours). For a typical exposure to TNF, 300 to 3000 units of TNF were added to 10 ml of cell suspension.
- Cells were pretreated with desired agents (ICE compounds, etc.) 10 minutes prior to ET-18-OCH_3 or TNF addition. ICE compounds stocks were in DMSO. 50 μl of compound or DMSO vehicle was added to the 10 ml treatment groups yielding the final concentration of compound and 0.5 % DMSO.

DNA Extraction:

- Cells were spun (400 x g, 5 min) and washed 2x in 10 mLs PBS.
- Cells were lysed by resuspending them into 200 µL of cold, sterile detergent buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.2% Triton X-100) and transferring the approximately 250 µL volume to sterile, 1.5 mL eppendorf tubes on ice. Then, tubes were incubated for 30 min. at 4°C, with mild shaking.
- Tubes were spun in a Microfuge for 15 min., the supernatant collected, taking care to avoid the cellular debris.
- The supernatants were incubated with 75 µg/mL RNAseA for 1 hr at 37°C then incubated with 200 µg/mL ProteinaseK and 0.5 % SDS [final] for 1 hr at 37°C.
- Ten µl of a 300 bp DNA was added as an internal standard to observe extraction efficiency.
- Supernatants were extracted twice with equal volume (200-300 µL) of cold, buffer saturated phenol (add phenol, vortex 15 seconds, microfuge 2 min., collect the top aqueous layer, avoiding the organic waste in between the two phases), once with 200 µL Phenol/Chloroform/Isoamyl alcohol 25:24:1 (v/v) and once with 100 µL Chloroform (100µL/sample is retrieved).
- Add 10 µL of sterile 3M NaCl (300 mM [final]) to the 100 µL sample and 200 µL of cold ethanol, vortex well and let stand overnight at -20°C.
- Samples were spun (Microfuge) 15 min and all but 25 µl of the ethanol was carefully removed. The DNA pellets were dried and resuspended in 30 µL of sterile 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA and 10 µL of gel loading Buffer. Load 20 µl/well.
- A DNA standard (for example, Sigma # D 5042, 123 bp ladder) was run on each gel.
- Samples were run on 1-2 % agarose gel with TBE buffer (5X TBE = 54 g Tris Base, 27.5 g Boric acid, 20ml 0.5 M EDTA, pH 8.0) with ethidium bromide added, for example for 90 - 120 min at 100 V, 50 mA.
- The resulting gels were visualized under UV light and the results recorded in a captured image.

Results

In HL-60 cells, treatment with ET-18-OCH_3 or TNF induced an apoptotic response that was prominent after 24 hours. Pretreatment with 50 µM of the compound of Example 3 was found to completely block the apoptotic response to both ET-18-OCH_3 or TNF (Table 1). Pretreatment with 5 µM of the compound of Example 3 was not effective over the 24 hour experiment. Addition of IL-1b (10 nM) had no effect on the ability of the compound of Example 3 to block apoptosis, suggesting that its primary mechanism of action is not inhibition of IL-1 production. These data support

that the compound of Example 3 blocks apoptosis by a novel mechanism of action, i.e., by inhibiting the activity of ICE and ICE-like proteases.

Table 1. Effect of Compound on Apoptosis

Drug	Concentration	Apoptotic Signal	Concentration	Presence of Apoptosis
None		ET-18-OCH_3	6 μM	yes
Example 3	50 μM	ET-18-OCH_3	6 μM	no
Example 3	5 μM	ET-18-OCH_3	6 μM	yes
None		TNF	270 U/ml	yes
Example 3	50 μM	TNF	270 U/ml	no

Assay II: Inhibition of ICE

Source of Enzyme

Human ICE was cloned and expressed in *E. coli* as its inactive precursor (p45) bearing a hexa-His flag on its *amino*-terminal end. Following harvesting, the cells were lysed, centrifuged, and the pellet containing the p45 solubilized with phosphate buffered 7 M urea at pH 7.5. The flagged p45 was applied to a Ni-nitrilo-acetic acid column, washed, and eluted with 300 mM imidazole. This yielded a highly enriched proenzyme preparation (390% pure p45). Catalytic autoproteolytic activation to p10/p20 dimer was achieved by concentrating the p45 on a Centricon ultrafiltration membrane (Amicon) at 10 _C for several hours. The formation of the catalytic subunits (p10 and p20) in activated samples was demonstrated by correlating time-dependent generation of ICE activity with p10/p20 signals in Western blots and by reversed-phase HPLC. Formation of authentic p10 and p20 was also confirmed by *N*-terminal sequence and MALD-mass spectral analyses of samples purified by reversed-phase HPLC. The activated enzyme was stored frozen at –80 _C.

Assay Protocol

ICE was assayed at 25 _C using the fluorogenic tetrapeptide substrate *N*-acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-7-amido-4-methylcoumarin (Ac-**YVAD**-AMC). The assays were conducted at pH 7.5 in a buffered system containing 25 mM Hepes, 10% sucrose, 0.1% CHAPS, and 2 mM DTT. The concentration of substrate was fixed at 25 uM. Fluorescence of the liberated 7-amino-4-methylcoumarin was continuously monitored at 460 nm following excitation at 335 nm.

Compound Testing

Compounds of Formula (I) were tested at a single dose of 100 uM following a 30 to 60-min preincubation with enzyme. The assay was initiated by the addition of 25 uM substrate (Ac-**YVAD**-AMC) and activity was monitored as described above.

Representative compounds of Formula (I), as exemplified by Examples 1 to 7 and 9 demonstrated positive inhibitiory activity in this assay ranging from about 36% to about 96%.

Assay III: Inhibition of ICE

ICE was assayed at 25 _C in 96-well plates using the fluorogenic tetrapeptide substrate *N*-acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-7-amido-4-methylcoumarin (Ac-**YVAD**-AMC). The assays were conducted at pH 7.5 in a buffered system containing 25 mM Hepes, 10% sucrose, 0.1% CHAPS, and 20-50 uM DTT. The concentration of substrate was fixed at 20 uM. Fluorescence of the liberated 7-amino-4-methylcoumarin was continuously monitored at 460 nm following excitation at 360 nm.

Compound Testing

Compounds were tested at a single dose of 50 to 100 uM. Activity was monitored as described above over a 30 to 60-minute time period following the simultaneous addition of substrate and inhibitor to initiate the reaction. The progress curves thus generated were fit by computer to Eq. 1 in order to assess potency and time-dependency:

$$v = \frac{(V_o(1 - e^{-k_{obs}t})}{k_{obs}} \qquad (1)$$

Representative compounds of formula (I) have demonstrated positive inhibitory activity in the above noted assay:

3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

tert-Butyl 7-alpha-methoxycephalosporanate sulfone

3,4-Dichlorobenzyl-(6R,7S)-3-(1-methyltetrazol-5-yl)thiomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-(phenylsulfonyl)methyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[2-methyl(1,3,4-oxadiazol-5-yl)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl (6R,7S)-3-(1,2,3-triazol-5-yl)thiomethyl-7-methoxy-3-cephem-4-carboxylate 1,1-dioxide

3,4-Dichlorobenzyl-5,5-dioxo-7-alpha-[2-hydroxyethyloxy]-cephalosporanate
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
Benzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
[(3,4)- and (2,3)-]Dimethylbenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide N-3,4-Dichlorobenzyl-N-methyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxamide-1,1-dioxide
(6R,7S)-4-Iodobenzyl--7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodo-4-methylbenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

METHODS OF TREATMENT

For therapeutic use the compounds of the present invention will generally be administered in a standard pharmaceutical composition obtained by admixture with a pharmaceutical carrier or diluent selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsule, ovules or lozenges either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The choice of form for administration as well as effective dosages will vary depending, inter alia, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

The compounds of the present invention, particularly those noted herein or their pharmaceutically acceptable salts which are active when given orally, can be formulated as liquids, for example syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerin, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule. Preferably the composition is in unit dose form such as a tablet or capsule.

Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

A typical suppository formulation comprises a compound or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycols, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For a patient this may be, for example, from about .001 to about 100mg/kg, preferably from about 0.001 to about 10mg/kg animal body weight. A daily dose, for a larger mammal is preferably from about 1 mg to about 1000 mg, preferably between 1 mg and 500 mg or a pharmaceutically acceptable salt thereof, calculated as the free base, the compound being administered 1 to 4 times per day. Unit dosage forms may contain from aobut 25μg to about 500mg of the compound.

There are many diseases and conditions in which dysregulation of apoptosis plays an important role. All of these conditions involve undesired, deleterious loss of specific cells with resulting pathological consequences.

Bone remodeling involves the initial resorption by osteoclasts, followed by bone formation by osteoblasts. Recently, there have been a number of reports of apoptotic events occurring during this process. Apoptotic events have been observed in both the bone forming and bone resorbing cells *in vitro* and indeed at the sites of these remodeling units *in vivo.*

Apoptosis has been suggested as one of the possible mechanisms of osteoclast disappearance from reversal sites between resorption and formation. TGF-ß1 induces apoptosis (approx. 30%) in osteoclasts of murine bone marrow cultures grown for 6 days *in vitro* . (Hughes, et al., *J. Bone Min. Res.* **9** , S138 (1994)). The anti-resorptive

bisphosphonates (clodronate, pamidronate or residronate) promote apoptosis in mouse osteoclasts *in vitro* and *in vivo* . (Hughes, et al., supra at S347). M-CSF, which has previously been found to be essential for osteoclast formation can suppress apoptosis, suggesting not only that maintenance of osteoclast populations, but also that formation of these multinucleated cells may be determined by apoptosis events. (Fuller, et al., *J. Bone Min. Res.* **8** , S384 (1993); Perkins, et al., *J. Bone Min. Res.* **8** , S390 (1993)). Local injections of IL-1 over the calvaria of mice once daily for 3 days induces intense and aggressive remodeling. (Wright, et al., *J. Bone Min. Res.* **9** , S174 (1994)). In these studies, 1% of osteoclasts were apoptotic 1 day after treatment, which increased 3 days later to 10%. A high percentage (95%) of these apoptotic osteoclasts were at the reversal site. This data suggests that ICE or ICE-like homologues are functionally very important in osteoclast apoptosis.

Therefore, one aspect of the present invention is the promotion of apoptosis in osteoclasts as a novel therapy for inhibiting resorption in diseases of excessive bone loss, such as osteoporosis, using compounds of Formula (I) as defined herein.

Apoptosis can been induced by low serum in highly differentiated rat osteoblast-like (Ros 17/2.8) cells (Ihbe, et al., (1994) *J. Bone Min. Res.* **9**, S167)). This was associated with a temporal loss of osteoblast phenotype, suggesting that maintenance of lineage specific gene expression and apoptosis are physiologically linked. Fetal rat calvaria derived osteoblasts grown *in vitro* undergo apoptosis and this is localized to areas of nodule formation as indicated by *in situ* end-labeling of fragmented DNA. (Lynch, et al., (1994) *J. Bone Min. Res.* **9**, S352). It has been shown that the immediate early genes c-fos and c-jun are expressed prior to apoptosis; c-fos and c-jun-Lac Z transgenic mice show constitutive expression of these transcription factors in very few tissues, one of which is bone (Smeyne, et al., (1992) *Neuron.* **8**, 13-23; and Morgan, J. (1993) Apoptotic Cell Death: Functions and Mechanisms. Cold Spring Harbor 13-15th October). Apoptosis was observed in these animals in the epiphyseal growth plate and chondrogenic zones as the petula ligament calcifies. Chondrogenic apoptosis has also been observed in PTHRP-less mice and these transgenics exhibit abnormal endochondral bone formation (Lee, et al., (1994) *J. Bone Min. Res.* **9**, S159). A very recent paper examined a human osteosarcoma cell line which undergoes spontaneous apoptosis. Using this cell line, LAP-4, but not ICE, could be detected and *in vitro* apoptosis could be blocked by inhibition or depletion of LAP-4 (Nicholson, et al., (1995) *Nature* **376**, 37-43). Thus, apoptosis may play a role in loss of osteoblasts and chondrocytes and inhibition of apoptosis could provide a mechanism to enhance bone formation.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to enhance bone formation using compounds of Formula (I) as defined herein.

Osteoarthritits (OA) is a degenerative disease characterized by progressive erosion of articular cartilage. Chondrocytes are the single cell-type found in articular cartilage and perturbations in metabolism of these cells may be involved in the pathogenesis of OA. Injury to cartilage initiates a specific reparative response which involves an increase in the production of proteoglycan and collagen in an attempt to reestablish normal matrix homeostasis. However, with the progress of the disease, the 3-dimensional collagen network is disrupted and cell death of chondrocytes occurs in OA lesions (Malemud, et al.: Regulation of chondrocytes in osteoarthritis. In: Adolphe, M. ed. Biological Regulation of Chondrocytes. Boca Raton:CRC Press, 1992, 295-319). It has been shown that in OA, chondrocytes adjacent to cartilage defects express high levels of bcl-2 (Erlacher, et al., (1995) *J. of Rheumatology*, 926-931). This represents an attempt to protect chondrocytes from apoptosis induced by the disease process.

Protection of chondrocytes during early degenerative changes in cartilage by inhibition of apoptosis may provide a novel therapeutic approach to this common disease. Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat osteoarthritis, using compounds of Formula (I) as defined herein.

Recent evidence shows that chronic, degenerative conditions of the liver are linked to hepatocellular apoptosis. These conditions include chemical-, infectious- and immune/inflammatory-induced hepatocellular degeneration. Apoptosis of liver cells has been observed in liver degenerative states induced by a variety of chemical agents, including acetaminophen (Ray, et a l.,(1993) *FASEB. J.* **7**, 453-463), cocaine (Cascales, et al., (1994) *Hepatology* **20**, 992-1001) and ethanol (Baroni, etal., (1994) *J. Hepatol.* **20**, 508-513). Infectious agents and their chemical components that have been shown to induce apoptosis include hepatitis ((Hiramatsu, et al., (1994) *Hepatology* **19**, 1354-1359; Mita, et al., (1994) *Biochem. Biophys. Res. Commun.* **204**, 468-474)), tumor necrosis factor and endotoxin . (Leist, et al., (1995) *J. Immunol.* **154**, 1307-1316; and Decker, K. (1993) *Gastroenterology* **28(S4)**, 20-25). Stimulation of immune / inflammatory responses by mechanisms such as allograft transplantation and hypoxia followed by reperfusion have been shown to induce apoptosis of hepatocytes (Krams, et al., (1995) *Transplant. Proc.* **27**, 466-467). Together, this evidence supports that hepatocellular apoptosis is central to degenerative liver diseases.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat degenerative liver diseases., using compounds of Formula (I) as defined herein.

Apoptosis is recognized as a fundamental process within the immune system where cell death shapes the immune system and effects immune functions. Apoptosis also is implicated in viral diseases (e.g AIDS). Recent reports indicate that HIV infection may produce an excess of apoptosis, contributing to the loss of $CD4^+$ T cells. Of additional interest is the observation that APO-1/Fas shares sequence homology with HIV-1 gp120.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat viral diseases, using compounds of Formula (I) as defined herein.

Additional therapeutic directions and other indications in which inhibition of apoptotic cysteine proteases is of therapeutic utility, along with relevant citations in support of the involvement for apoptosis in each indication, are presented below in Table 1.

Table 1: Therapeutic Indications Related to Apoptosis

Indication	Citations
Ischemia / reperfusion	Barr et al., (1994) *BioTechnology* **12**, 487-493; Thompson, C. B. (1995) *Science* **267**, 1456-1462
Stroke	Barr et al supra; and Thompson, C., supra
Polycystic kidney disease	Barr et al., supra; and Mondain, et al., (1995) *ORL J. Otorhinolaryngol. Relat. Spec.* **57**, 28-32
Glomerulo-nephritis	Barr et al., supra
Osteoporosis	Lynch et al., (1994) *J. Bone Min. Res.* **9**, S352; Nicholson et al., (1995) *Nature* **376**, 37-43
Erythropoiesis / Aplastic anemia	Thompson, C., supra; Koury et al., (1990) *Science* **248**, 378-381

Chronic liver degeneration	Thompson, C., supra; Mountz et al., 1994) *Arthritis Rheum.* **37**, 1415-1420; Goldin et al., (1993) *Am. J. Pathol.* **171**, 73-76
T-cell death	Thompson, C., supra; Ameison et al., (1995) *Trends Cell Biol.* **5**, 27-32
Osteoarthritis - chondrocytes	Ishizaki et al., (1994) *J. Cell Biol.* **126**, 1069-1077; Blanco et al., (1995) *Am. J. Pathol.* **146**, 75-85
Male pattern baldness	Mondain et al., supra; Seiberg et al., (1995) *J. Invest. Dermatol.* **104**, 78-82; Tamada et al., (1994) *Br. J. Dermatol.* **131**, 521-524
Alzheimer's disease	Savill, J.,(1994) *Eur. J. Clin. Invest.* **24**, 715-723; Su et al., (1994) *Neuroreport* **5**, 2529-2533; Johnson, E., (1994) *Neurobiol. Aging* **15 Suppl. 2**, S187-S189
Parkinson's disease	Savill, J., supra; Thompson, C., supra
Type I diabetes	Barr et al., supra

The IL-1 and TNF inhibiting effects of compounds of the present invention are determined by the following *in vitro* assays:

Interleukin - 1 (IL-1)

Human peripheral blood monocytes are isolated and purified from either fresh blood preparations from volunteer donors, or from blood bank buffy coats, according to the procedure of Colotta *et al*, J Immunol, **132**, 936 (1984). These monocytes ($1x10^6$) are plated in 24-well plates at a concentration of 1-2 million/ml per well. The cells are allowed to adhere for 2 hours, after which time non-adherent cells are removed by gentle washing. Test compounds are then added to the cells for about 1hour before the addition of lipopolysaccharide (50 ng/ml), and the cultures are incubated at 37°C for an additional 24 hours. At the end of this period, culture super-natants are removed and clarified of cells and all debris. Culture supernatants are then immediately assayed for IL-1 biological activity, either by the method of Simon *et al.*, J. Immunol. Methods, **84**, 85, (1985) (based on ability

of IL-1 to stimulate a Interleukin 2 producing cell line (EL-4) to secrete IL-2, in concert with A23187 ionophore) or the method of Lee *et al.*, J. ImmunoTherapy, **6** (1), 1-12 (1990) (ELISA assay).

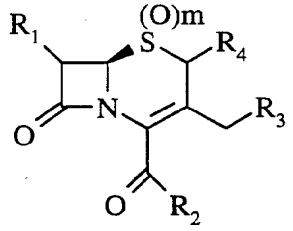
Tumour Necrosis Factor (TNF):

Human peripheral blood monocytes are isolated and purified from either blood bank buffy coats or platelet pheresis residues, according to the procedure of Colotta, R. *et al.*, J Immunol, **132**(2), 936 (1984). The monocytes are plated at a density of $1x10^6$ cells/ml medium/well in 24-well multi-dishes. The cells are allowed to adhere for 1 hour after which time the supernatant is aspirated and fresh medium (1ml, RPMI-1640, Whitaker Biomedical Products, Whitaker, CA) containing 1% fetal calf serum plus penicillin and streptomycin (10 units/ml) added. The cells are incubated for 45 minutes in the presence or absence of a test compound at 1nM-10mM dose ranges (compounds are solubilized in dimethyl sulfoxide/ethanol, such that the final solvent concentration in the culture medium is 0.5% dimethyl sulfoxide/0.5% ethanol). Bacterial lipopoly-saccharide (*E. coli* 055:B5 [LPS] from Sigma Chemicals Co.) is then added (100 ng/ml in 10 ml phosphate buffered saline) and cultures incubated for 16-18 hours at 37°C in a 5% CO_2 incubator. At the end of the incubation period, culture supernatants are removed from the cells, centrifuged at 3000 rpm to remove cell debris. The supernatant is then assayed for TNF activity using either a radio-immuno or an ELISA assay, as described in WO 92/10190 and by Becker *et al.*, J Immunol, 1991, **147**, 4307.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the are can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound of the formula:



(I)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, -OC(O)R_5, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof; excluding the compounds tert-Butyl 7-alpha-methoxycephalosporanate sulfone; tert-Butyl 7-beta-methoxycephalosporanate sulfone; Methyl (6R,7S)-7-Methoxy-3-acetoxymethyl-3-cephem-4-carboxylic acid -1,1-dioxide; and Benzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide.

2. The compound according to Claim 1 wherein R_a is a benzyl moiety optionally substituted independently one or more times by hydroxy, halogen, alkyl, or alkoxy.

3. The compound according to Claim 1 wherein R_a is methyl or t-butyl.

4. The compound according to Claim 1 wherein the R_1 moiety is an optionally substituted alkoxy moiety.

5. The compound according to Claim 4 wherein the R_1 alkoxy is methoxy or 2-hydroxyethoxy.

6. The compound according to Claim 1 wherein m is 2.

7. The compound according to Claim 1 wherein R_3 is $S(O)_n R_6$.

8. The compound according to Claim 7 wherein R_6 is a heteroaryl which is an optionally substituted tetrazole, triazole, or oxadiazole.

9. The compound according to Claim 1 wherein R_3 is hydrogen.

10. The compound according to Claim 1 which is:
tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

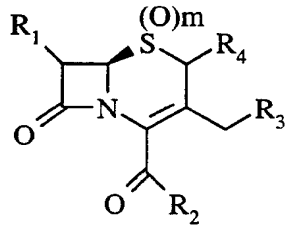
3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

11. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable carrier or diluent.

12. A pharmaceutical composition comprising a compound according to Claim 10 and a pharmaceutically acceptable carrier or diluent.

13. A method of blocking excess or inappropriate apoptosis in a mammal in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of the formula:



(Ia)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and that only one of R_3 and R_4 can be bromine;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;
n is 0, or an integer having a value of 1 or 2;
or a pharmaceutically acceptable salt thereof.

14. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs in Alzheimer disease.

15. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs in viral infections.

16. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs during infarction or reperfusion injury.

17. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs during ischemia.

18. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in excessive bone loss.

19. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in the disease of osteoarthritis.

20. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in hepatocellular degeneration.

21. The method according to Claim 13 wherein the compound is:
tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide

3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

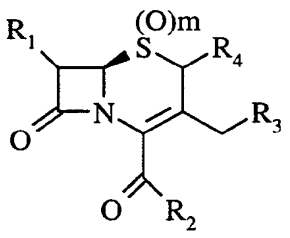
3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

22. A method for the treatment of diseases or disorders associated with excessive IL-1b convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of the formula:



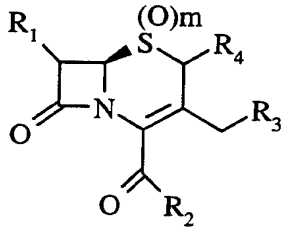
(Ia)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;
R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;
R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and provided that only one of R_3 and R_4 can be bromine;
R_4 is hydrogen;
R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;
R_6 is optionally substituted aryl, or optionally substituted heteroaryl;
m is an integer having a value of 1 or 2;
n is 0, or an integer having a value of 1 or 2;
or a pharmaceutically acceptable salt thereof.

23. A method of blocking or decresing the production of IL-1b and/or TNF, in a mammal in need of such treatment, which method comprises administering to said mammal an effective amount of a compound of the formula:



(Ia)

wherein
R_1 is hydrogen, an optionally substituted alkoxy or halogen;
R_2 is OR_a;
R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;
R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and that only one of R_3 and R_4 can be bromine;
R_4 is hydrogen;
R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;
R_6 is optionally substituted aryl, or optionally substituted heteroaryl;
m is an integer having a value of 1 or 2;
n is 0, or an integer having a value of 1 or 2;
or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13967

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 501/00; A61K31/545
US CL :Please See Extra Sheet.
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. : 540/215, 226, 229, 230;
514/204, 208, 209, 200

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)

STN-CAS-on line
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 -- Y	US, A,5,446,037 A(MAITI et al) 29 August 1995, column 3 and claim 1 when R4, here, is other than hydrogen.	1-23 1-23
X -P Y	Chemical Abstract, vol. 124, no.5. 29 January 1996(Columbus,OH,USA)page 1203,column 1,the abstract no.55675h. Alpengiani et al. WIPO document no. 94/28003, 08 December 1994.	1-23
X -- Y	Chemical Abstract, vol. 122,no.3.16 January 1995(Columbus OH. USA)page 905,column 1,the abstract no 31161n, Alpegiani et al., "Cephem Sulfones as Inactivators of Human Leukocyte Elastase", J. Med. Chem. (1994), vol. 37(23), pages 4003-19.	1-23 1-23

☐ 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 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 12 DECEMBER 1996	Date of mailing of the international search report 03 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN M. FORD aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13967

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

540/215, 226, 229, 230;
514/204, 208, 209, 200

Electronic Acknowledgement Receipt

EFS ID:	14287044
Application Number:	11330868
International Application Number:	
Confirmation Number:	9998
Title of Invention:	BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS
First Named Inventor/Applicant Name:	Jason Edward Brittain
Customer Number:	46347
Filer:	Stephanie A. Barbosa/Viantinna Campana Bordas
Filer Authorized By:	Stephanie A. Barbosa
Attorney Docket Number:	CP391
Receipt Date:	21-NOV-2012
Filing Date:	12-JAN-2006
Time Stamp:	11:41:14
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	Transmittal Letter	CEPH-4391_SIDS_Trans_11-21-12.PDF	103831 e1a6d3dfc23324ab026dacc52baf5daa46caa7a2	no	3

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	CEPH-4391_SIDS_1449_11-21-12.PDF	121564 eca834bff97f85ad8420df394c4d3db7b7e5a5eb	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	EP_0780386.PDF	4879857 f206388169ee852d734596624362ead5bde06ea3	no	86
Warnings:					
Information:					
4	Foreign Reference	WO_97-08174.PDF	1928731 314ad9926d94be44d9567d2029bb6d5d53ba5721	no	40
Warnings:					
Information:					
5	Non Patent Literature	DHHS_FDA_ICH_GuidanceOnImpurities-ResidualSolvents_FederalRegister_1997_67377-67388.PDF	161538 76ab07042edb5580fd0fffde78b76895d07a213b	no	12
Warnings:					
Information:					
		Total Files Size (in bytes):	7195521		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
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.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
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.

DOCKET NO.: CEPH-4391 / CP391 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Jason Edward Brittain **Confirmation No.: 9998**

Application No.: 11/330,868 **Group Art Unit: 1617**

Filing Date: January 12, 2006 **Examiner: Soroush, Ali**

For: BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

Filed Via EFS

INFORMATION DISCLOSURE STATEMENT

Pursuant to 37 CFR § 1.56 and in accordance with 37 CFR §§ 1.97-1.98, information relating to the above-identified application is hereby disclosed. Inclusion of information in this statement is not to be construed as an admission that this information is material as that term is defined in 37 CFR § 1.56(b).

☒ **IDS Filed Under 37 CFR 1.97(b)**

In accordance with § 1.97(b), since this Information Disclosure Statement is being filed either within three months of the filing date of the above-identified application, within three months of the date of entry into the national stage of the above identified application as set forth in § 1.491, before the mailing date of a first Office Action on the merits of the above-identified application, or before the mailing date of a first Office Action after the filing of request for continued examination under § 1.114, no additional fee is required.

☐ **IDS filed Under 37 CFR 1.97(c)**

In accordance with § 1.97(c), this Information Disclosure Statement is being filed after the period set forth in § 1.97(b) above but before the mailing date of either a Final Action under § 1.113 or a Notice of Allowance under § 1.311, or before an action that otherwise closes prosecution in the application, therefore:

☐ Certification in Accordance with § 1.97(e) is attached; or

☐ The fee of **$180.00** as set forth in § 1.17(p) is attached.

☐ **IDS filed Under 37 CFR 1.97(d)**

In accordance with § 1.97(d), this Information Disclosure Statement is being filed after the mailing date of either a Final Action under § 1.113 or a Notice of Allowance under § 1.311 but before, or simultaneously with, the payment of the Issue Fee, therefore included are: Certification in Accordance with § 1.97(e); and the submission fee of **$180.00** as set forth in § 1.17(p).

CONTENT OF IDS PURSUANT TO 37 CFR 1.98

☐ Copies of reference numbers listed on the attached Form 1449/PTO or Substitute for Form 1449/PTO are not required to be submitted pursuant to 37 CFR § 1.98(a)(2)(iii).

☒ Copies of reference numbers 95-97 listed on the attached Form 1449/PTO or Substitute for Form 1449/PTO are enclosed herewith.

☐ Copies of reference numbers are not being submitted because they were previously cited by or submitted to the U.S. Patent and Trademark Office in patent application number , filed for which a claim for priority under 35 U.S.C. § 120 has been made in the instant application.

☐ The month of publication for reference numbers is not available. However, the year of publication for these references is sufficiently earlier than the effective US filing date and any foreign priority date so that the particular month of publication is not in issue pursuant to 37 CFR § 1.98(b).

REFERENCES IN A LANGUAGE OTHER THAN ENGLISH

☐ The following documents are not in the English language. Accordingly, a concise explanation of the relevance of the document was incorporated in the specification passages identified below, the document was identified in a foreign communication as identified below or an English language counterpart application has been provided as indicated below.

Foreign Language Document	Cite No.	Pages of Reference in Specification or Relevance of Document

Foreign Language Document	Cite No.	English Language Counterpart	Cite No.

☐ **CERTIFICATION IN ACCORDANCE WITH § 1.97(e)**

I hereby certify that:

☐ Each item of information contained in this information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

☐ No item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in this information disclosure statement was known to any individual designated in § 1.56(c) more than three months prior to the filing of this information disclosure statement.

Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

Date: November 21, 2012

/Stephanie A. Barbosa/
Stephanie A. Barbosa
Registration No. 51,430

WOODCOCK WASHBURN LLP
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2929 Arch Street, 12th Floor
Philadelphia, PA 19104-2891
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

46347 7590 02/04/2013
WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891

EXAMINER
SOROUSH, ALI

ART UNIT	PAPER NUMBER
1617	

DATE MAILED: 02/04/2013

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/330,868	01/12/2006	Jason Edward Brittain	CP391	9998

TITLE OF INVENTION: BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1770	$300	$0	$2070	05/06/2013

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

46347 7590 02/04/2013
WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/330,868	01/12/2006	Jason Edward Brittain	CP391	9998

TITLE OF INVENTION: BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1770	$300	$0	$2070	05/06/2013

EXAMINER	ART UNIT	CLASS-SUBCLASS
SOROUSH, ALI	1617	548-304700

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/330,868	01/12/2006	Jason Edward Brittain	CP391	9998

46347 7590 02/04/2013

WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891

EXAMINER	
SOROUSH, ALI	
ART UNIT	**PAPER NUMBER**
1617	

DATE MAILED: 02/04/2013

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 802 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 802 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No.	Applicant(s)	
	11/330,868	BRITTAIN ET AL.	
	Examiner	Art Unit	
	ALI SOROUSH	1617	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the IDS submissions of 11/15/2012 and 11/21/2012*.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *83-91*.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 11152012, 11212012
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/ALI SOROUSH/
Primary Examiner, Art Unit 1617

DETAILED ACTION

Claim Status

Claims 83-91 are pending.

Claims 31, 32, and 78-82 are cancelled and 1-30 and 33-77 were previously cancelled.

Claims 83-91 have been examined.

Claims 83-91 are rejected.

Priority

Priority to application 60/644,354 filed on 01/14/2005 is acknowledged.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 11/15/2012 and 11/21/2012 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements have been considered by the examiner.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: the prior art teaches a formulation of bendamustine and mannitol to be lyophilized. The prior art also teach a combination of mannitol, tertiary-butyl alcohol, water, and an anti-neoplastic agent can be lyophilized. The prior art suggests using a combination of mannitol and tertiary-butyl alcohol with bendamustine to produce a formulation to be lyophilized. However, Applicant has unexpectedly found that the addition of tertiary-butyl alcohol stabilizes the formulation such that bendamustine degradation is negiligable (no more than 0.5% formation of bendamustine ethyl ester). Therefore, claims 83-91 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Claims 83-91 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALI SOROUSH whose telephone number is (571)272-9925. The examiner can normally be reached on M-F (9am-6pm).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on (571)272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ALI SOROUSH/
Primary Examiner, Art Unit 1617

January 27, 2013

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11330868	BRITTAIN ET AL.
	Examiner	Art Unit
	ALI SOROUSH	1616

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
34	284	08/20/2012	AS
548	304.7	08/20/2012	AS

SEARCH NOTES		
Search Notes	Date	Examiner
see search history printouts	08/20/2012	AS
Inventor/Assignee search EAST/PALM (Jason Edward Brittain, Joe Craig Franklin, Cephalon, Inc.)	08/20/2012	AS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
34	284	08/20/2012	AS
548	304.7	08/20/2012	AS

/ALI SOROUSH/ Primary Examiner.Art Unit 1617	

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BIB DATA SHEET

CONFIRMATION NO. 9998

SERIAL NUMBER	FILING or 371(c) DATE / RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/330,868	01/12/2006	548	1617	CP391

APPLICANTS

Jason Edward Brittain, El Cajon, CA;
Joe Craig Franklin, Tulsa, OK;

**** CONTINUING DATA ***************************

This appln claims benefit of 60/644,354 01/14/2005

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

02/27/2006

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /ALI SOROUSH/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CA	6	78	21

ADDRESS

WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891
UNITED STATES

TITLE

BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

FILING FEE RECEIVED		
0.00	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	2	treanda	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:41
S2	0	bendamustine same (lyophilize lyphilized)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:41
S3	10	bendamustine and (lyophilize lyphilized)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:42
S4	46	bendamustine and (lyophilize lyphilized freeze$1dried)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:42
S5	3	bendamustine same (lyophilize lyphilized freeze$1dried)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:42
S6	88851	lyophilize lyophilization freeze$dry freeze$dried free$1drying	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:56
S7	22	S6 same (alkylating adj agent)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 19:57
S8	2	bendamustine same (aqueous adj	US-PGPUB;	OR	ON	2010/08/14

		solution) same unstable	USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			20:03
S9	0	"cephalon.in"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:04
S10	563	cephalon.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:05
S11	11	S10 and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:05
S12	4	bendamustine same (aqueous adj solution)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:06
S13	458	bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:06
S14	30	bendamustine adj hydrochloride	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:06
S15	58	bendamustine same injection	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:07
S16	18	bendamustine same solid	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	2010/08/14 20:12

			IBM_TDB			
S17	2	bendamustine same unstable	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:13
S18	2	"0656211"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/14 20:29
S19	0	"0656211"	EPO	OR	ON	2010/08/14 20:29
S20	610	ku.in.	EPO	OR	ON	2010/08/14 20:29
S21	1	S20 and thiotepa	EPO	OR	ON	2010/08/14 20:30
S22	0	"5330835".pn.	EPO	OR	ON	2010/08/17 12:07
S23	2	"5330835".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/17 12:08
S24	3	"4145400".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/17 12:10
S25	3	"4145440".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/17 12:10
S26	1	10/417631.app.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2010/08/24 13:13
S27	0	benadmustine with mannitol with alcohol	EPO	OR	ON	2011/04/22 20:07
S28	0	benadmustine	EPO	OR	ON	2011/04/22 20:07
S29	11	bendamustine ribomustin treanda "SDX-105" bendamustin Cytostasan "IMET 3393" "Zimet 3393" "4-[5-[Bis(2-chloroethyl)amino]-1-	EPO	OR	ON	2011/04/22 20:20

		methylbenzimidazol-2-yl]butanoic acid" "16506-27-7"				
S30	775	bendamustine ribomustin treanda "SDX-105" bendamustin Cytostasan "IMET 3393" "Zimet 3393" "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" "16506-27-7"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:20
S31	10	S30 with mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S32	13	S30 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S33	13	S30 with alcohol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:21
S34	22	S30 same alcohol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:22
S35	23	S30 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:24
S36	345	S30 and mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:36
S37	52	S36 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:38
S38	108	(mannitol "(2R,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol" Osmitrol Osmofundin) with (t-Butanol 2-Methyl-	US-PGPUB; USPAT; USOCR;	OR	ON	2011/04/22 20:44

		2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	FPRS; EPO; JPO; DERWENT; IBM_TDB			
S39	31	S38 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:44
S40	2	"5362718".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:52
S41	1	S30 same (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:55
S42	15	S30 and (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:55
S43	18	S30 with rapamycin	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 20:56
S44	23	S30 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:01
S45	6	S30 same (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:01
S46	132	S30 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	2011/04/22 21:01

		propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	JPO; DERWENT; IBM_TDB			
S47	299	(mannitol "(2R,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol" Osmitrol Osmofundin) same (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:02
S48	7	S47 and S30	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:02
S49	65	cyclophosphamide with mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:07
S50	17	S49 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:07
S51	0	S50 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:12
S52	17166	(nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S53	113050	S52 sme (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S54	6	S52 same (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR;	OR	ON	2011/04/22 21:14

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S55	2335	S52 and (lyophilization lyophilize freeze$1dry freeze$1drying)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:14
S56	4	S35 and (t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:15
S57	3	S30 same tablet	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:18
S58	60242	(t-Butanol 2-Methyl-2-propanol ((t-Butyl tert-Butyl tertiary-Butyl) adj alcohol) 1,1-Dimethylethanol Dimethylethanol tert-Butanol 2-Methyl-propan-2-ol (Trimethyl adj carbinol) Trimethylcarbinol 2-metilpropan-2-ol Trimethylmethanol "2-Propanol, 2-methyl-")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:22
S59	81388	lyophilization lyophilize freeze$1dry freeze$1drying	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:22
S60	477	S58 same S59	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:22
S61	52	S60 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:23
S62	7	chlorambucil same lyophilization	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	2011/04/22 21:41

			IBM_TDB			
S63	49972	freeze$1dry freez$1drying lyophilisation lyophilization cryodesiccation	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:45
S64	82	S63 and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:45
S65	6	S38 and S64	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:46
S66	13	S30 with water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:48
S67	10	fishman.in. and K4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:50
S68	0	fishman.in. and S30	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:50
S69	2	"20020102215"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 21:53
S70	986	brittain.in. franklin.in. and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/22 22:53
S71	2	(brittain.in. franklin.in.) and bendamustine	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	2011/04/22 22:54

			JPO; DERWENT; IBM_TDB			
S72	0	"4670262".pn.	EPO	OR	ON	2011/04/25 11:15
S73	2	"4670262".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:15
S74	626	jenapharm.as. ribosepharm.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:43
S75	0	S74 and (freeze$1dry freez$1drying lypholization lyophilize)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:44
S76	28	S74 and (powder)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 11:44
S77	396	GlOlA.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 15:35
S78	0	S77 and dinitroalanine	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 15:35
S79	4	S77 and dinitroaniline	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2011/04/25 15:35
S80	12	bendamustine "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" Treakisym Ribomustin Treanda "SDX-105"	EPO	OR	ON	2012/08/20 17:07
S81	1158	bendamustine "4-[5-[Bis(2-	US-PGPUB;	OR	ON	2012/08/20

		chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" Treakisym Ribomustin Treanda "SDX-105"	USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			17:09
S82	17	S81 near5 water	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:09
S83	15	S81 near5 (mannitol "(2R,3R,4R,5R)-Hexan-1,2,3,4,5,6-hexol")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:10
S84	19	S81 with (mannitol "(2R,3R,4R,5R)-Hexan-1,2,3,4,5,6-hexol")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:10
S85	203678	"tert-Butanol" "2-methyl-2-propanol" "tertiary-butyl alcohol" "2-Methylpropan-2-ol" "Dimethylethanol" "1,1-Dimethylethanol" ""tert-butyl alcohol" "t-butyl alcohol" ""1,1-Dimethyl ethanol" "trimethyl carbinol" "t-butyl hydroxide" "trimethyl methanol" "dimethyl ethanol" "methyl-2-propanol"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:15
S86	165922	(mannitol "(2R,3R,4R,5R)-Hexan-1,2,3,4,5,6-hexol")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:15
S87	24	S85 near5 S86	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
S88	107	S85 with S86	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
S89	2	S88 and S81	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	2012/08/20 17:16

			JPO; DERWENT; IBM_TDB			
S90	364	S85 same S86	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
S91	7	S90 and S81	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
S92	7	S81 near5 S85	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:16
S93	8	S81 with S85	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:17
S94	183540	Freeze$1drying lyophilisation lyophilization cryodesiccation lyophilized lyophilize	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:22
S95	516	S94 and S81	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:23
S96	22	S94 same S81	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:23
S97	93	Mundipharma.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:29
S98	0	Mundipharma.as. and S81	US-PGPUB; USPAT;	OR	ON	2012/08/20 17:30

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			
S99	34	S81 same mannitol	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:36
S100	1160	bendamustine "4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid" Treakisym Ribomustin Treanda "SDX-105" "IMET 3393"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 17:53
S101	273	34/284.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
S102	0	34/284.ccls. and S81	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
S103	273	34/284.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:32
S104	2	"5977129".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 18:39
S105	904	548/304.4.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:00
S106	11	S105 and (nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:01

S107	593	548/304.7.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:06
S108	14	S107 and (nitrogen adj mustard)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:06
S109	9	(brittain.in. franklin.in. cephalon.as.) and bendamustine.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/08/20 19:08

1/27/2013 9:18:13 PM
C:\Users\asoroush\Documents\EAST\Workspaces\11330868.wsp

Substitute for 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(use as many sheets as necessary)*				***Complete if Known***	
				Application Number	11/330,868
				Filing Date	January 12, 2006
				First Named Inventor	Jason Edward Brittain
				Art Unit	1616
				Examiner Name	Ali Soroush
Sheet	1	of	1	**Attorney Docket Number**	CEPH-4391 (CP391US)

U. S. PUBLICATION AND PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number Number – Kind Code (if known)	Publication or Grant Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document
/A.S./	**1**	5,192,743	03-09-1993	Hsu et al.
/A.S./	**2**	5,183,746	02-02-1993	Shaked et al.

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document Country Code- Number -Kind Code (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	T
/A.S./	**3**	WO 2006/065392	06-22-2006	Cephalon, Inc.	

Examiner Signature	/Ali Soroush/	**Date Considered**	01/27/2013

Substitute for 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(use as many sheets as necessary)*				***Complete if Known***	
				Application Number	11/330,868
				Filing Date	January 12, 2006
				First Named Inventor	Jason Edward Brittain
				Art Unit	1617
				Examiner Name	Soroush, Ali
Sheet	1	of	1	**Attorney Docket Number**	CEPH-4391 / CP391

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document Country Code- Number -Kind Code (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	T
/A.S./	**95**	EP 0780386	06-25-1997	F. Hoffmann-La Roche AG	
/A.S./	**96**	WO 97/08174	03-06-1997	Smithkline Beecham Corporation	

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author, title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), Volume-issue Number(s), publisher, city and/or country where published.	T
/A.S./	**97**	Department of Health and Human Services, Food and Drug Administration, “International Conference on Harmonisation; Guidance on Impurities: Residual Solvents,” Federal Register, December 24, 1997, 62(247), 67377-67388	

Examiner Signature	/Ali Soroush/	**Date Considered**	01/27/2013

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11330868	BRITTAIN ET AL.
	Examiner	**Art Unit**
	ALI SOROUSH	1617

CPC		
Symbol	**Type**	**Version**

CPC Combination Sets				
Symbol	**Type**	**Set**	**Ranking**	**Version**

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION	
CLASS	**SUBCLASS**	**CLAIMED**	**NON-CLAIMED**
548	304.7	C 0 7 D 235 / 04 (2006.01.01)	

CROSS REFERENCE(S)					
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**				
34	284				

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/ALI SOROUSH/ Primary Examiner.Art Unit 1617	01/27/2013	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11330868	BRITTAIN ET AL.
	Examiner	**Art Unit**
	ALI SOROUSH	1617

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/ALI SOROUSH/ Primary Examiner.Art Unit 1617	01/27/2013	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11330868	BRITTAIN ET AL.
	Examiner	**Art Unit**
	ALI SOROUSH	1617

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☐ **T.D.** ☐ **R.1.47**

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	83														
2	84														
3	85														
4	86														
5	87														
6	88														
7	89														
8	90														
9	91														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 9	
/ALI SOROUSH/ Primary Examiner.Art Unit 1617 (Primary Examiner)	01/27/2013 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

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CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

46347 7590 02/04/2013
WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891

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Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/330,868	01/12/2006	Jason Edward Brittain	CP391	9998

TITLE OF INVENTION: BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1770	$300	$0	$2070	05/06/2013

EXAMINER	ART UNIT	CLASS-SUBCLASS
SOROUSH, ALI	1617	548-304700

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 Woodcock Washburn LLP

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Cephalon, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Frazer, PA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual XXX Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

XXX Issue Fee

XXX Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

XXX The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number 233050 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature /Stephanie A. Barbosa/ Date April 5, 2013

Typed or printed name Stephanie A. Barbosa Registration No. 51,430

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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Electronic Patent Application Fee Transmittal

Application Number:	11330868
Filing Date:	12-Jan-2006
Title of Invention:	BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS
First Named Inventor/Applicant Name:	Jason Edward Brittain
Filer:	Stephanie A. Barbosa/Ann Trevisani
Attorney Docket Number:	CP391

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	1780	1780
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**2080**

Electronic Acknowledgement Receipt

EFS ID:	15445344
Application Number:	11330868
International Application Number:	
Confirmation Number:	9998
Title of Invention:	BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS
First Named Inventor/Applicant Name:	Jason Edward Brittain
Customer Number:	46347
Filer:	Stephanie A. Barbosa/Ann Trevisani
Filer Authorized By:	Stephanie A. Barbosa
Attorney Docket Number:	CP391
Receipt Date:	05-APR-2013
Filing Date:	12-JAN-2006
Time Stamp:	14:41:41
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$2080
RAM confirmation Number	1008
Deposit Account	233050
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Issue_Fee_Transmittal_CP391_US.PDF	1396391 44ddff871ce138ec506000c60267ec1ac137ea70	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32091 81dd640a75a8bd6cb31296f1a80d4a9246dbafb3	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1428482		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
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.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
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.

FORM PTO-1449 **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** List of Patent and Publications Cited by Applicant	Attorney Docket	CP391
	Application Number	11/330,868
	Filing Date	January 12, 2006
	First Named Inventor	Brittain
	Group Art Unit	1616
	Examiner Name	A. Soroush
	Sheet	2 of 5

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Document No.	Date	Country	Translation YES	Translation NO
/A.S./	B1	DD 159289	Jun. 1, 1981	Germany	x	
	B2	DD 159877	Apr. 13, 1983	Germany	x	
	B3	DD 293808	Sep. 12, 1991	Germany		x
	B4	DE 80967	Jun. 1, 1970	Germany	x	
	B5	DE 10016077	Dec. 13, 2001	Germany		x
	B6	DE 10304403	Aug. 5, 2004	Germany	A27	
	B7	DE 10306724	Sep. 18, 2003	Germany		x
	B8	EP 1354952	Oct. 22, 2003	Germany		
	B9	EP 1444989	Aug. 11, 2004	Italy		
	B10	WO 96/28148	~~Mar. 13, 1998~~	~~Australia~~		
	B11	WO 03/066027 A1	Feb. 7, 2003	PCT		
	B12	WO 03/081238	Oct. 2, 2003	PCT		
	B13	WO 03/086470 A3	May 6, 2004	PCT		
	B14	WO 03/094990	Mar. 11, 2003	PCT	A26	

Change(s) applied to document, /N.W.S./ 10/2/2012

1996-09-19

OTHER DOCUMENTS Non-Patent Literature Documents

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), Title of Article, Title of Journal (book, magazine, catalog, etc.) Date, Pertinent Pages, Volume-Issue Number, publisher, city and/or country where published.
/A.S./	C1	AIVADO, MANUEL et al., *Bendamustine in the treatment of chronic lymphocytic leukemia: Results and future perspectives*, Seminars in Oncology, **2002**, pp. 19-22, Vol. 29 No. 4, Suppl. 13.
/A.S./	C2	BARMAN BALFOUR, JULIA A. et al., *Bendamustine*, Drugs, **2001**, pp. 631-638, Vol. 61(5), Auckland, New Zealand
/A.S./	C3	BREMER, KARL, *High rates of long-lasting remissions after 5-day bendamustine chemotherapy cycles in pre-treated low-grade non-hodgkin's-lymphomas*, Journal of Cancer Research and Clinical Oncology, **2002**, pp.603-609, Vol. 128(11).

Examiner's Signature	/Ali Soroush/	**Date:**	08/14/2009

FORM PTO-1449

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

List of Patent and Publications Cited by Applicant

Attorney Docket	CP391
Application Number	11/330,868
Filing Date	January 12, 2006
First Named Inventor	Brittain
Group Art Unit	1616
Examiner Name	A. Soroush
Sheet	1 of 5

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number	Name	Date of Publication	Class	Subclass	
/A.S./	A1	US-5204335	Sauerbier et al.	Apr. 20, 1993			
	A2	US-5227373	Alexander et al.	Jul. 13, 1993			
	A3	US-5750131	Wichert et al.	May 12, 1998			
	A4	US-5770230	Teagarden et al.	Jun. 23, 1998			
	A5	US-5776456	Anderson et al.	Jul. 7, 1998			
	A6	US-5955504	Wechter et al.	Sep. 21, 1999			
	A7	US-5972912	Marek et al.	Oct. 26, 1999			
	A8	US-6034256	~~Masferrer~~	Mar. 7, 2000			Carter, et al.
	A9	US-6077850	~~Masferrer~~	Jun. 20, 2000			Carter, et al.
	A10	US-6090365	Kaminski et al.	Jul. 18, 2000			
	A11	US-6271253 B1	~~Masferrer~~	Aug. 7, 2001			Carter, et al.
	A12	US-6380210	Desimone et al.	Apr. 30, 2002			
	A13	US-6492390 B2	~~Masferrer~~	Dec. 12, 2002			Carter, et al.
	A14	US-6545034 B1	Carson et al.	Apr. 8, 2003			
	A15	US-6569402	Cheesman et al.	May 27, 2003			
	A16	US-6573292 B1	Nardella	Jun. 3, 2003			
	A17	US-6613927 B1	Kwok	Sep. 2, 2003			
	A18	US-2003/0232874	Nardella	Dec 18, 2003			
	A19	US-2004/0053972	Nara	Mar. 18, 2004			
	A20	US-2004/0058956	Akiyama et al.	Mar. 25, 2004			
	A21	US-2004072889	Masjerrer	Apr. 15, 2004			
	A22	US-2004/0096436 A1	Carson et al.	May 20, 2004			
	A23	US-2004152672	Carson et al.	Aug. 5, 2004			
	A24	US-2004/0247600	Leoni	Dec. 9, 2004			
	A25	US-2005/0060028 A1	Horres et al.	Mar. 17, 2005			
	A26	US-2005/0176678	Horres et al.	Aug 11, 2005			
	A27	US-2006/0051412	Petereit et al.	Mar 9, 2006			

Change(s) applied to document, /N.W.S./ 10/2/2012

Examiner's Signature	/Ali Soroush/	Date:	08/14/2009

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/330,868	05/07/2013	8436190	CP391	9998

46347 7590 04/17/2013

WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STRET
PHILADELPHIA, PA 19104-2891

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 1748 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Jason Edward Brittain, El Cajon, CA;
Joe Craig Franklin, Tulsa, OK;

AO 120 (Rev. 08/10)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/26/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
CEPHALON, INC.	GLENMARK PHARMACEUTICALS LTD., GLENMARK GENERICS LTD., GLENMARK GENERICS S.A. and GLENMARK GENERICS INC., USA,

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	CEPHALON, INC.
2	8,436,190	5/7/2013	CEPHALON, INC.
3			
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

Copy 1—Upon initiation of action, mail this copy to Director **Copy 3—Upon termination of action, mail this copy to Director**
Copy 2—Upon filing document adding patent(s), mail this copy to Director **Copy 4—Case file copy**

AO 120 (Rev. 08/10)

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DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/26/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
CEPHALON, INC.	HOSPIRA, INC.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	US 8,445,524 B2	5/21/2013	CEPHALON, INC.
2	US 8,436,190 B2	5/7/2013	CEPHALON, INC.
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AO 120 (Rev. 08/10)

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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/26/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
CEPHALON, INC.	SUN PHARMA GLOBAL FZE, SUN PHARMACEUTICAL INDUSTRIES LTD., and SUN PHARMACEUTICAL INDUSTRIES, INC.,

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	US 8,445,524 B2	5/21/2013	CEPHALON, INC.
2	US 8,436,190 B2	5/7/2013	CEPHALON, INC.
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AO 120 (Rev. 08/10)

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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/31/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
Cephalon, Inc.	Sandoz Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	Cephalon, Inc.
2	8,436,190	5/7/2013	Cephalon, Inc.
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

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AO 120 (Rev. 08/10)

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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/20/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
Cephalon, Inc.	Dr. Reddy's Laboratories, Ltd. and Dr. Reddy's Laboratories, Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	Cephalon, Inc.
2	8,436,190	5/7/2013	Cephalon, Inc.
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AO 120 (Rev. 08/10)

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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/20/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
Cephalon, Inc.	Innopharma, Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	Cephalon, Inc.
2	8,436,190	5/7/2013	Cephalon, Inc.
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DECISION/JUDGEMENT

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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	12/20/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
Cephalon, Inc.	Agila Specialties Inc. f/k/a/ Strides, Inc. and Onco Therapies Limited

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	Cephalon, Inc.
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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	1/31/2014	for the District of Delaware

PLAINTIFF	DEFENDANT
CEPHALON, INC.	ACTAVIS LLC, f/k/a ACTAVIS INC. AND ACTAVIS ELIZABETH LLC

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	US 8,445,524 B2	5/21/2013	Cephalon, Inc.
2	US 8,436,190 B2	5/7/2013	Cephalon, Inc.
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
13-2095-GMS	12/26/2013	for the District of Delaware

PLAINTIFF	DEFENDANT
CEPHALON, INC.	ACCORD HEALTHCARE, INC. and INTAS PHARMACEUTICALS LTD.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,445,524	5/21/2013	CEPHALON, INC.
2	8,436,190	5/7/2013	CEPHALON, INC.
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY
4/9/2014	☑ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	8,609,863	12/17/2013	CEPHALON, INC.
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

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