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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07C 229/36, 237/20, 271/22, C07K 7/06, 14/00, A61K 38/08, 38/16

A1

(11) International Publication Number: WO 96/30332

(43) International Publication Date: 3 October 1996 (03.10.96)

(21) International Application Number: PCT/US96/04311

(22) International Filing Date: 29 March 1996 (29.03.96)

(30) Priority Data:
08/414,520 31 March 1995 (31.03.95) US

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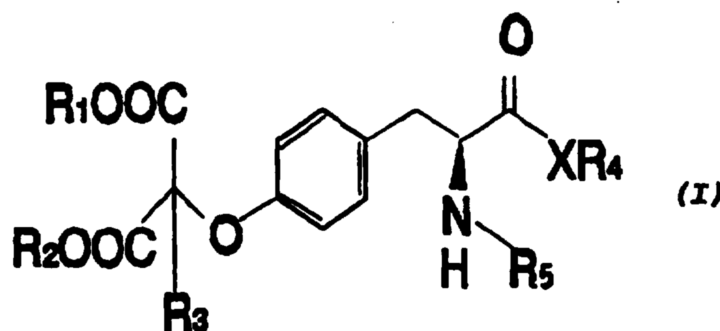
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: O-MALONYLTYROSYL COMPOUNDS, O-MALONYLTYROSYL COMPOUND-CONTAINING PEPTIDES, AND USES THEREOF



(57) Abstract

The present invention relates to non-phosphorous containing O-malonyltyrosyl compounds of general formula (I), derivatives thereof, uses of the O-malonyltyrosyl compounds in the synthesis of peptides, and O-malonyltyrosyl compound-containing peptides. The O-malonyltyrosyl compounds and O-malonyltyrosyl compound-containing peptides of the present invention are uniquely stable to phosphatases, capable of crossing cell membranes, suitable for application to peptide synthesis of O-malonyltyrosyl compound-containing peptides, and amenable to prodrug derivatization for delivery into cells. The present invention also provides for O-malonyltyrosyl compound-containing peptides which exhibit inhibitory potency against binding interactions of receptor domains with phosphotyrosyl-containing peptide ligands. In formula (I), R_1 and R_2 are independentl[illegible] drogen, alkyl, aralkyl, alkaryl, aryl and heteroaryl; R_3 is hydrogen, halogen, amino, hydroxy, and alkoxy; X is nitrogen or oxygen; R_4 [illegible] rogen, alkyl, aralkyl, alkaryl, optionally substituted aryl, and heteroaryl; R_5 is hydrogen, fluorenyl methoxy carbonyl (FMOC), tert-b[illegible] arbonyl (BOC), and carbobenzoxy (CBZ), carbamoyl, alkyl, amido, aryl, and heteroaryl; with the proviso that substituents of formula (I[illegible] hich can be substituted are optionally substituted.

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TITLE OF THE INVENTION

O-MALONYLTYROSYL COMPOUNDS, O-MALONYLTYROSYL COMPOUND-CONTAINING PEPTIDES, AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to non-phosphorus containing O-malonyltyrosyl compounds, derivatives thereof, O-malonyltyrosyl compound-containing peptides, pharmaceutical compositions comprising said peptides, and their use as pharmaceutically active agents. The invention also provides for O-malonyltyrosyl compound-containing peptides which exhibit inhibitory potency against binding interactions of receptor domains and against protein-tyrosine phosphatases.

BACKGROUND OF THE INVENTION

Aberrant cellular signal transduction can cause or accentuate a variety of disease processes including immune dysfunction, cancer, and diabetes. For this reason, cell signaling pathways have become targets for the development of new therapeutic agents (Brugge, J.S., Science 260:918-919 (1993); Brunton, V.G. and Workman, P.; Cancer Chemother Pharmacal 32:1-19 (1993)). One of the most intensely studied areas of cellular signal transduction is the area of phosphotyrosyl-dependent pathways. Particularly important in phosphotyrosyl dependent pathways is the strategic role of phosphotyrosyl (pTyr) residues, which appear to serve as molecular switches that can both activate and inactivate downstream signaling processes. (Panayotou, G. and Waterfield, M.D., Bioessays 15:171-177 (1993)). Binding of ligands to the extracellular domain of growth factor receptors, including the insulin receptor, triggers their intercellular protein-tyrosine kinase (PTK) domains resulting in substrate phosphorylation on tyrosine and further signal transduction. (Brunton, V.G. and Workman, P.; Cancer Chemother Pharmacal 32:1-19 (1993)).

Specifically, signal transduction by pTyr-dependent mechanisms relies on a complex triad of interactions. This

includes PTKs, which generate pTyr residues, frequently in response to external stimuli, such as binding of growth hormones to cell surface receptors. A second signaling component is assumed by protein-tyrosine phosphatases (PTPs), which remove pTyr phosphates, and may play either positive or negative roles in the overall signal transduction. The third leg of the triad is assumed by Src homology 2 (SH2) domain-mediated binding of secondary signaling proteins to pTyr residues contained within protein structures. The actions of PTKs are counterbalanced by protein-tyrosine phosphatases (PTPs) which hydrolyze pTyr phosphate esters (Walton, K.M.; Dixon, J.E., Ann. Rev. Biochem. 62:101-120 (1993), and which would conceptually be expected to act as inhibitory regulators of PTK-mediated signaling. It has previously been suggested that PTPs may also be positive signal effectors in several systems (Tan, Y.H., Science 262:376-377 (1993)). For example, PTPα, CD45 and p80cdc25 are PTPs which can activate the PTKs, $p60^{src}$ and $p56^{lcks}$ and the serine-threonine kinase p34cdc2 (Morla, A.O., et al., Cell 58:193-203 (1989)) respectively, perhaps by dephosphorylating inhibitory pTyr residues. PTPs also appear to be required for the mitogenic effects of some cytokines, such as interleukin-4 (IL-4) (Miresluis, A.R.; Thorpe, R., J Biol Chem 266:18113-18118 (1991)) and for some interferons (Igarashi, K., et al., Mol Cell Biol 13:3984-3989 (1993)). PTPs may also contribute directly to disease processes, as exemplified by the insulin receptor PTK, which is activated by autophosphorylation following binding of insulin to the extracellular ligand-binding domain, and where hydrolysis of these activating pTyr residues by PTPs could potentially exacerbate diabetic conditions (Sale, G.J., Advances in Protein Phosphatases 6:159-186 (1991)).

In spite of the potential value which PTP inhibitors may present for the study of signal transduction pathways and for therapeutic intervention, relatively little has been reported on the development of such agents. Several metal-

containing PTP inhibitors are known, including vanadate, oxovanadium complexes (Posner, B.I., et al., J Biol Chem 269:4596-4604 (1994); Watanabe, H., et al., J. Med. Chem. 37:876-877 (1994)) and gallium nitrate (Berggren, M.M., et al., Cancer Res 53:1862-1866 (1993)) as well as large, highly charged molecules such as suramin (Ghosh, J.; Miller, R.A., Biochem Biophys Res Commun, 194: 36-44 (1993)) and melittin (Errasfa, M.; Stern, A., Eru J. Pharmacal 247:73-80 (1993)). All of these agents would be expected to act in a fairly nonspecific fashion. The search for small molecule inhibitors has recently yielded the nitrosoamine-containing fermentation product "dephostatin" (Imoto, M., et al., J. Antibiot 46:1342-1346 (1993)) and the irreversible suicide inhibitors, 4-difluoromethyl phenylphosphate (Wange, R.L., et al., J. Biol. Chem., 270:944-948 (1995)), however both of these latter compounds could potentially generate highly toxic metabolites.

Another approach toward the design of PTP inhibitors relies on the replacement of pTyr residues in PTP peptide substrates with non-hydrolyzable phosphate mimetics. Phosphonic acids are isosteric with parent phosphates, yet are chemically and enzymatically resistant to P-C bond cleavage, making them valuable phosphate mimetics in a variety of biologically relevant contexts (Blackburn, G.M., Chem. Ind. (London) 134-138 (1981); Engel, R. Phosphonic acids and phosphonates as antimetabolites, in the role of phosphonates in living systems. R.L. Hilderbrand, Editor. 1983, CRC Press, Inc: Boca Raton, Fl. p. 97-138). Phosphonomethyl phenylalanine (Pmp) is a phosphonate-based surrogate of pTyr in which the phosphate ester oxygen has been replaced by a methylene unit. Pmp-containing peptides have previously been shown to act as competitive PTP inhibitors (Chatterjee, S., et al., Peptides: Chemistry and Biology, J.E. Rivier and J.A. Smith, Editor. 1992, Escom Science Publishers: Leiden, Netherland, p. 553-555; Zhang, Z.Y., et al., Biochemistry 33:2285-2290 (1994)). Pmp-

bearing peptides also bind to Src homology 2 (SH2) domains similar to the native pTyr-containing peptides, yet with reduced affinity (Domchek, S.M., et al., Biochemistry 31:9865-9870 (1992)). A Pmp derivative, phosphonodifluoromethyl phenylalanine (F_2Pmp), was previously developed which bears two fluorines substituted at the alpha methylene (Burke, T.R., Jr., et al., J. Org. Chem. 58:1336-1340 (1993); Burke, T.R., Jr., et al., Tetrahedron Lett. 34:4125-4128 (1993); Smyth, M.S., et al., Tetrahedron Lett. 33:4137-4140 (1992)). It has also been shown that the F_2Pmp-containing peptides exhibit enhanced inhibitory potency in PTP assays relative to their Pmp counterparts (Burke, T.R., Jr., et al., Biochem. Biophys. Res. Commun. 204:1148-1153 (1994)). However, F_2Pmp-containing peptides are inadequate for use in pharmaceutical compounds, for although the F_2Pmp moiety is a valuable new motif for the preparation of PTP inhibitors, its di-ionized character at physiological pH makes it resistant to crossing cell membranes. Because of the inability of F_2Pmp-containing peptides to cross cell membranes, tedious microinjections (Xiao, S., et al, J Biol Chem 269:21244-21248 (1994)) or cell permeabilizations (Wange, R.L., et al., J. Biol. Chem., 270:944-948 (1995)) techniques are required. Methods have been reported for the bio-reversible protection of phosphates (for example, Srivastva, D.N.; Farquhar, D., Bioorganic chemistry 12: 118-129 (1984)) and phosphates (for example, Iyer, R.P., et al., Tetrahedron Letters 30:7141-7144 (1989); Freeman, S., et al., J. Chem. Soc. Chem. Commun. 875-877; (1991); Mitchell, A.G., et al., J. Chem. Soc. Perkin Trans. I (1992); Lombaert, S.D., et al., J. Med. Chem. 37:498-511 (1994)), such "prodrug" derivatization is frequently difficult to accomplish and not readily suitable for application to peptide synthesis. Thus, the lability to phosphatases, and the low penetration across cell membranes of the phosphate-containing pTyr pharmacophore, provide two significant limitations to the

therapeutic or pharmacological utility of pTyr-based agents. There is therefore a great need for pTyr mimetics which can be prepared as prodrugs amenable for solid-phase peptide synthesis, and which maintain PTP inhibitory potency when substituted into appropriate peptides.

The use of a malonate pharmacophore to mimic phosphate functionality has been described in an unrelated enzyme system (*See* Marzabadi, M.R., et al., Bioorg. Med. Chem. Lett. 2:1435-40 (1992); Corey, S.D., et al., Bioorg. Med. Chem. Lett. 3:2857-2862 (1993); Miller, M.J., et al., Bioorg. Med. Chem. Lett. 7:1435-1440 (1993); Sikorski, J.A., et al., Phosphorus, Sulfur Silicon Relat. Elem. 76:115-118 (1993)). However, these compounds were not directed at pTyr or related signaling.

One means of modulating PTK dependent signaling is by inhibition of Src homology 2 (SH2) domain binding interactions. Small pTyr containing peptides are able to bind to SH2 domains and compete with larger pTyr peptides or native protein pTyr ligands. Such pTyr peptides are limited in their utility as SH2 domain inhibitors *in vivo* due to their hydrolytic liability to PTPs and poor cellular penetration of the ionized phosphate moiety. The phosphonate-based pTyr mimetics Pmp and F_2Pmp, mentioned above, have been successfully employed for the preparation of PTP-resistant SH2 domain inhibitor peptides, however the problem of cellular penetration remains unsolved for these phosphonate-based compounds.

SH2 domains are homologous sequences of approximately 100 amino acids found in a variety of important signal transducing molecules, where they facilitate a key component of PTK mediated cellular signaling by promoting protein-protein associations. (Margolis, B., Growth Differ 3:73-80 (1993); Panayotou, G., et al., Bioessays 15:171-177 (1993); and Pawson, T., et al., Curr Biol 3:434-442 (1993)). The central roles played by PTKs in a large number of mitogenic signaling cascades (Fantl, W.J., et al., Ann. Rev. Biochem.,

453-481 (1993); Fry, M.J., et al., Protein Sci. 2:1785-1797 (1993)), and the involvement of aberrant or over-expression of PTKs with several cancers and proliferative diseases (Cantley, L.C., Cell 64:281-302 (1991)), has made the development of inhibitors which specifically block the binding of SH2 domains desirable both as biological tools and as potential therapeutic agents. (Burke, T.R., et al., Drugs of the Future 17:119-131 (1992); Brugge, J.S., Science 260:918-919 (1993)). For SH2 binding domains, interactions are frequently dependent on the presence of a pTyr residue in the bound protein. Among different classes of SH2 domains, a secondary ligand specificity resides within the amino acid sequence neighboring the pTyr residue, particularly in residues toward the C-terminal side, thereby allowing families of SH2 domains to "recognize" specific binding sites on target proteins. Small pTyr-bearing peptides modeled after these target sequences also bind with high affinity and moderate selectivity to the appropriate SH2 domains, thereby providing a potential means of competitively inhibiting specific SH2 signaling pathways. (Fantl, W.J., et al., Cell 69:413-423 (1992); Songyang, Z., et al., Cell 72:767-778 (1993).

Accordingly, the present invention overcomes the obstacles of the prior art by providing for the preparation and use of new O-malonyltyrosyl compounds and O-malonyltyrosyl compound-containing peptides which are stable to phosphatases, capable of crossing cell membranes, suitable for application in peptide synthesis, and amenable to prodrug derivatization for delivery into cells. The present invention also provides for O-malonyltyrosyl compounds and derivatives thereof which can be protected in the neutral diester form for enhanced delivery across cell membranes and subsequent esterase-mediated liberation of the active dicarboxylic acid once inside the cell.

The present invention further provides for use of the O-malonyltyrosyl compound-containing compounds in the

synthesis of peptides, and for O-malonyltyrosyl compound-containing peptides which exhibit inhibitory potency against binding interactions of receptor domains with pTyr-containing peptide ligands, whose advantages and uses will become apparent from the following objectives of the invention and disclosure.

SUMMARY OF THE INVENTION

The present invention relates to O-malonyltyrosyl compounds and derivatives thereof, the application of O-malonyltyrosyl compounds in peptide synthesis, and O-malonyltyrosyl compound-containing peptides and uses thereof. The O-malonyltyrosyl compounds, derivatives thereof, and O-malonyltyrosyl compound-containing peptides of the present invention are stable to phosphatases, suitable for application to peptide synthesis of O-malonyltyrosyl compound-containing peptides, and amenable to prodrug derivatization for delivery into cells.

Specifically, the present invention provides for the preparation and use of O-malonyltyrosyl compounds herein designated "OMT". The present invention further provides for the design and preparation of OMT compounds protected in the carboxylic acid diester form, suitable for incorporation into peptides.

The present invention further provides for the design and synthesis of derivatives of OMT, such as, for example, *O,O*-bis(*tert*-butyl)-N-Fmoc OMT and monofluoro-OMT.

The present invention also provides for OMT compounds which exhibit inhibitory activity against protein-tyrosine phosphatases (PTPs).

The present invention additionally provides for OMT-containing peptides which exhibit inhibitory potency against protein-tyrosine phosphatase (PTP), or Src homology 2 (SH2) domain binding interactions with phosphotyrosyl-containing ligands.

Specifically, the present invention provides for the preparation and use of OMT-containing peptides which exhibit inhibitory potency against P1-3 kinase C-terminal p85 SH2 domain binding interactions with phosphotyrosyl-containing peptide ligands.

Further, the present invention provides for methods of preparation and use of OMT-containing peptides directed against Src domain binding interactions with phosphotyrosyl-containing ligands.

The present invention further provides the preparation and use of OMT-containing peptides directed against Grb SH2 domain binding interactions with phosphotyrosyl-containing ligands.

The present invention further provides for methods of preparation and use of OMT-containing peptides directed against N-terminal SH-PTP 2 SH2 domain binding interactions with phosphotyrosyl-containing ligands.

Further, the present invention provides methods and compositions for treating or preventing disease processes, such as those associated with immune dysfunction, cancer, and diabetes.

The present invention also provides methods of preventing or treating a disease, such as immune dysfunction, cancer, and diabetes, by the administration of a therapeutically effective amount of an OMT-containing peptide.

The present invention also provides methods of preventing or treating cancers by the administration of a therapeutically effective amount of an OMT-containing peptide in combination with toxins, cytotoxic drugs, or irradiation.

The present invention also provides for pharmaceutical compositions for use in the methods described herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B. Figures 1A and 1B set forth the structures and relative binding sites of (A) arylphosphate and (B) arylmalonate pharmacophores to the p56^{lck} SH2 domain.

Figures 2A and 2B. Figures 2A and 2B set forth the energy minimized structures of $HCF_2PO(O-)_2$ (Figure 2A) and $CH_2(CO_2-)_2$ (Figure 2B) bound within the protein tyrosine phosphatase 1B (PTP-1B) catalytic site.

Figure 3. Figure 3 sets forth the effect of the OMT-peptide Ac-Asp-Ala-Asp-Glu-[L-OMT]-Leu-amide on PTP 1B catalyzed insulin receptor dephosphorylation using ^{32}P-labeled intact insulin receptor as substrate.

Figure 4: Figure 4 sets forth the effect of the FOMT-peptide Ac-Asp-Ala-Asp-Glu-[L-FOMT]-Leu-amide on PTP 1B catalyzed insulin receptor dephosphorylation using ^{32}P labeled intact insulin receptor as substrate.

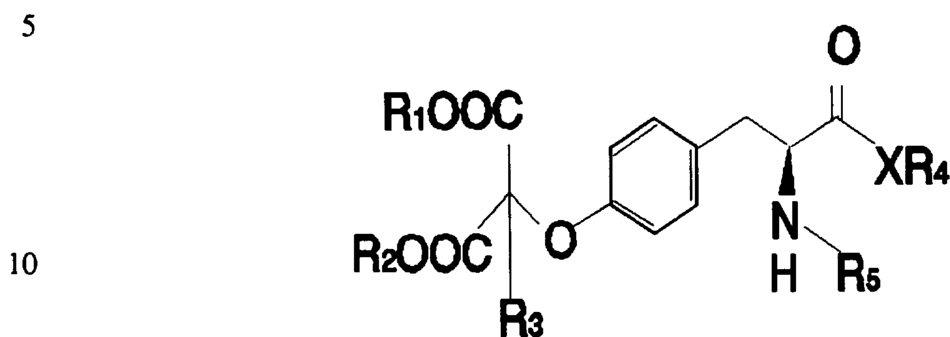
DETAILED DESCRIPTION OF THE INVENTION

The present invention generally provides novel O-malonyltyrosyl compounds, novel O-malonyltyrosyl compound-containing peptides, pharmaceutical compositions comprising said peptides, and their use as pharmaceutically active agents.

More particularly, the present invention provides for the preparation and use of novel O-malonyltyrosyl compounds and derivatives thereof which are stable to phosphatases and capable of crossing cell membranes. The present invention also provides that the new O-malonyltyrosyl compounds and derivatives thereof are amenable to prodrug derivatization for delivery into cells. Derivatization herein refers to derivatization of the O-malonyltyrosyl compound to a

diester, thus converting the compound to a cell permeable form.

The present invention relates to O-malonyltyrosyl compounds of the Formula (I):



I

wherein R_1 and R_2 are independently hydrogen, alkyl, aralkyl, alkaryl, aryl, and heteroaryl;

wherein R_3 is hydrogen, halogen, amino, hydroxy, and alkoxy;

wherein X is nitrogen or oxygen;

wherein R_4 is hydrogen, alkyl, aralkyl, alkaryl, optionally substituted aryl, and heteroaryl;

wherein R_5 is hydrogen, fluorenyl methoxy carbonyl (FMOC), tert-butoxy carbonyl (BOC), and carbobenzoxy (CBZ), carbamoyl, alkyl, amido, aryl, and heteroaryl; with the proviso that substituents of Formula (I) which can be substituted are optionally substituted.

Preferred R_1 and R_2 substituents of Formula I, can be, for example, tert-butyl, phenyl, and benzyl.

Preferred R_4 substituents of Formula I, can be, for example, tert-butyl, benzyl, and pentafluorophenyl.

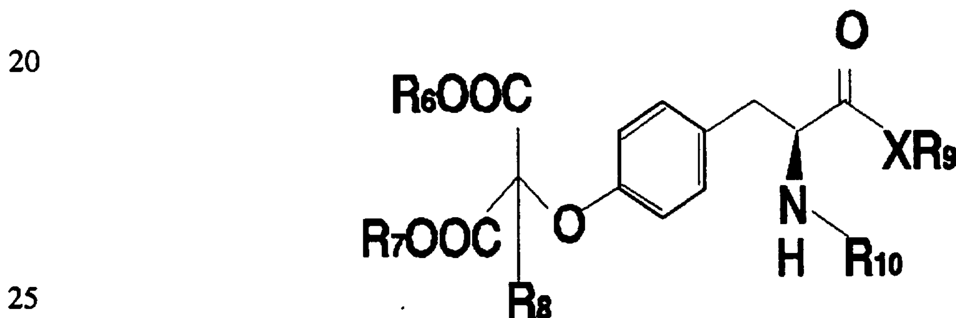
Alkyls occurring in Formula I can be alkyls which are C_{1-20} alkyls.

When halogens occur in Formula I, the halogens can be chlorine, bromine, or fluorine, and the preferred halogen is fluorine.

When substituted alkyls occur in Formula I, examples of suitable substituents are hydroxy, halogen, alkoxy, haloalkoxy, and alkoxyalkyl; and wherein the alkyl groups and the alkyl groups of the alkaryl and aralkyl groups herein are linear or branched chain, or cyclic having up to 10 carbon atoms.

When substituted heteroaryl groups occur in Formula I, examples of suitable substituents are halogen, nitro, cyano, or haloalkyl groups; and wherein the alkyl, haloalkyl, alkenyl, haloalkenyl, alkoxy, and haloalkoxy groups herein are linear or branched chains, having less than 10 carbon atoms, preferably less than 5 carbon atoms, and the halo substitution in all these groups consists of one or more halogen atoms, which are the same or different, from mono substitution up to complete poly substitution.

The present invention further relates to the application of the compounds of Formula (II) for use in the synthesis of peptides:



(II)

wherein R_6 and R_7 are independently hydrogen, alkyl, aryl, alkaryl, and ethylenethioalkyl;

wherein R_8 is hydrogen, halogen, alkoxy, haloalkoxy, nitro, amido, and substituted amino groups;

wherein X_2 is nitrogen or oxygen;

wherein R_9 is hydrogen, alkyl, aralkyl, alkaryl, optionally substituted aryl, and heteroaryl;

wherein R_{10} is hydrogen, fluorenyl methoxy carbonyl (FMOC), tert-butoxy carbonyl (BOC), and carbobenzoxy (CBZ), carbamoyl, alkyl, amido, aryl, and heteroaryl; with the proviso that substituents of Formula (II) which can be substituted are optionally substituted.

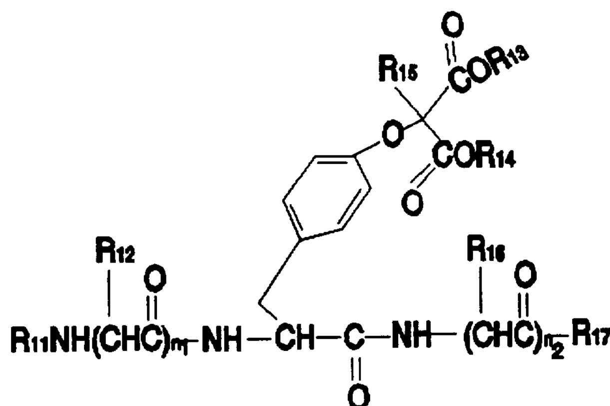
Preferred R_9 substituents of Formula II can be, for example, tert-butyl, benzyl, and pentafluorophenyl.

Alkyls occurring in Formula II can be alkyls which are C_{1-20} alkyl and C_{1-6} alkyl. Aryls occurring in Formula II can be aryls which are C_{6-10} aryl.

When substituted alkyls occur in Formula II, examples of suitable substituents are hydroxy, halogen, alkoxy, haloalkoxy, and alkoxyalkyl; and wherein the alkyl groups and the alkyl groups of the alkaryl and aralkyl groups herein are linear or branched chain, or cyclic having up to 10 carbon atoms.

When substituted heteroaryl groups occur in Formula II, examples of suitable substituents are halogen, nitro, cyano, or haloalkyl groups; and wherein the alkyl, haloalkyl, alkenyl, haloalkenyl, alkoxy, and haloalkoxy groups herein are linear or branched chains, having less than 10 carbon atoms, preferably less than 5 carbon atoms, and the halo substitution in all these groups consists of one or more halogen atoms, which are the same or different, from mono substitution up to complete poly substitution.

The present invention further relates to peptides of the Formula (III):



(III)

wherein R_{11} is hydrogen, acetyl, alkanoyl, alkyl, aryl, aralkyl, alkaryl, or polyethyleneoxy;

wherein R_{12} and R_{16} are residues of amino acids selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine, or derivatives thereof, and also a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II;

wherein R_{13} and R_{14} are independently hydrogen, alkyl, and ethylenethioalkyl;

wherein R_{15} is hydrogen, halogen, alkoxy, haloalkoxy, nitro, amido, and substituted amino groups;

wherein R_{17} is hydroxy, NH_2, O-alkyl, O-aryl, O-aralkyl, O-alkaryl, N-polyethyleneoxy; and

wherein n_1 and n_2 may be the same or different, are zero, or 1-10, but wherein n_1 and n_2 are not zero at the same time;

with the proviso that substituents of Formula (III) which can be substituted are optionally substituted.

Alkyls occurring in Formula III can be alkyls which are C_{1-20} alkyl.

Aryls occurring in Formula III can be aryls which are C_{6-10} aryl.

When substituted alkyls occur in Formula III, examples of suitable substituents are hydroxy, halogen, alkoxy, haloalkoxy, and alkoxyalkyl; and wherein the alkyl groups and the alkyl groups of the alkaryl and aralkyl groups herein are linear or branched chain, or cyclic having up to 10 carbon atoms.

When substituted heteroaryl groups occur in Formula III, examples of suitable substituents are halogen, nitro, cyano, or haloalkyl groups; and wherein the alkyl, haloalkyl, alkenyl, haloalkenyl, alkoxy, and haloalkoxy groups herein are linear or branched chains, having less

than 10 carbon atoms, preferably less than 5 carbon atoms, and the halo substitution in all these groups consists of one or more halogen atoms, which are the same or different, from mono substitution up to complete poly substitution.

When R_{12} and R_{16} of Formula III are independently amino acids or derivatives thereof, and one or more of the amino acids is aspartic acid and/or glutamic acid, the preferred sidechains of the aspartic acid and/or glutamic acid moieties are independently *n*-butyl ester, *n*-alkyl, or aryl.

When R_{12} and R_{16} of Formula III are independently peptides, the amino acid sequence may be a linear or branched chain, and may consist of any number of amino acids, usually from about three to thirty amino acids, the preferred length being 5-10 amino acids. The amino acid sequence of the peptide depends upon the particular use of the peptide. For example, the design of a peptide for use as an inhibitor of receptor binding will be directed toward the amino acid sequence of the particular receptor domain, and may vary greatly between receptors.

Specific but not limiting examples of peptides of Formula III useful in the present invention include the following:

(1) peptide D-X-V-P-M-L (SEQ ID NO. 1), directed towards inhibition of the binding of the P1-3 kinase C-terminal p85 SH2 domain with phosphotyrosyl-containing peptide ligands, wherein X is the residue of a compound of Formula II;

(2) peptide Q-X-E-E-I-P (SEQ ID NO. 2), directed towards inhibition of the binding of the Src SH2 domain with phosphotyrosyl-containing peptide ligands, wherein X is the residue of a compound of Formula II;

(3) peptide N-X-V-N-I-E (SEQ ID NO. 3), directed towards inhibition of the binding of the Grb2 SH2 domain with phosphotyrosyl-containing peptide ligands, wherein X is the residue of a compound of Formula II; and

(4) peptide L-N-X-I-D-L-D-L-V (SEQ ID NO. 4), directed towards inhibition of the binding of the N-terminal SH-PTP2 SH2 domain with phosphotyrosyl-containing peptide ligands, wherein X is the residue of a compound of Formula II.

The O-malonyltyrosyl compounds and O-malonyltyrosyl compound-containing peptides of the present invention may exist in a free, i.e. unprotected, or a protected form. The protected form herein refers to compounds wherein one or more reactive groups, e.g. N-terminal amino groups or -OH groups, are covered by a protecting group. Suitable protecting groups are any of those known in the art of peptide chemistry, such as N-, carboxy-, and O- protecting groups. The preferred form of the O-malonyltyrosyl compound-containing peptides of the present invention is the diester form, wherein the carboxyl groups are in a neutral state, allowing for passage through cell membranes. The carboxylic acid diester form, alkyl or other suitable prodrug esters, may be considered "prodrugs", i.e. protected forms of the compound which are useful as pharmaceuticals.

The peptides of the present invention, whether they are in free or protected form, may exist as salts or as complexes. Acid addition salts may be formed with organic acids, polymeric acids, and inorganic acids, for example. Such acid addition salt forms include *inter alia* the hydrochlorides and acetates. Complexes are herein defined as compounds of known type, formed on addition of inorganic substances, such as inorganic salts or hydroxides such as Ca- and Zn- salts, and/or on addition of polymeric organic substances.

The present invention further provides methods and compositions for preventing or treating diseases. Particular non-limiting examples of diseases include immune dysfunction, cancer, and diabetes. Specifically, this invention provides for the use of the compounds and compositions of the present invention to inhibit binding interactions of receptor domains with phosphotyrosyl-

containing ligands for treating or preventing the disease processes associated with immune dysfunction, cancer, or diabetes. This invention also provides pharmaceutical compositions comprising the same.

More particularly, the present invention provides methods of treating diabetes by administration of a therapeutically effective amount of an O-malonyltyrosyl compound-containing peptide which, for example, exhibits inhibitory potency against the binding interactions of the SH2 domain with phosphotyrosyl-containing ligands.

The present invention further provides methods of preventing or treating a disease by the administration of a therapeutically effective amount of an O-malonyltyrosyl compound-containing peptide.

The present invention also provides methods of preventing or treating diseases by the administration of a therapeutically effective amount of an O-malonyltyrosyl compound-containing peptide in combination with chemotherapeutic agents, toxins, or irradiation. Examples of chemotherapeutic agents are known to those skilled in the art and include, but are not limited to, bleomycin, mitomycin, cyclophosphomide, doxorubicin, paclitaxel, and cisplatin.

In one embodiment of the invention, the O-malonyltyrosyl compound-containing peptides are administered in a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier encompasses any of the standard pharmaceutical carriers such as sterile solution, tablets, coated tablets and capsules. Such carriers may typically contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stensic acid, talc, vegetable fats or olis, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives and other ingredients.

The administration of the compound may be effected by any of the well known methods, including but not limited to,

oral, intravenous, intramuscular, and subcutaneous administration. The preferred method of administration is intravenous.

In the practice of the methods of this invention, the amount of the O-malonyltyrosyl compound-containing peptide incorporated in the composition may vary widely. Methods for determining the precise amount depend upon the subject being treated, the specific pharmaceutical carrier, the route of administration being employed, the frequency with which the compound is to be administered, and whether the composition is administered in conjunction with a chemotherapeutic agent and/or irradiation treatment.

The present invention provides novel O-malonyltyrosyl compounds and O-malonyltyrosyl compound-containing peptides which do not exhibit the problems which presently exist in compounds which have similar applications.

The phosphate-containing pTyr pharmacophore demonstrates lability to phosphatases and low penetration across cell membranes, which are two significant limitations to the therapeutic and pharmacological utility of pTyr-based agents which the practice of the present invention has overcome.

Previously, PTP inhibitors were designed for replacement of the pTyr residue in PTP-substrate peptides. This was accomplished by modifying peptide substrates so as to render them incapable of undergoing chemical transformation by PTP. Replacement of the pTyr residue in PTP-substrate peptides, with the non-hydrolyzable pTyr mimetic, Pmp 2 (Burke, T.R., Jr. et al., Synthesis 11:1019-1020 (1991)), resulted in peptides which are competitive PTP inhibitors (Chatterjee, S., et al., Peptides: Chemistry and Biology, J.E. Rivier and J.A. Smith, Editor, 1992, Escom Science Publishers: Leiden, Netherlands. p. 553-555; Zhang, Z.Y., et al., Biochemistry 33:2285-2290 (1994)). Pmp differs from pTyr in having a methylene substituted for the tyrosyl 4'-ester oxygen. It was previously shown that

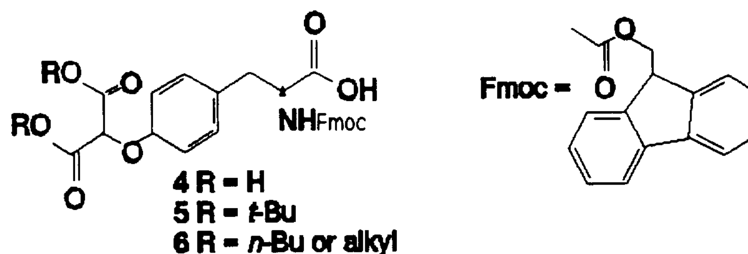
substitution of the Pmp residue in one such hexameric inhibitor peptide with F_2Pmp 3 (Burke, T.R., Jr., et al., J. Org. Chem. 58:1336-1340 (1993); Burke, T.R., Jr., Tetrahedron Lett. 34:4125-4128 (1993); Smyth, M.S. and Burke, T.R., Jr., Tetrahedron Lett. 35: 551-554 (1994)) resulted in a 1000-fold increase in inhibitory potency (IC_{50} = 100 nM against PTP 1B) (Burke, T.R., et al., Biochem. Biophys. Res. Commun. 204:129-134 (1994)). Because the difluorophosphonate moiety is di-ionized at physiological pH (Smyth, M.S., et al., Tetrahedron Lett. 33:4137-4140 (1992)), transport across cell membranes was compromised and cellular studies of F_2Pmp-containing peptides resorted to membrane permeabilization (Wange, R.L., et al., J. Biol. Chem. 270:944-948 (1995)) or microinjection (Xiao, S., et al., J. Biol. Chem. 269:21244-21248 (1994)) techniques. While prodrug protecting groups have been developed for phosphates (Srivastva, D.N. and Farquhar, D., Bioorganic Chemistry 12:118-129 (1984); McGuigan, C., et al., Bioorg. Med. Chem. Lett. 2:701-704 (1992); Perigaud, C., et al., Bioorg. Med. Chem. Lett 3:2521-2526 (1993); Farquhar, D., et al., J. Med. Chem. 38:488-495 (1995)) and phosphonates (Freeman, S., et al., J. Chem. Soc. Chem. Commun. 875-877 (1991); Lombaert, S.D., et al., J. Med. Chem. 37:498-511 (1994)), these protecting groups have not yet been extended to F_2Pmp-containing peptides, where synthetic challenges exist.

The present invention overcomes the deficiencies of the fluorine-containing peptides of the prior art by providing for the design and synthesis of nonhydrolyzable non-phosphorus-based O-malonyltyrosyl compounds. For example, Pmp 2 and its monofluoro (FPmp 3) and difluoro (F_2Pmp 4) analogues have been shown to retain SH2 domain binding potency when substituted into appropriate peptides, yet are not hydrolyzed by phosphatases. (See Domchek, S.M., et al., Biochemistry 31:9865-9870 (1992); Burke, T.R., Jr., et al., Biochemistry 33:6490-6494 (1994)). Since the phosphonate

group is ionized at physiological pH, (Smyth, M.S.,et al., Tetrahedron Lett. 33:4137-4140 (1992)) these peptides show limited penetration of cell membranes. F_2Pmp-containing peptides have been successfully used in cell-based systems, however only when cells which were made permeable (Wange, R.L., et al., J. Biol. Chem. 270:944-948 (1995)) or microinjection techniques (Xiao, S., et al., J. Biol. Chem. 269:21244-21248 (1994)) were employed for these studies. Accordingly, there is a need for synthetic peptides which are both nonhydrolyzable and able to penetrate cell membranes.

The literature contains several examples of bio-reversible protection of phosphate (Srivastva, D.N. and Farquhar, D., Bioorganic Chemistry 12: 118-129 (1984)) and phosphonates, (Iyer, R.P., et al., Tetrahedron Letters 30:7141-7144 (1989); Freeman, S., et al., J. Chem. Soc. Chem. Commun. 875-877 (1991); Mitchell, A.G., et al., J. Chem. Soc. Perkin Trans. I (1992); Lombaert, S.D., et al., J. Med. Chem. 37:498-511 (1994)) however such "prodrug" derivatization is frequently difficult to accomplish, not readily applicable to peptide synthesis and has not yet been extended to F_2Pmp species. Based on prior findings of Sikorski, et al. that the malonate moiety can mimic a phosphate structure in EPSP (5-enolpyruvoyl-shikimate-3-phosphate) synthase inhibitors, (*See* Miller, M.J., et al., Bioorg. Med. Chem. Lett. 4:2605-2608 (1994)), the compound *L-O*-malonyltyrosine (L-OMT) was designed and synthesized by the methods of the present invention. L-OMT improves upon the prior compounds used to replace pTyr in that L-OMT is amenable to prodrug derivatization. L-OMT was designed by replacing the phosphate group of the pTyr residue with a malonate dicarboxylic acid structure. As stated previously, the advantage of L-OMT over former phosphonate-based analogues such as F_2Pmp is that preparation of OMT as its carboxylic acid diester affords one potential means of prodrug protection. The malonyl structure of OMT contains

two carboxylic acids instead of the phosphate group and, as such, it can be readily protected as the di-ester for delivery across cell membranes. One inside the cell, esterase-mediated cleavage of the esters liberate the active di-acid form.



The present invention further provides for analogues of OMT having the formulas **5** and **6**, shown above, suitably protected for incorporation into peptides by solid-phase synthesis. The present invention demonstrates that peptide synthesis using *O,O*-bis(tert-butyl)-N-Fmoc OMT formula **5**, as described in Example 6, resulted in simultaneous removal of tert-butyl groups during acid-catalyzed cleavage from the resin, and provides OMT-peptides in which the malonate carboxyls are in the free, biologically active acid form. Alternatively, solid-phase peptide synthesis using *n*-butyl or alkyl esters in place of tert-butyl esters (compounds of formula **6**) resulted in final peptides which retain the malonate diester protection. This diester form may be considered a "prodrug" of OMT in that the carboxyl groups are in a neutral form, allowing passage through cell membranes. Liberation of the biologically active, free acid form can then occur by esterase-mediated hydrolysis of the ester functionalities inside the cell.

The present invention also provides for an analogue of OMT, herein designated monofluoro-OMT (FOMT), the synthesis of which is described in Example 7. The present invention further describes a novel use for L-OMT in the synthesis of SH2 domain inhibitory peptides. The process of designing and synthesizing OMT containing, SH2 domain inhibitory

peptides of the present invention initially involved the examination of phosphatase resistant amino acid analogues which could serve as a mimetic of pTyr in SH2 binding interactions. The initial examination was based on the knowledge of the hydrolytic lability of tyrosine phosphate to cellular PTPs (*See* Walton, K.M., et al., Ann. Rev. Biochem. 62:101-120 (1993)). Pmp is a phosphonate homologue of pTyr wherein the phosphate ester oxygen has been replaced by a methylene. A protected form of Pmp suitable for incorporation into peptides by solid phase synthesis was developed (*See* Burke, T.R., et al., Synthesis 11:1019-1020 (1991); Shoelson, S.E., et al., Tetrahedron Lett. 32:6061-6064 (1991)). The SH2 inhibitory potency of a Pmp-containing peptide corresponding to the sequence surrounding Tyr-315 of the mouse polyoma mT antigen prepared with this reagent was then examined. A two-fold loss of potency was observed for the protected form of Pmp (ID_{50}= 7.2 μM) as compared with the corresponding pTyr-containing peptide (ID_{50}= 3.6 μM) (*See* Domchek, S.M., et al., Biochemistry 31:9865-9870 (1992)). Based on this determination, new analogues of Pmp were designed which bore either one fluorine (FPmp) or two fluorines (F_2Pmp) on the methylene bridge. (*See* Burke, T.R., et al., J. Org. Chem. 58:1336-1340 (1993); Burke, T.R., et al., Tetrahedron Lett. 34:4125-4128 (1993); Otaka, A., et al., Tetrahedron Lett. 34:7039-7042 (1993); and Smyth, M.S., et al., Tetrahedron Lett. 35:551-554 (1994)). The inhibitory potencies of the fluorinated Pmps were enhanced relative to the parent Pmp. (*See* Burke, T.R., et al., Biochemistry 33:6490-6494 (1994)). The F_2Pmp-containing peptides were studied in intact cells. (*See* Xiao, S., et al., J. Biol. Chem. 269:21244-21248 (1994); Wange, R.L., et al., J. Biol. Chem., 270:944-948 (1995)). Because the fluorinated Pmp-containing peptides do not have the ability to cross cell membranes, artificial means were required to introduce them into the cell. This lack of cell permeability again emphasizes the significant limitation of

phosphonate-based SH2 domain inhibitors as pharmacological tools and as therapeutics. The practice of the present invention improves upon the fluorinated Pmps by providing for a non-phosphorus-based mimetic of pTyr, herein designated O-malonyltyrosyl (OMT), which uses a malonate group in place of the phosphonate or phosphate portion.

The present invention further provides for the preparation and use of OMT-containing peptides which exhibit PTP or SH2 inhibitory potency against binding interactions of receptor domains with phosphotyrosyl-containing ligands. Specific examples of peptides exhibiting PTP or SH2 domain inhibitory potency include, but are not limited to, OMT-containing peptides exhibiting inhibitory potency against the P1-3 kinase C-terminal p85 SH2 domain, the Src SH2 domain, the Grb SH2 domain, and the N-terminal SH-PTP2 SH2 domain.

Generally, the SH2 inhibiting peptides of the present invention were prepared by incorporating OMT into each of four SH2 domain inhibitory peptides using solid-phase peptide techniques and the protected analogue (L)-N^{α}-fmoc-O'-[(O",O"-(tertbutyl)malonyl] tyrosine (*See* Ye, B. and Burke, T.R., Jr., Tetrahedron Lett. (in review)). OMT-residues can potentially be protected in the neutral diester form for delivery across cell membranes and subsequent esterase-mediated liberation of the active dicarboxylic acid once inside the cell. Example 8 describes the preparation and synthesis of four OMT-containing peptides against the following SH2 domains; The PI-3 kinase C-terminal p85 SH2 domain (Ac-D-[L-OMT]-V-P-M-L-amide; IC_{50} = 14.2 μM (SEQ. ID. NO. 1)); the Src SH2 domain (AC-Q-[L-OMT]-X-E-E-I-P-amide; IC_{50} > 200 μM (SEQ. ID. NO. 2)); the Grb2 SH2 domain (Ac-N-[L-OMT]-V-N-I-E-amide; IC_{50} > 600 μM (SEQ. ID. NO. 3)) and the N-terminal SH-PTP2 SH2 domain (Ac-L-N-[L-OMT]-I-D-L-D-L-V-amide; IC_{50} = 22.0 μM (SEQ. ID. NO. 4)). SH2 domain binding assays were conducted for each of the four peptides, generating the IC_{50} values set forth above. The IC_{50} values

indicate a significant degree of selectivity between SH2 domains, with the OMT-containing peptides having reasonable affinity for the p85 and SH-PTP2 SH2 domains but not for the Src and Grb SH2 domains. The IC_{50} value for the SH-PTP2 SH2 domain is equivalent to the previously observed for the corresponding F_2Pmp-containing peptide.

Table 1 sets forth the inhibition constants of peptides identical to the four OMT-containing peptides referred to above, except either pTyr or L-F^2Pmp was substituted in place of the OMT moiety. The values demonstrate that OMT-peptide Nos. 7 and 8 are essentially inactive in Src or Grb2 binding assays, respectively. Alternatively, moderate affinity for 6 is indicated against the p85 SH2 domain (IC_{50} = 14.2μM), although potency is significantly reduced relative to the corresponding pTyr-peptide (IC_{50} = 0.15μM) or L-F_2Pmp-peptide (IC_{50} = 0.17μM). (*See* Burke, T.R., Jr., et al., Biochemistry 33:6490-6494 (1994)).

Table 1 also indicates the potency of OMT-peptide no. 9 against the SH-PTP2 SH2 domain. While the absolute magnitude of inhibition (IC_{50} = 22μM) is of the same order as that seen with the p85 directed L-OMT peptide no. 6, in this latter example the L-OMT peptide suffered a 200 fold loss of potency relative to either the pTyr or F_2Pmp peptides. On the other hand SH-PTP2:peptide no. 9 shows no loss of potency relative to the corresponding L-F_2Pmp peptide (IC_{50} = 23μM). (*See* Xiao, S., et al., J Biol Chem 269:21244-21248 (1994)). This is significant as the ability of the L-F_2Pmp peptide to block SH-PTP2 mediated mitogenic signaling in rat 1 fibroblasts was previously demonstrated (*See* Xiao, S., et al., J Biol Chem 269:21244-21248 (1994) suggesting that the L-OMT peptide no. 9 may also possess sufficient potency to elicit a measurable effect in cellular assays.

The interaction of the SH2 domain of the OMT residue of the present invention was compared with that of a native pTyr pharmacophore using molecular modeling studies. (See Example 2 and Figures 1A and 1B). Although the phosphate

and malonate structures are chemically quite different, and the malonate group occupies approximately 18% more volume than the phosphate (See Figures 1A and 1B), their interactions with the SH2 domain are remarkably similar, and both structures can be accommodated while maintaining nearly identical SH2 domain geometries, demonstrating that OMT is able to bind to SH2 domains in a manner similar to pTyr residues.

The disparity observed in binding potencies of OMT peptides directed against the different SH2 domains may indicate that the OMT residue is bound differently in the pTyr pockets of the respective SH2 domains. Potency differences may also reflect larger discrepancies in the overall mode of binding of peptide ligands. For example, binding of pTyr peptides to SH2 domains of the Src family have been shown to employ pronounced "two pronged" interactions between the SH2 domain and pTyr and a second hydrophobic pocket located 3 residues C-terminal to the pTyr residue. It could be anticipated that the high contribution of the pTyr binding to the overall peptide-SH2 domain interaction would amplify any loss of potency brought about by a pTyr mimetic. Alternatively, SH2 domains such as SH-PTP2 appear to exhibit peptide-SH2 domain binding interactions distributed over a more extended region. Since the contribution of the pTyr binding to the total binding of the peptide ligand may be less important, loss of affinity at the pTyr binding site may be better tolerated, and a higher retention of potency may be observed for peptides employing pTyr mimetics.

Specificity is a desirable attribute in the development of SH2 domain inhibitors. It has been previously reported that depending on the SH2 domain, peptides bearing the pTyr mimetic F_2Pmp 3 can exhibit either enhanced or reduced potency relative to parent pTyr-bearing peptides. (*See* Burke, T.R., Jr., et al., Biochemistry 33:6490-6494 (1994)).

This potentially indicated that a measure of selectivity may be achieved by differences in binding at the pTyr site.

A second desired feature of SH2 domain inhibitors is bioavailability. Prodrug delivery of the diester-protected O-malonyltyrosyl compound-containing peptides of the present invention through cell membranes will contribute significantly to the development of cell-permeable inhibitors.

EXAMPLES

The Examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the scope of the invention in any way.

EXAMPLE 1

Peptide Synthesis

The tyrosine phosphate mimicking amino acid X = L-OMT was incorporated into the EGFR~~88 993~~ segment, D-A-D-E-X-L, using solid-phase synthesis with Fmoc chemistry. The amino acid Fmoc-*L*-OMT(tert-butyl)$_2$-OH was synthesized according to a published method (Ye, B. and Burke, T.R., Jr., Tetrahedron Lett. (in review)). The peptide was prepared using PAL resin (Albericio, F., et al., J. Org. Chem. 55:3730-3743 (1990)), DIPCDI/HOBT coupling reagents, and 20% piperidine/DMF for Fmoc deprotection. The resin-bound protected peptides were acetylated with 10% 1-acetylimidazole/DMF. The peptide Ac-D-A-D-E-[*L*-OMT]-L-amide was obtained in one step by simultaneous cleavage from the resin and deprotection with TFA containing 5% each (v/v) of ethanedithiol, m-cresol, thioanisole and water. The peptides were purified by reverse phase HPLC under the following conditions: Vydac C_{18} column (10x250 mm); solvent gradient: A:0.05% TFA in H_2O, B: 0.5% TFA in 90% acetonitrile in H_2O, gradient (B%): 10-55% over 30 minutes; flow rate: 2.5 mL/minute; UV detector: 220 nm; retention time: 14.5 minutes. FABMS (M+H)+ 868.3 (calcd. 868.3). Amino acid analysis: Asp (1.98), Glu

(1.00), Ala (1.01), Leu (1.02); OMT could not be determined by this analysis.

EXAMPLE 2

Molecular Modelling

Structures of a difluoromethylphosphonate group [$HCF_2PO(O-)_2$] and a malonate group [$CH_2(CO_2-)_2$], complexed within the catalytic site of the PTP 1B enzyme (Figures 2A and 2B, respectively) were minimized by an ab initio method using a 3-21G basis set on a CONVEX mainframe computer using GAUSSIAN 92 (GAUSSIAN 92, Gaussian, Inc., Carnegie Office Park, Building 6, Pittsburgh, PA 15106). The geometry of the binding site and mode of binding of the phosphonate were derived from X-ray crystallographic data of a difluorophosphonate-containing inhibitor bound within the PTP 1B catalytic site. During the minimization of the difluoromethylphosphonate-enzyme complex, the geometry of the binding site was fixed relative to the X-ray structure, and the geometry parameters and position of the phosphonate were optimized. Figures 2A and 2B set forth the energy minimized structures of $HCF_2PO(O-)_2$ (Figure 2A) and $CH_2(CO_2-)_2$ (Figure 2B) bound within the protein tyrosine phosphatase 1B (PTP-1B) catalytic site. The minimized geometry of the phosphonate is shown in Figure 2A. In minimizing the complex of the PTP 1B with the malonate structure not only the geometry parameters and position of the malonate were optimized, but also the geometry of the enzyme structure within the binding site during the first 50 hours of CPU time. The minimized malonate complex is shown in Figure 2B. The overall geometry of binding was based on the X-ray structure of an aryl difluorophosphonate inhibitor complexed to PTP-1B.

EXAMPLE 3

Tissue Culture Cell Line

Either of the following cell lines which overexpress human insulin receptors may be used for the assay of insulin receptor dephosphorylation by recombinant PTP 1B:
5 (1) L6: rat skeletal muscle myoblasts (may be obtained from the ATCC under ATCC Accession No. CRL1458); and
(2) HEPG2: human hepatocellular carcinoma (may be obtained from the ATCC under ATCC Accession No. HB8065).

The cells were maintained in F-12 medium containing 10%
10 fetal bovine serum and were cultured to confluence.

EXAMPLE 4

Preparation of Partially Purified Human Insulin Receptors

Membranes from the cultured cells, overexpressing human
15 insulin receptors were isolated and solubilized with Triton X-100, essentially as described by Liotta et al. (Liotta, A.S., et al., J. Biol. Chem. 269:22996-23001 (1994)). In brief, cells were scraped off the dishes in an isotonic homogenization buffer that contained 10 mM HEPES, pH 7.5,
20 0.25 M sucrose, 5 mM EDTA, 20 μg/ml aprotinin, 10 μg/ml leupeptin, 0.2 mM phenylmethylsulfonyfluoride (PMSF), and pelleted by centrifugation at 300 x g for 10 minutes. The cell pellet was resuspended in homogenization buffer (43 x 10^6 cells/ml) and homogenized twice using a Polytron
25 homogenizer (Brinkman) at a setting of 7, for 15 seconds each time. The homogenate was centrifuged at 12,000 x g for 20 minutes at 4°C, and the pellet containing nuclei, debris, and mitochondria was discarded. The supernatant was centrifuged at 100,000 x g for 60 minutes at 4°C, and the
30 resulting crude membrane pellets were washed and frozen at -70°C. When needed, the membrane pellet was resuspended in solubilization buffer containing 50 mM HEPES, pH 7.5, 0.25 M sucrose, 20 μg/ml aprotinin, 10 μg/ml leupeptin, 0.2 mM PMSF and 1% Triton X-100 (w/v) (3 to 5 mg protein/mL
35 solubilization buffer.) After a 30 minute incubation on ice

with occasional stirring, the mixture was centrifuged at 100,000 x g for 60 minutes at 4°C, and the insoluble material discarded. Purified insulin receptors from solubilized membranes were obtained after passing through a wheat germ agglutin (WGA) (obtained from Vector Laboratories, Inc., Burlingame, CA) column following the method of Brillon et al. (Brillon, D.J., et al., Endocrinology 123:1837-1847 (1988). The WGA eluate that contained purified receptors was divided into 100 μl aliquots and stored -70°C.

EXAMPLE 5

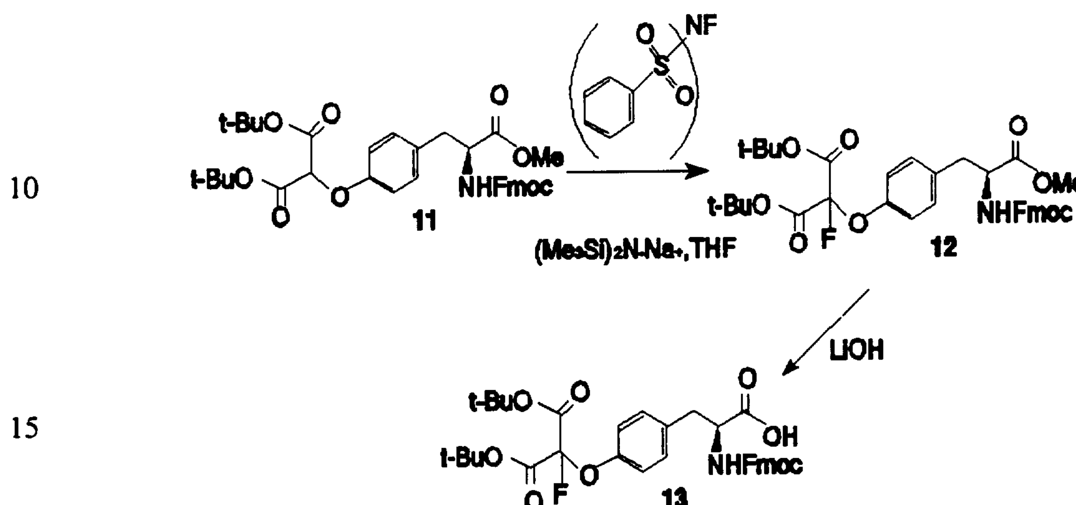
Assay of Insulin Receptor Dephosphorylation by Recombinant PTP 1B

WGA-purified human insulin receptors were autophosphorylated with [γ-^{32}P]ATP as previously described (Liotta, A.S., et al., J. Biol. Chem. 269:22996-23001 (1994)), and this ^{32}P-labeled insulin receptor was used as substrate for the assay of PTP 1B activity, essentially following the method described by Burke et al. (Burke, T.R. Jr., Biochem. Biophys. Res. Commun. 204:129-134 (1994)). In brief, ^{32}P-labeled autophosphorylated insulin receptors (10 μg/ml) were incubated with 0.5 μg/ml recombinant PTP 1B at 22°C in a 100 μl reaction containing 50 mM HEPES, pH 7.5, 0.1 mg/ml BSA, 5 mM DTT, 5 mM EDTA, 0.05% Triton X-100, in the absence or presence of various peptides at the indicated concentrations. The assay was terminated at various intervals by transferring an aliquot of the reaction mixture to a tube that contained 1 volume of 2-fold concentrated Laemmli sample buffer (36); samples were heated at 95°C for 5 minutes prior to electrophoresis in 7.5% SDS-polyacrylamide gels under reducing conditions. The ^{32}P remaining in the 95 kDa insulin receptor β-subunit was quantified by Betagen counting of the fixed and dried gels.

EXAMPLE 6

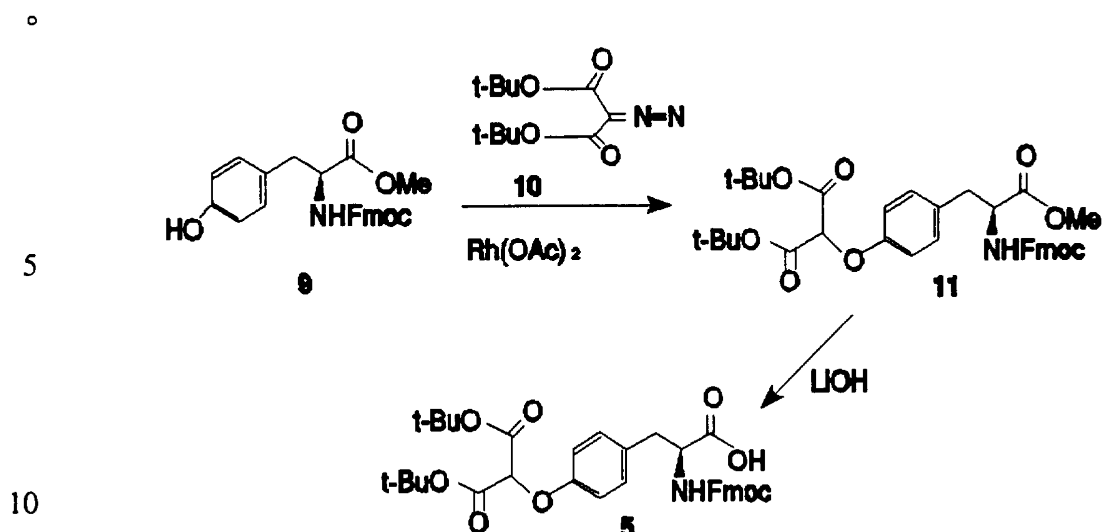
Preparation of monofluoro-OMT

Monofluoro-OMT (FOMT) (formula no. **13**), a derivative of OMT, was prepared from formula no. **11** by electrophilic fluorination, yielding formula no. **12**, which was then demethylated, yielding formula no. **13**.



EXAMPLE 7

The synthesis of O,O-bis(tert-butyl)-N-Fmoc OMT (formula no. **5**) is set forth in the schematic below using synthetic methodology previously reported. Starting from known (formula no. **9**) reaction with diazo di-tertbutyl malonate (formula no. **10**) in the presence of rhodium diacetate gave protected OMT derivative formula no. **11**. Hydrolysis of the methyl ester provided the desired formula no. **5**.



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The n-butyl or alkyl diesters formula no. **6** can be prepared as above using the corresponding diazo di-(n-butyl) malonate (or dialkyl ester) and N^{α}-fmoc tyrosinate tert-butyl ester, followed by treatment with TPA. Alternatively, compound **11** can be treated with acid (trifluoroacetic acid) to hydrolyze the tert-butyl groups, then re-esterified with the desired ester group. Selective hydrolysis of the OMe ester with LiOH would then provide final products of formula no. **6**.

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EXAMPLE 8

Preparation of SH2 domain inhibitory peptides.

L-O-malonyltyrosine (L-OMT 4), a non-phosphorus containing pTyr mimetic, was incorporated into SH2 domain inhibitory peptides using solid-phase peptide techniques and the protected analogue (L)-N^{α}-fmoc-O'-[(O",O"-(tert-butyl)malonyl] tyrosine (Ye, B.E. and Burke, T.R., Jr., Tetrahedron Lett. (in review)).

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The sequences of these peptides are identical to pTyr and F_2Pmp containing peptides which were previously shown to exhibit high affinity to the desired SH2 domain constructs. (*See* Burke, T.R., Jr., et al., Biochemistry 33:6490-6494

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(1994); and Smyth, M.S., et al., Tetrahedron Lett. 33:4137-4140 (1992)).

A. Preparation of the peptide Ac-Asp-[*L*-OMT]-Val-Pro-Met-Leu-amide (SEQ. ID. NO. 1) against the P1-3 kinase C-terminal p85 SH2 domain.

The OMT-containing peptide exhibiting inhibitory potency against the SH2 domain was derived from Tyr751 of the PDGF receptor for inhibition of the PI3-kinase p85 C-terminal SH2 domain (*See* Piccione, E., et al., Biochemistry 32:3197-3202 (1993)), and synthesized by introducing the pTyr mimetic *L*-O-malonyl tyrosine (*L*-OMT) into the peptide Ac-Asp-[*L*-OMT]-Val-Pro-Met-Leu-amide by solid phase peptide techniques using the Di-*tert*-Butyl protected Fmoc-OMT.

Competition assays were performed to determine the relative SH2 domain affinities for the peptide vs. high affinity phosphopeptide ligands, as set forth in Example 9. Table 1 sets forth the inhibition constants of the peptide Ac-Asp-[*L*-OMT]-Val-Pro-Met-Leu-amide (peptide no. 6) against the p1-3 kinase C-terminal p85 SH2 domain construct. Also shown in Table 1 are the inhibition constants of identical peptides having either pTyr or L-F^2Pmp substituted in place of the L-OMT moiety.

B. Preparation of the peptide Ac-Gln-[*L*-OMT]-Glu-Glu-Ile-Pro-amide (SEQ. ID. NO. 2) against the Src SH2 domain.

The peptide Ac-Gln-[*L*-OMT]-X-Glu-Glu-Ile-Pro-amide was derived from Tyr324 of the hamster polyoma virus middle T antigen for inhibition of the Src SH2 domain (*See* Payne, G., et al., Proc. Natl. Acad. Sci. USA 90:4902-4906 (1993); and Songyang, Z., et al., Cell 72:767-778 (1993)). Competition assays were performed to determine the relative SH2 domain affinities for the peptide vs. high affinity phosphopeptide ligands, as set forth in Example 9. Table 1 sets forth the inhibition constants of the peptide Ac-Gln-[*L*-OMT]-Glu-Glu-Ile-Pro-amide (peptide no. 7) against the Src SH2 domain

construct. Also shown in Table 1 are the inhibition constants of identical peptides having either pTyr or L-F^2Pmp substituted in place of the L-OMT moiety.

C. Preparation of the peptide Ac-Asn-[*L*-OMT]-Val-Asn-Ile-Glu-amide (SEQ. ID. NO. 3) against the Grb2 SH2 domain.

The peptide Ac-Asn-[*L*-OMT]-Val-Asn-Ile-Glu-amide was derived from Tyr895 of IRS-1 for inhibition of the Grb2 SH2 domain (*See* Sun, S.J., et al., Mol. Cell. Biol. 13:7428-7428 (1993)). Competition assays were performed to determine the relative SH2 domain affinities for the peptide vs. high affinity phosphopeptide ligands, as set forth in Example 9. Table 1 sets forth the inhibition constants of the peptide Ac-Asn-[*L*-OMT]-Val-Asn-Ile-Glu-amide (peptide no. 8) against the Grb2 SH2 domain construct. Also shown in Table 1 are the inhibition constants of identical peptides having either pTyr or L-F^2Pmp substituted in place of the L-OMT moiety.

D. Preparation of the peptide Ac-Leu-Asn-[*L*-OMT]-Ile-Asp-Leu-Asp-Leu-Val-amide (SEQ. ID. NO. 4) against the SH-PTP2 SH2 domain.

The peptide Ac-Leu-Asn-[*L*-OMT]-Ile-Asp-Leu-Asp-Leu-Val-amide was derived from Tyr1172 of IRS-1 for inhibition of the SH-PTP2 (also known as Syp) N-terminal SH2 domain (*See* Sun, S.J., et al., Mol. Cell. Biol. 13:7428-7428 (1993)). Competition assays were performed to determine the relative SH2 domain affinities for the peptide vs. high affinity phosphopeptide ligands, as set forth in Example 9. Table 1 sets forth the inhibition constants of the peptide Ac-Leu-Asn-[*L*-OMT]-Ile-Asp-Leu-Asp-Val-amide (peptide no. 9) against the SH-PTP2 SH2 domain construct. Also shown in Table 1 are the inhibition constants of identical peptides having either pTyr or L-F^2Pmp substituted in place of the L-OMT moiety.

Table 1

Inhibition Constants of Peptide Inhibitors

			IC50 ± S.E.		
No.	Peptide	SH2 Domain	X=pTyr[a]	*L*-F2Pmp[a]	*L*-OMT
6	Ac-D-X-V-P-M-L-amide	p85 (C-terminal)	0.15 ± 0.03	0.17 ± 0.02	14.2 ± 1.3
7	Ac-Q-X-E-E-I-P-amide	Src	5.7 ± 0.7	1.0 ± 0.2	>200
8	Ac-N-X-V-N-I-E-amide	Grb2	0.9 ± 0.1	4.7 ± 0.7	>600
10	Ac-L-N-X-I-D-L-D-L-V-amide	SH-PTP2 (N-teminal)	4[b]	23[b]	22.0 ± 1.4

a Except where indicated, inhibition constants have previously been reported. (See Burke, et al., Biochemistry 33:6490-6494 (1994)).

b Previously reported. (See Xiao, S., et al., J. Biol. Chem. 269:21244-21248 (1994)).

EXAMPLE 9

SH2 Domain Binding Assays

Details of the SH2 domain competition assay are described by Piccione, E., et al., Biochemistry 32:3197-3202 (1993). Briefly, four distinct assays were used to determine relative SH2 domain affinities for pTyr analogues vs. high affinity phosphopeptide ligands. In each assay a glutathione S-transferase (GST)/SH2 domain fusion protein was paired with an appropriate high-affinity [^{125}I]Bolton-Hunter radiolabeled phosphopeptide, and varying concentrations of unlabeled peptides were added as competitors. The C-terminal SH2 domain of PI 3-kinase p85 was paired with IRS-1pY628, GNGDpYMPMSPK (SEQ ID NO. 5) (*See* Piccione, E., et al., Biochemistry 32:3197-3202 (1993)), the Src SH2 domain was paired with hmT pY324, KEPQpYEEIPIYL (SEQ ID NO. 6) (*See* Payne, G., et al., Proc. Natl. Acad. Sci. USA 90:4902-4906 (1993), the PLCγ-C SH2 domain was paired with PDGF pY1021, DNDpYIIPLPDPK (SEQ ID NO. 7) (*See* Piccione, E., et al., Biochemistry 32:3197-3202 (1993)), and the Lck SH2 domain was paired with the hmTpY324 sequence similar to assays conducted on the Src SH2 domain. An underline denotes the position of the [^{125}I]Bolton-Hunter modified lysine. GST/SH2 domain fusion proteins (0.5-1.0 μM, estimated by Bradford assay), 35 fmol of HPLC-purified, [^{125}I]Bolton-Hunter-treated phosphopeptide (67 nCi), and varying concentrations of pTyr analogues were combined in 200 μl total volume of 20 mM Tris-HCl, 250 mM NaCl, 0.1% bovine serum albumin, 10mM dithiothreitol, pH 7.4, and vortexed. Glutathione-agarose (25 μl of a 1:4 aqueous slurry, Molecular Probes) was added and the samples were incubated overnight at 22° C with constant mixing. Following centrifugation for 5 min at 12,000g, supernatant solutions were removed by aspiration and [^{125}I]radioactivity associated with the unwashed pellets was determined with a γ-counter.

EXAMPLE 10

Molecular Modeling Studies

In order to compare the SH2 domain interaction of the OMT residue of the present invention with that of a native pTyr pharmacophore, molecular modeling studies were conducted using a previously reported X-ray structure of a high affinity pTyr peptide bound to the p56[lck] SH2 domain. Figures 1A and 1B set forth the structures and relative binding sites of (A) arylphosphate and (B) arylmalonate pharmacophores to the p56[lck] SH2 domain. Complexation of the pTyr phenyl phosphate pharmacophore within this SH2 domain is shown in Figure 1A. Binding of the corresponding OMT pharmacophore is shown in Figure 1B. The phosphate and malonate oxygen atoms are shaded. The structure of Figure 1A shows complexation of the pTyr phenyl phosphate pharmacophore within the SH2 domain, and was derived from the previously reported X-ray structure of a bound high affinity pTyr-peptide (See Eck, M.J., et al. Nature 362:87-91 (1993)), while the structure of Figure 1B, which shows binding of the corresponding OMT pharmacophore, was obtained by molecular modeling as herein described. Although the phosphate and malonate structures are chemically quite different, and the malonate group occupies approximately 18% more volume than the phosphate, their interactions with the SH2 domain are remarkably similar, and both structures can be accommodated while maintaining nearly identical SH2 domain geometries.

EXAMPLE 11

Preparation of the peptide Ac-D-A-D-E-OMT-L-amide (SEQ. ID. NO. 8) and testing against PTB-1B.

The peptide Ac-D-A-D-E-OMT-L-amide (SEQ. ID. NO. 8) was prepared by solid-phase peptide techniques using the Di-*tert*-butyl protected Fmoc-OMT 2.

The peptide Ac-D-A-D-E-OMT-L-amide (SEQ. ID. NO. 8) was then examined for inhibitor potency against PTB-1B

dephosphorylation of phosphorylated insulin receptor. Briefly, this assay was conducted as follows: ^{32}P-labeled autophosphorylated insulin receptors were incubated with recombinant PTP-1B in the absence or the presence of Ac-D-A-D-E-OMT-L-amide (SEQ. ID. NO. 8) at various concentrations. The assay was terminated at different intervals and examined by electrophoresis in 7.5% SDS-polyacrylamide gels under reducing conditions. The ^{32}P remaining in the 95 kDa insulin receptor β-subunit was quantified by Betagen counting of the fixed and dried gels. Under identical conditions, the F_2Pmp-containing peptide and the Pmp-containing peptide showed inhibition constants of 100 nM and 200 μM, respectively. (*See* Burke, et al., Biochem. Biophys. Res. Commun. 204:129-134 (1994)). The peptide Ac-D-A-D-E-OMT-L-amide (SEQ. ID. NO. 8) exhibited an inhibition constant of approximately 10 μM.

The effect of the OMT-peptide Ac-D-A-D-E-[L-OMT]-L-amide (SEQ. ID. NO. 8) on PTP 1B catalyzed insulin receptor dephosphorylation using ^{32}P-labeled intact insulin receptor as substrate is set forth in Figure 3.

A similar set of experiments was performed using the peptide Ac-D-A-D-E-(L-FMOT)-L-amide (SEQ. ID. NO. 8). An IC_{50} value of 1μM was obtained, demonstrated graphically in Figure 4.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

5

(ii) TITLE OF INVENTION: O-MALONYLTYROSYL COMPOUNDS, O-MALONYLTYROSYL COMPOUND-CONTAINING PEPTIDES AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

10

- (A) ADDRESSEE: MORGAN & FINNEGAN, L.L.P.
- (B) STREET: 345 PARK AVENUE
- (C) CITY: NEW YORK
- (D) STATE: NEW YORK
- (E) COUNTRY: USA
- (F) ZIP: 10154

15

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: FLOPPY DISK
- (B) COMPUTER: IBM PC COMPATIBLE
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: ASCII

(vi) CURRENT APPLICATION DATA:

20

- (A) APPLICATION NUMBER: TO BE ASSIGNED
- (B) FILING DATE: 29 MARCH 1996
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 08/414,520
- (B) FILING DATE: 31 MARCH 1995
- (C) CLASSIFICATION:

25

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: WILLIAM S. FEILER
- (B) REGISTRATION NUMBER: 26,728
- (C) REFERENCE/DOCKET NUMBER: 2026-4187PCT

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (212) 758-4800

30

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(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6
- (B) TYPE: AMINO ACID
- (C) STRANDNESS: UNKNOWN

35

- (D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Wherein Xaa is a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asp-Xaa-Val-Pro-Met-Leu

(2) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Wherein Xaa is a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gln-Xaa-Glu-Glu-Ile-Pro

(2) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Wherein Xaa is a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asn-Xaa-Val-Asn-Ile-Gln

(2) INFORMATION FOR SEQ ID NO: 4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Wherein Xaa is a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Leu-Asn-Xaa-Ile-Asn-Leu-Asp-Leu-Val

(2) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: The C-terminal Lys is an [I^{125}] Bolton-Hunter modified lysine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Gly-Asn-Gly-Asp-Tyr-Met-Pro-Met-Ser-Pro-Lys

(2) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: The N-terminal Lys is an [I^{125}] Bolton-Hunter modified lysine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Lys-Glu-Pro-Gln-Tyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu

(2) INFORMATION FOR SEQ ID NO: 7

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: The C-terminal Lys is an [I^{125}] Bolton-Hunter modified lysine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp-Asn-Asp-Tyr-Ile-Ile-Pro-Leu-Pro-Asp-Pro-Lys

(2) INFORMATION FOR SEQ ID NO: 8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: AMINO ACID
(C) STRANDNESS: UNKNOWN
(D) TOPOLOGY: UNKNOWN

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Wherein Xaa is a residue of a unit derived from the O-malonyltyrosyl compounds of Formula II.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asp-Ala-Asp-Glu-Xaa-Leu

o

What is claimed is:

1. O-malonyltyrosyl compounds of the Formula (I):

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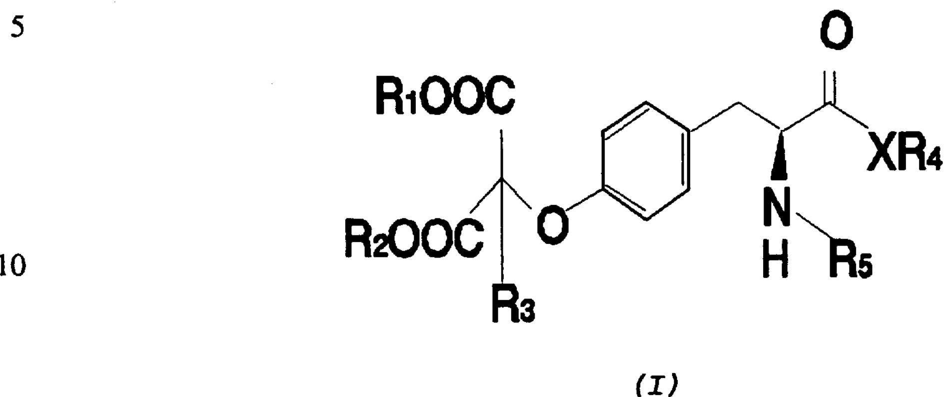

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(I)

wherein R_1
15 and R_2 are independently hydrogen, alkyl, aralkyl, alkaryl, aryl, and heteroaryl;

wherein R_3 is hydrogen, halogen, amino, hydroxy, and alkoxy;

wherein X is nitrogen or oxygen;

20 wherein R_4 is hydrogen, alkyl, aralkyl, alkaryl, optionally substituted aryl, and heteroaryl;

wherein R_5 is hydrogen, fluorenyl methoxy carbonyl (FMOC), tert-butoxy carbonyl (BOC), and carbobenzoxy (CBZ), carbamoyl, alkyl, amido, aryl, and heteroaryl; with the
25 proviso that substituents of Formula (I) which can be substituted are optionally substituted.

2. The O-malonyltyrosyl compound of Formula (I), claim 1, wherein R_1 and R_2 are independently tert-butyl, n-butyl, or
30 hydrogen.

3. The O-malonyltyrosyl compound of Formula (I), claim 1, wherein R_3 is hydrogen or fluorine.

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4. The O-malonyltyrosyl compound of Formula (I), claim 1, wherein R_4 is hydrogen, methyl, tert-butyl, or benzyl.

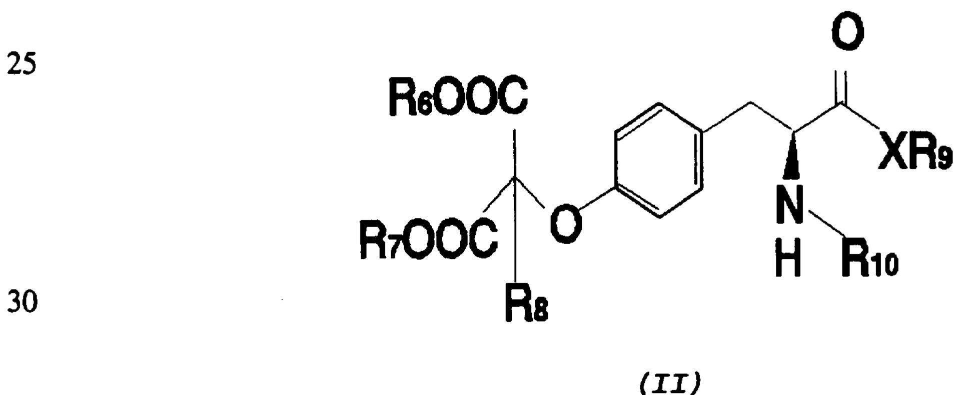
5. The O-malonyltyrosyl compound of Formula (I), claim 1, wherein R_5 is flurenyl methoxy carbonyl, tert-butoxy carbonyl, or carbobenzoxy.

6. A method for affecting signal transduction pathways of cells, comprising contacting the cells with at least one compound of Formula (I) of claim 1 in an amount effective to affect signal transduction.

7. A composition for affecting signal transduction pathways of cells, comprising at least one compound of Formula (I) of claim 1 in an amount effective to affect signal transduction pathways, and a suitable carrier.

8. A method for the treatment or prevention of diseases in a patient which comprises administering to said patient a therapeutically effective amount of the composition of claim 7, or a pharmaceutically acceptable salt thereof.

9. O-malonyltyrosyl compounds of the Formula (II):



(II)

wherein R_6 and R_7 are independently hydrogen, alkyl, aryl, alkaryl, and ethylenethioalkyl;

wherein R_8 is hydrogen, halogen, alkoxy, haloalkoxy, nitro, amido, and substituted amino groups;

wherein X_2 is nitrogen or oxygen;

wherein R_9 is hydrogen, alkyl, aralkyl, alkaryl, optionally substituted aryl, and heteroaryl;

wherein R_{10} is hydrogen, fluorenyl methoxy carbonyl (FMOC), tert-butoxy carbonyl (BOC), and carbobenzoxy (CBZ), carbamoyl, alkyl, amido, aryl, and heteroaryl; with the proviso that substituents of Formula (II) which can be substituted are optionally substituted.

10. O-malonyltyrosyl compounds of Formula (II) of claim 9 wherein R_8 is a halogen selected from the group consisting of fluorine, bromine or chlorine.

11. O-malonyltyrosyl compounds of Formula (II) of claim 9 wherein R_6 and R_7 are independently hydrogen.

12. O-malonyltyrosyl compounds of Formula (II) of claim 9 wherein R_6 and R_7 are independently *tert*-butyl or *n*-butyl; R_8 is hydrogen; R_9 is hydrogen; and R_{10} is fluorenyl methoxy carbonyl, tert-butoxy carbonyl, or carbobenzoxy.

13. O-malonyltyrosyl compounds of Formula (II) of claim 9 wherein R_6 and R_7 are independently *tert*-butyl, *n*-butyl, or alkyl; R_8 is fluorine; R_9 is hydrogen; and R_{10} is fluorenyl methoxy carbonyl, tert-butoxy carbonyl, or carbobenzoxy.

14. O-malonyltyrosyl compound-containing peptides of Formula (III):

(III)

wherein R_{11}
is hydrogen, acetyl, alkanoyl, alkyl, aryl, aralkyl, alkaryl, or polyethyleneoxy;

wherein R_{12} and R_{16} are residues of amino acids selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine, or derivatives thereof, and also a residue of a unit derived from the O-malonyltyrosyl compounds of Formula (II);

wherein R_{13} and R_{14} are independently hydrogen, alkyl, and ethylenethioalkyl;

wherein R_{15} is hydrogen, halogen, alkoxy, haloalkoxy, amido, and substituted amino groups;

wherein R_{17} is hydroxy, NH_2, O-alkyl, O-aryl, O-aralkyl, O-alkaryl, N-polyethyleneoxy;

with the proviso that substituents of Formula (III) which can be substituted are optionally substituted; and

wherein n_1 and n_2 may be the same or different, are zero, or 1-10, but wherein n_1 and n_2 are not zero at the same time.

15. A composition for the treatment or prevention of a disease comprising Formula (III) of claim 14 and a suitable carrier.

16. A method for the treatment or prevention of diseases in a patient which comprises administering to said patient a therapeutically effective amount of the composition of claim 15, or a pharmaceutically acceptable salt thereof.

17. The method of claim 16 wherein the disease is cancer, diabetes, or immune dysfunction.

18. The method of claim 17 wherein the disease is cancer and the method further comprises administering the composition in conjunction with a chemotherapeutic agent.

19. The method of claim 17 wherein the disease is cancer and the method further comprises administering the composition in conjunction with irradiation treatment.

20. A peptide according to claim 14, wherein said peptide is of the formula D-X-V-P-M-L (SEQ ID NO. 1), and wherein X is the residue of a compound of Formula (II).

21. A peptide according to claim 14, wherein said peptide is of the formula Q-X-E-E-I-P (SEQ ID NO. 2), and wherein X is the residue of a compound of Formula (II).

22. A peptide according to claim 14, wherein said peptide is of the formula N-X-V-N-I-E (SEQ ID NO. 3), and wherein X is the residue of the compound of Formula (II).

23. A peptide according to claim 14, wherein said peptide is of the formula L-N-X-I-D-L-D-L-V (SEQ ID NO. 4), and wherein X is the residue of the compound of Formula (II).

24. A composition for inhibiting binding interactions of the P1-3 kinase C-terminal p85 SH2 domain with ligands comprising at least one peptide according to claim 14 and a suitable carrier.

25. A composition for inhibiting binding interactions of the Src SH2 domain with ligands comprising at least one peptide according to claim 14 and a suitable carrier.

26. A composition for inhibiting binding interactions of the Grb2 SH2 domain with ligands comprising at least one peptide according to claim 14 and a suitable carrier.

27. A composition for inhibiting binding interactions of the N-terminal SH-PTP2 SH2 domain with ligands comprising at least one peptide according to claim 14 and a suitable carrier.

28. A method for the treatment or prevention of diseases in a patient where the inhibition of P1-3 kinase C-terminal p85 S2 domain binding interactions is desirable, which comprises administering to said patient a therapeutically effective amount of the composition of claim 24, or a pharmaceutically acceptable salt thereof.

29. A method for the treatment or prevention of diseases in a patient where inhibition of Src SH 2 domain binding interactions is desirable, which comprises administering to said patient a therapeutically effective amount of the composition of claim 25, or a pharmaceutically acceptable salt thereof.

30. A method for the treatment or prevention of diseases in a patient where inhibition of Grb2 SH2 domain binding interactions is desirable, which comprises administering to said patient a therapeutically effective amount of the

composition of claim 26, or a pharmaceutically acceptable salt thereof.

31. A method for the treatment or prevention of diseases in a patient where inhibition of N-terminal SH-PTP2 SH2 domain binding interactions is desirable, which comprises administering to said patient a therapeutically effective amount of the composition of claim 27, or a pharmaceutically acceptable salt thereof.

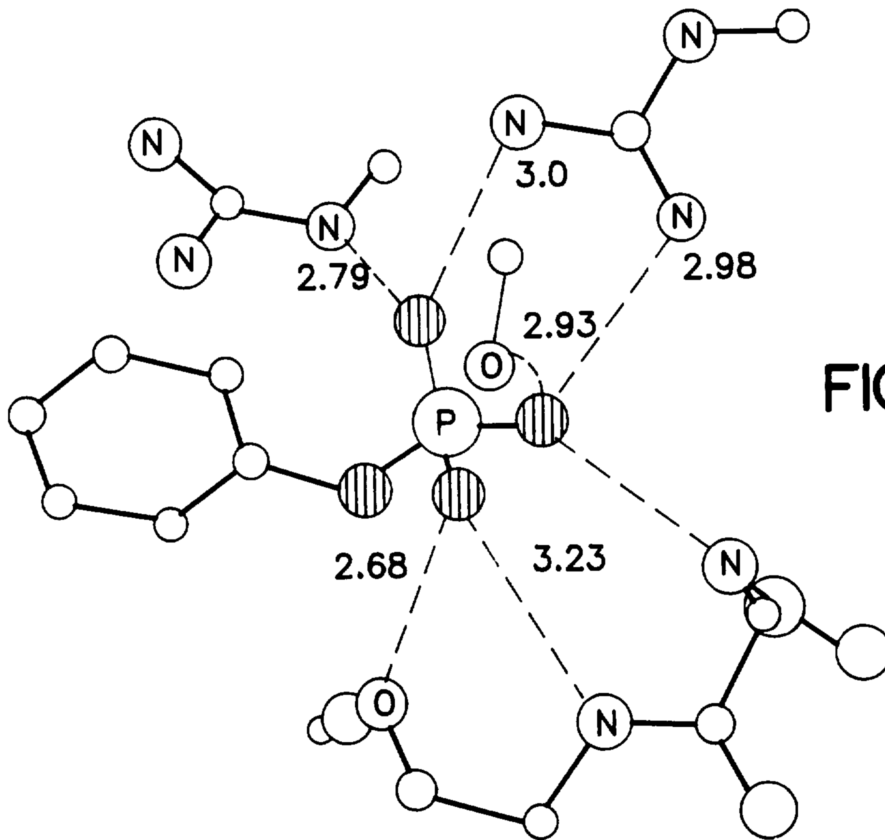


FIG. 1A

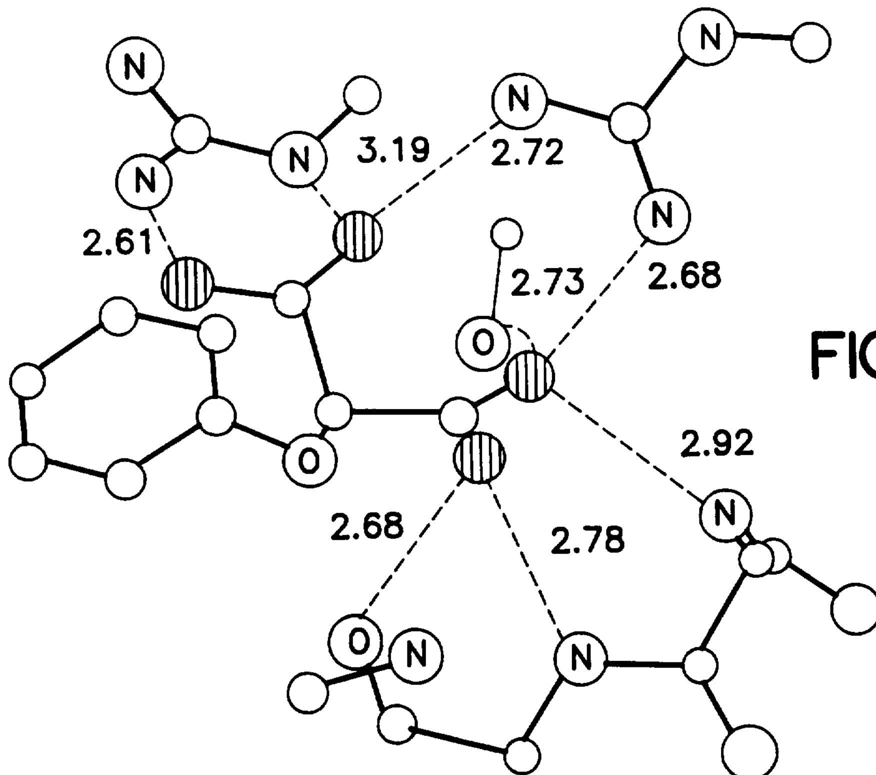


FIG. 1B

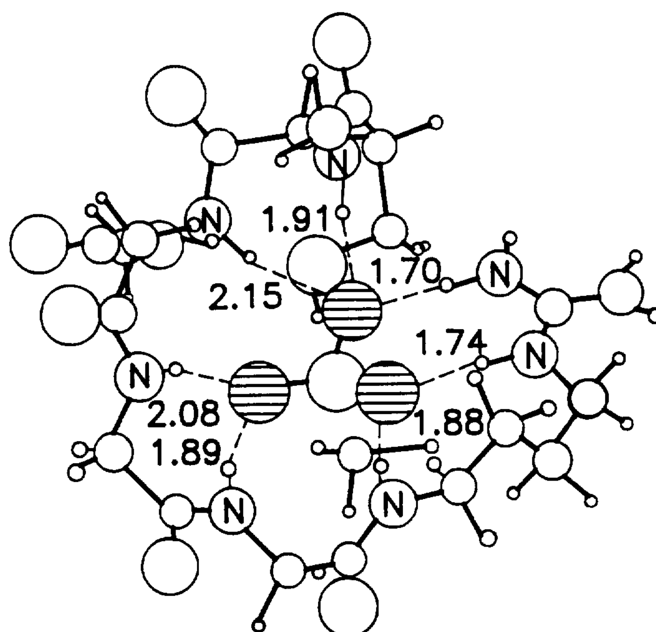


FIG. 2A

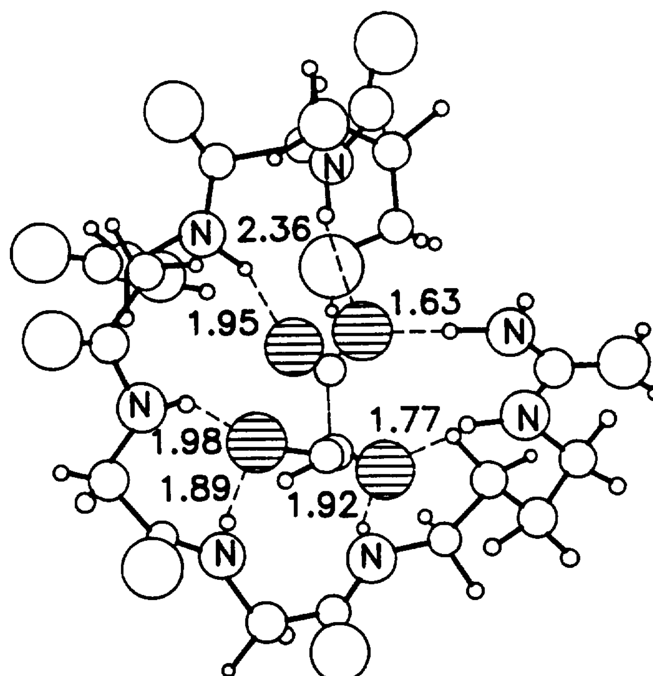


FIG. 2B

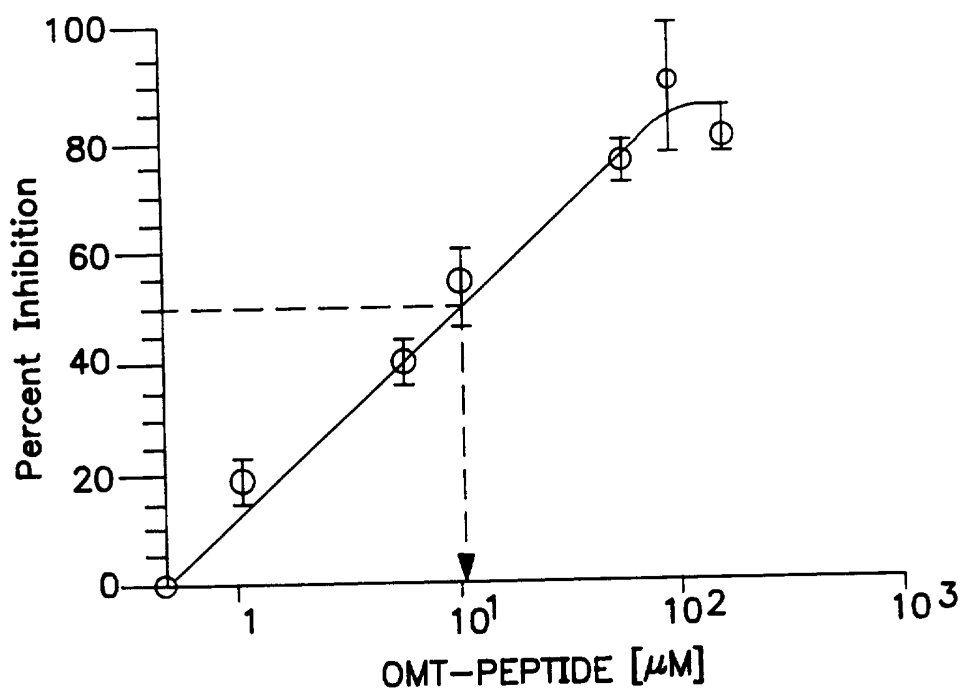


FIG. 3

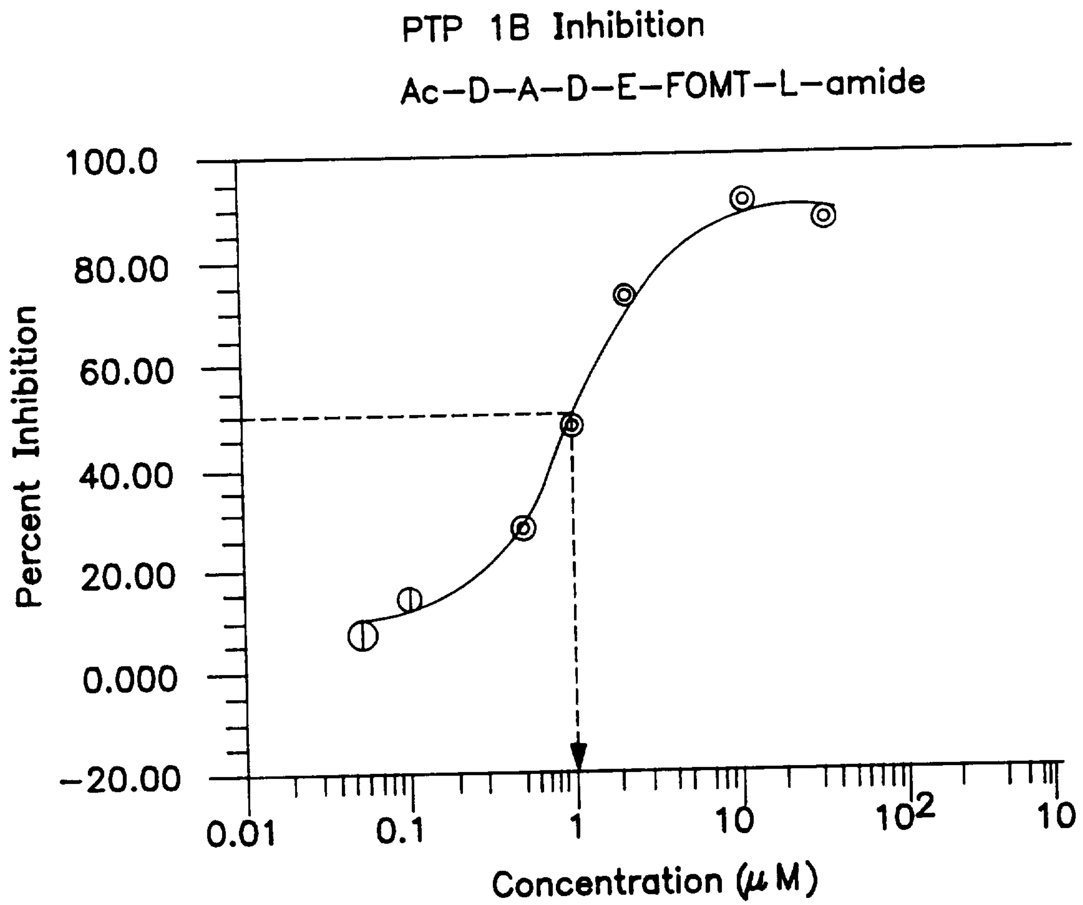


FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/04311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C229/36 C07C237/20 C07C271/22 C07K7/06 C07K14/00
A61K38/08 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 25561 (US HEALTH) 23 December 1993 see page 5, line 1 - line 20; claims 9-17,22,24 ---	1-5,9-19
Y	BIOORG. MED. CHEM. LETT. (1994), 4(21), 2605-8, XP000576247 MILLER, MICHAEL J. ET AL: "EPSP synthase inhibitors design. IV. New aromatic substrate analogs and symmetrical inhibitors containing novel 3- phosphate mimics" see the whole document ---	1-5,9-19
A	WO,A,94 07913 (WARNER LAMBERT CO) 14 April 1994 see claims; examples --- -/--	14-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

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

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

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

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

"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

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

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 1996	25.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seufert, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/04311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 08600 (JOSLIN DIABETES CENTER INC) 28 April 1994 see claims; examples	14-31
P,X	CHEMICAL ABSTRACTS, vol. 124, no. 13, 25 March 1996 Columbus, Ohio, US; abstract no. 169152, KIMURA, TOSHIRO ET AL: "Quantitative Structure-Activity Relationships of the Synthetic Substrates for Elastase Enzyme Using Nonlinear Partial Least Squares Regression" XP002008491 see RN 174097-31-5, Propanedioic acid, [4-(2-amino-2-carboxyethyl)phenoxy]-, (S)- & J. CHEM. INF. COMPUT. SCI. (1996), 36(2), 185-9,	1-4,9,11
P,X	TETRAHEDRON LETT. (1995), 36(27), 4733-6, XP002008488 YE, BIN ET AL: "L-O-(2-Malonyl)tyrosine (L-OMT), a new phosphotyrosyl mimic suitably protected for solid-phase synthesis of signal transduction inhibitory peptides" see the whole document	1-5,9, 11,12, 14,24-27
P,X	J. MED. CHEM. (1995), 38(21), 4270-5, XP002008489 YE, BIN ET AL: "L-O-(2-Malonyl)tyrosine: A New Phosphotyrosyl Mimetic for the Preparation of Src Homology 2 Domain Inhibitory Peptides" see the whole document	1-5,9, 11,12, 14,19-27
P,X	BIOCHEM. BIOPHYS. RES. COMMUN. (1995), 209(3), 817-22, XP002008490 KOLE, HERMANTA K. ET AL: "Protein-tyrosine phosphatase inhibition by a peptide containing the phosphotyrosyl mimetic, L-O-malonyltyrosine" see the whole document	1,3, 9-11,14

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INTERNATIONAL SEARCH REPORT	In[illegible]ational application No. PCT/US96/04311

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 8,16-19,28-31 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interna   l Application No
PCT/US 96/04311

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9325561	23-12-93	US-A-	5264607	23-11-93
		AU-B-	4630193	04-01-94
		US-A-	5475129	12-12-95
WO-A-9407913	14-04-94	AU-B-	5136093	26-04-94
WO-A-9408600	28-04-94	NONE		