US007329644B2

(12) **United States Patent**
Saviano et al.

(10) **Patent No.: US 7,329,644 B2**
(45) **Date of Patent: Feb. 12, 2008**

(54) **PREPARATION AND USE OF CYCLIC AND BRANCHED PEPTIDES AND THEIR LABELLED DERIVATIVES AS THERAPEUTIC AGENTS, CHOLECYSTOKININ AGONISTS OR ANTAGONISTS, AND DIAGNOSTIC AGENTS TO IDENTIFY AND LOCATE TUMOURS**

(75) Inventors: **Michele Saviano**, Milan (IT); **Stefania De Luca**, Milan (IT); **Giancarlo Morelli**, Milan (IT); **Diego Tesauro**, Milan (IT); **Carlo Pedone**, Milan (IT)

(73) Assignee: **Bracco Imaging S.p.A.**, Milan (IT)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 188 days.

(21) Appl. No.: **10/479,096**

(22) PCT Filed: **May 21, 2002**

(86) PCT No.: **PCT/EP02/05562**

§ 371 (c)(1),
(2), (4) Date: **Jul. 16, 2004**

(87) PCT Pub. No.: **WO02/094873**

PCT Pub. Date: **Nov. 28, 2002**

(65) **Prior Publication Data**

US 2004/0254339 A1 Dec. 16, 2004

(30) **Foreign Application Priority Data**

May 22, 2001 (IT) MI2001A1057

(51) **Int. Cl.**
A61K 38/00 (2006.01)

(52) **U.S. Cl.** **514/9**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

DE	276482	10/1988
EP	0960939	1/1999
WO	WO92/18627	10/1992
WO	WO97/44341	11/1997

OTHER PUBLICATIONS

Robert T. Jensen, "Involvement of Cholecystokinin/Gastrin-Related Peptides and their Receptors in Clinical Gastrointestinal Disorders," Pharmacology & Toxicology, vol. 91, p. 333.*

Rudinger (J. Rudinger. In: Peptide Hormones, JA Parsons, Ed. (1976) 1-7).*

Sigma (Sigma. Designing Custom Peptides. http://www.sigma-genosys.com/peptide_design.asp (Accessed Dec. 16, 2004), 2 pages).*

Berendsen (H..J.C. Berendsen. A Glimpse of the Holy Grail? Science (1998) 282, pp. 642-643).*

Voet (D. Voet and J.G. Voet. Biochemistry, 2nd Edition. (1995), pp. 235-241).*

Robert T. Jensen, "Involvement of Cholecystokinin/Gastrin-Related Peptides and their Receptors in Clinical Gastrointestinal Disorders," Pharmacology & Toxicology, vol. 91, p. 333.*

Sporn et at, "Chemoprevention of Cancer," Carcinogenesis, vol. 21 (2000), 525-530.*

Gura, "Cancer Models: Systems for Identifying New Drugs Are Often Faulty," Science vol. 278 (1997), 1041-1042.*

Kwekkeboom, et al., "Cholecystokinm receptor imaging using an octapeptide DTPA-CCK analouge in patients with medullary thyroid carcinoma", European Journal of Nuclear Medicine, vol. 27, No. 9, Sep. 2000, pp. 1312-1317.*

PCT International Search Report for PCT/EP02/05562 dated Mar. 31, 2003.

PCT International Preliminary Examination Report for PCT/EP02/05562 dated Jul. 4, 2003.

Romani, S., et al.; "Synthesis of the trypsin fragment 10-25/75-88 of mouse nerve growth factor", Int. J. Peptide Protein Res. 29, 1987, 101-117.

Schaffhausen, et al.; "Antibody to the Nonapeptide Glu-Glu-Glu-Gly-Tyr-Met-Pro-Met-Glu Is specific for Polyoma Middle T Antigen and Inhibits in Vitro Kinase Activity", The Journal of Biological Chemistry, vol. 257, No. 21, Nov. 10, 1982, pp. 12467-12470.

Kwekkeboom, et al.; "Cholecystokinin receptor imaging using an octapeptide DTPA-CCK analouge in patients with medullary thyroid carcinoma", European Journal of Nuclear Medicine, vol. 27, No. 9, Sep. 2000, pp. 1312-1317.

Behr, et al.; "Targeting of cholecystokinin-B/gastrin receptors in vivo: preclinical and initial clinical evaluation of the diagnostic and therapeutic potential of radiolabelled gastrin", European Journal of Nuclear Medicine, vol. 25, No. 4, Apr. 1998, pp. 424-430.

* cited by examiner

Primary Examiner—Anish Gupta
Assistant Examiner—Thomas S. Heard
(74) *Attorney, Agent, or Firm*—Kramer Levin Naftalis & Frankel LLP

(57) **ABSTRACT**

This patent application describes the preparation of cyclic and branched peptides of general formula (I) and their conjugated derivatives labelled with a paramagnetic or radioactive metal. The compounds of the present invention are used as diagnostic agents to identify and locate primary tumours and their metastases which over-express type A and/or B cholecystokinin receptors, and as therapeutic agents and cholecystokinin agonists or antagonists.

18 Claims, 3 Drawing Sheets

MAIA Exhibit 1010

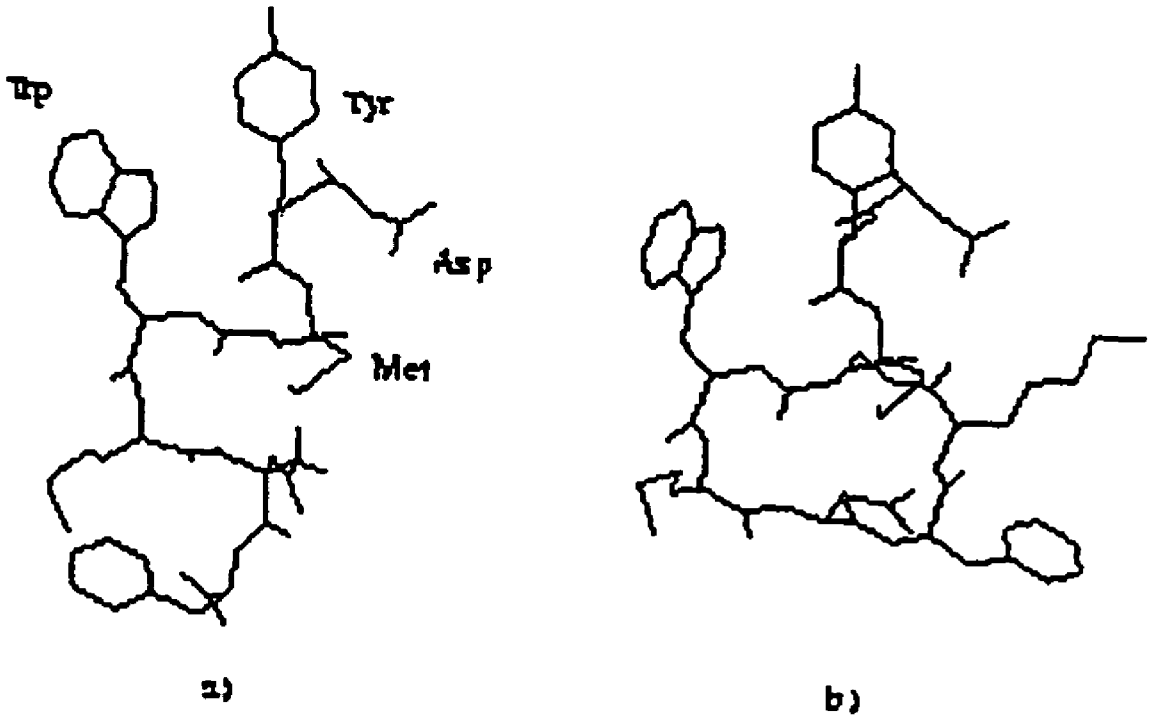


Figure 1

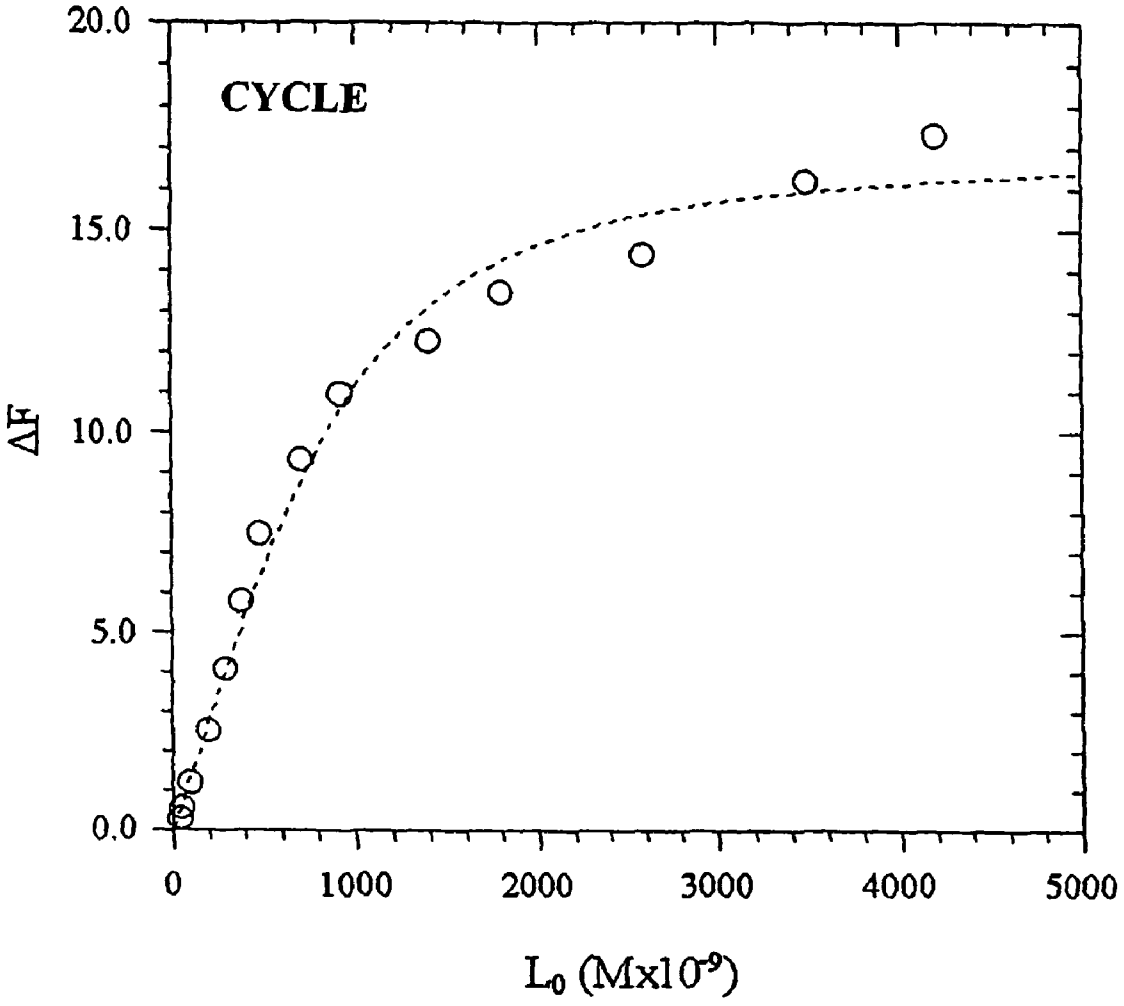


Figure 2

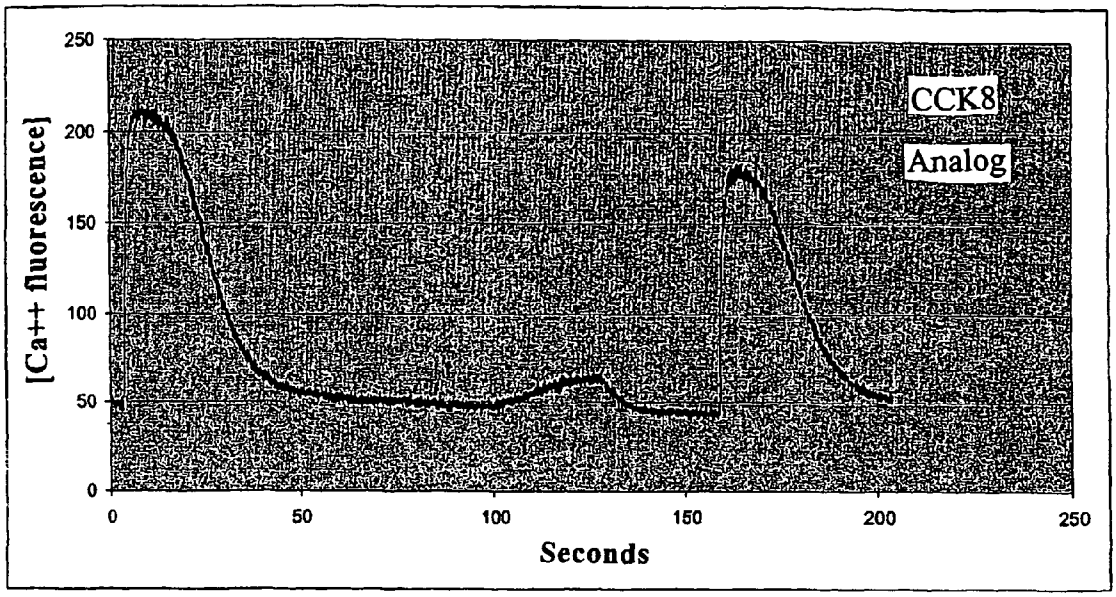


Figure 3

PREPARATION AND USE OF CYCLIC AND BRANCHED PEPTIDES AND THEIR LABELLED DERIVATIVES AS THERAPEUTIC AGENTS, CHOLECYSTOKININ AGONISTS OR ANTAGONISTS, AND DIAGNOSTIC AGENTS TO IDENTIFY AND LOCATE TUMOURS

The present invention relates to cyclic and branched peptides of general formula (I) and their derivatives conjugated with a spacer molecule Y and a chelating agent C, labelled with a paramagnetic or radioactive metal.

The compounds of the invention are used as diagnostic agents to identify and locate primary human tumours and their metastases which over-express type A and/or type B cholecystokinin receptors, and as therapeutic agents and cholecystokinin agonists or antagonists.

Cholecystokinins (CCKs) are a family of peptide molecules whose biological action is performed as a hormone and a neurotransmitter. All the CCKs originate from a process of fragmentation which takes place on a pre-hormone consisting of 115 amino acid residues, followed by a post-translational process of alpha-amidation of the C-terminal phenylalanine residue and sometimes, sulphation of the tyrosine residue contained in the C-terminal portion. The cholecystokinins therefore exist in various molecular forms; the most important ones have a sequence of 58, 39, 33 or 8 amino acid residues, and they all have the same C-terminal sequence of 8 amino acid residues:

Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-amide.

The form containing this sequence only is known as CCK8.

The biological activity of cholecystokinin depends on the type of receptor with which it interacts. Two types of receptor are known: type A and type B. In non-pathological situations, type A receptor is present in the tissues of peripheral organs such as the stomach, gall bladder, intestine and pancreas. The most important physiological actions due to the interaction of the CCK peptide hormone with type A receptor are contraction of the gall bladder, secretion of pancreatic enzymes, regulation of secretion, and absorption into the gastrointestinal tract. Type B receptor is mainly present in the central nervous system, where the interaction with cholecystokinin causes analgesia, satiety and anxiety, and regulates the release of dopamine.

Both the cholecystokinin receptors belong to the class of G-Protein Coupled Receptors (GPCRs), membrane receptors with seven transmembrane helixes joined by intra- and extra-cellular loops with an extracellular N-terminal arm and an intracellular C-terminal part. Both receptors have high affinities for the various forms of cholecystokinin; however, type A receptor has a greater affinity for the sulphated forms of cholecystokinin, namely the ones which contain a sulphuric group on the Tyr 27 residue, while type B receptor has a high affinity for the various forms of non-sulphated cholecystokinin and for gastrin. A series of peptide and non-peptide cholecystokinin-analog molecules with agonist or antagonist activity for type A and type B receptors are known (P. De Tullio, Current Medicinal Chemistry, 6, 433, 1999; F. Noble, Progress in Neurobiology, 58, 349, 1999). No pharmacological application has been found for any of the known molecules due to their low bioavailability and low solubility or high enzymatic degradation.

Cholecystokinin receptors have recently been identified in primary human tumours and metastases (J. C. Reubi, Cancer

(Biochemical Journal, 89, 114-123, 1963), ^{111}In or ^{115}In, used in nuclear medicine to visualise human tumours, is described in particular in that article and in the patent cited by J. C. Reubi.

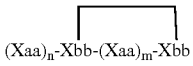
Type A receptor in particular is over-expressed in pancreatic and oesophageal tumours, while type B receptor has been found to be over-expressed in small lung cell tumour, tumours of the colon and gastrointestinal tract, medullary thyroid tumours, astrocytomas and ovarian stromal tumours.

Some peptides deriving from cholecystokinin modified with chelating agents of radioactive or paramagnetic metals have been studied in clinical trials. In particular, CCK8 derivatives containing the chelating agents DTPA or DOTA which complex radioactive metals like ^{111}In and ^{90}Y, and their application to identify and treat tumours that over-express type B cholecystokinin receptor, have been reported (M. De Jong, Journal of Nuclear Medicine, 40, 2082, 1999).

The NMR structure of the complex between the non-sulphated peptide CCK8 and the N-terminal part of type A cholecystokinin receptor, responsible for the interaction with the peptide hormone, was recently published (M. Pellegrini, Biochemistry, 38, 14775, 1999). The N-terminal part of the receptor (receptor fragment) consists of 47 amino acids, and represents the extracellular N-terminal arm and the first part of transmembrane helix 1 of the type A receptor. This fragment does not contain the residue of Arg 197, present on the transmembrane loop, which is responsible for the interaction with the sulphuric group of Tyr 27 of CCK 8, with the result that peptide CCK8 is not used in the sulphated form (V. Gigoux, Protein Science, 8, 2347, 1999). In addition to the detailed structural information indicated in the NMR study, a recent study performed by observing the variations in fluorescence of the tryptophan residues present on the receptor fragment and the peptide confirmed the binding (R. Ragone, Biopolymers, 47-53, 56, 2001, publication pending), and enabled the affinity constant between non-sulphated CCK8 and the receptor fragment to be determined.

DESCRIPTION OF THE INVENTION

The compounds object of the present invention are cyclic peptides of general formula (I):



(I)

wherein:

Xaa, independently of each other, is any amino acid;

Xbb is an alpha or beta amino acid containing at least three functional groups selected from the group consisting of:

—COOH, —NH_2, —SH and —OH,

n is between 0 and 15, and

m is between 2 and 12.

Xbb is preferably selected from the group consisting of:

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.