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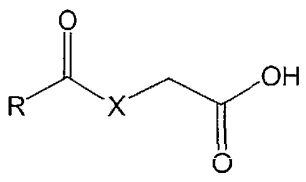
(54) Title: ENZYME INHIBITORS



(I)

(57) Abstract: A compound of Formula (I) wherein R is a biomolecular residue, or derivative thereof, of a *C*-terminal amide biomolecule which is activated by the action of PAM; and X is O or CH^2 or a salt, or prodrug thereof.

ENZYME INHIBITORS

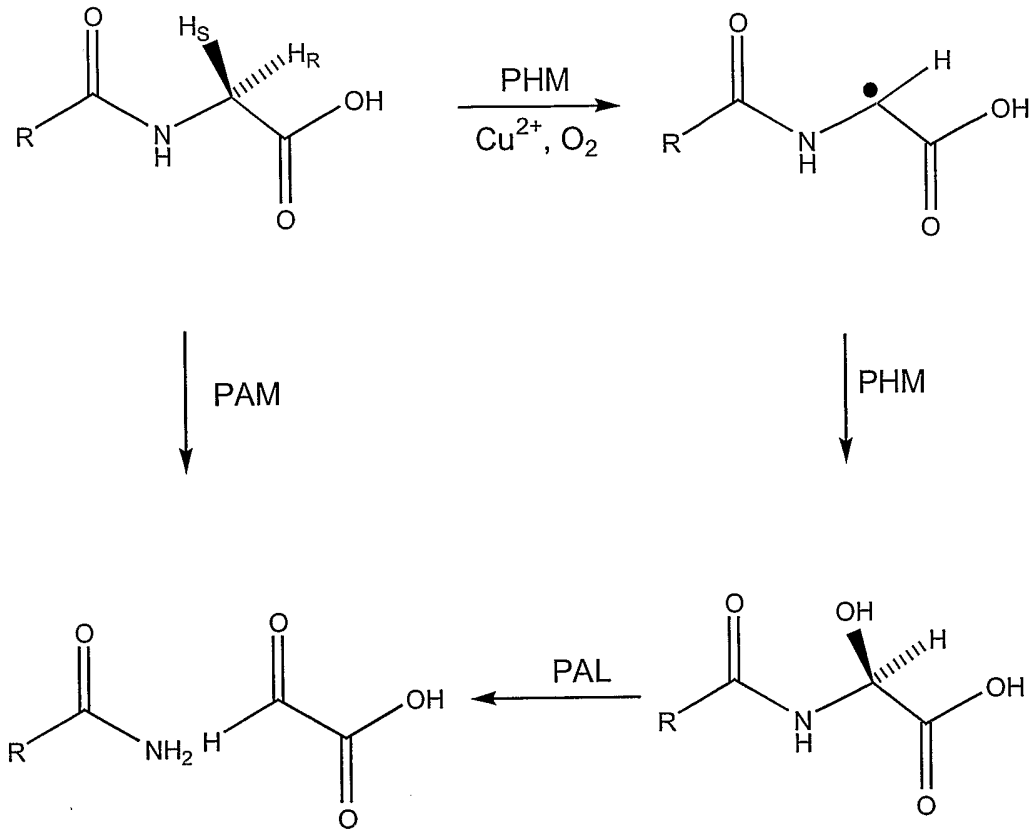
FIELD OF THE INVENTION

The present invention relates to inhibitors of peptidylglycine α-amidating monooxygenase (PAM) and their use in therapy. In particular, the invention relates to glycolate ester and the corresponding methylene equivalent analogues of peptide hormones implicated in disease states or conditions in mammals, their use in therapy and compositions containing them.

BACKGROUND

Carboxy terminal amidation is a required post-translational modification for the bioactivation of many glycine-extended peptides which are derived from direct gene translation products, the presence of an α-amide group on the carboxy terminal end of the peptide chain being essential for bioactivity. The resultant *C*-terminal amides are known for their role in cellular communication, in particular as neuropeptide and growth hormones, and are implicated in a variety of pathological conditions, including asthma, inflammation and tumours.

Peptidylglycine α-amidating monooxygenase catalyses the formation of *C*-terminal amides by oxidative cleavage of the corresponding *C*-terminal glycine extended precursors. PAM consists of two independent enzymatic domains; peptidylglycine α-hydroxylating monooxygenase (PHM, E.C. 1.14.17.3), and peptidylamidoglycolate lyase (PAL, E.C. 4.3.2.5).[1,2] PHM catalyses the molecular oxygen, copper and ascorbate dependent hydroxylation of a *C*-terminal glycine residue. The hydroxyglycine produced is then hydrolysed to give the amide product and glyoxylate, a process which is catalysed by PAL at physiological pH. In the key step, mediated by PHM, a copper bound superoxide radical abstracts the pro-*S* hydrogen from the glycine residue, to give an α-glycyl radical (Scheme 1). It is believed that more than one form of PAM exists, each form having a selectivity towards the *C*-terminal amidation of particular glycine extended biomolecules.



Scheme 1

The *C*-terminal amidation of other glycine extended biomolecules, including fatty acids[3,4], bile acids[5], nicotinic acids[6], and aspirin[7], has also been attributed to PAM. Fatty acid amides are known to affect a number of neurochemical communication pathways, including sleep regulation, while the amidation of aspirin may be important in its metabolic processing.

The importance of the role of PAM in pathological conditions has led to interest in its regulation, and the development of a number of classes of inhibitors. One of the first inhibitors was phenylbutenoic acid, which has been demonstrated to be effective *in vivo* in reducing serum PAM activity, as well as showing anti-inflammatory and analgesic activity. Other olefinic substrate analogues have since been identified as suicide inhibitors, including α,β-unsaturated acids, a terminal vinylglycine peptide, and diastereomers of a terminal styrylglycine peptide, all of which show turnover dependent inactivation of PAM,

although are not potent inhibitors. A number of other inhibitors have also been reported, including inorganic sulfite, benzyl hydrazine, *N*-formylamides, unsaturated thioacetic acids and *N*-substituted homocysteine analogues.

In view of the role of PAM, in particular the PHM mediated radical formation, in the formation of *C*-terminal amides which are implicated in a wide range of pathological conditions, there remains a need for new inhibitors of PAM, preferably inhibitors which exhibit a selectivity of action and/or have improved inhibitory activity.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

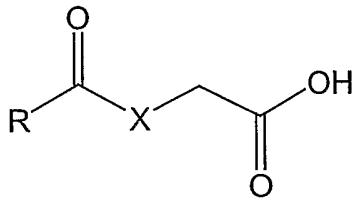
The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that the prior art forms part of the common general knowledge in Australia.

It has now been found that analogues of PAM substrates which cannot form a stable radical may provide inhibitors of PAM and thus may have utility in therapeutic applications, in particular in the treatment of a disease or condition in which the action of PAM on a substrate is implicated. The present invention thus relates to inhibitors which may be useful in therapy by inhibiting PAM such that excessive, undesirable or inappropriate *C*-terminal amide biomolecule (eg peptide hormones) formation is prevented or reduced.

Thus, the present invention provides analogues of PAM substrates, said analogue being a molecule wherein the group -C(O)-NH-CH_2-CO_2H of a PAM substrate is replaced by a moiety unable to form a stable radical. In particular, glycine terminated glycolate ester

analogues (-C(O)-O-CH_2-CO_2H), or their methylene equivalents, (-C(O)-CH_2-CH_2-CO_2H) of PAM substrates may provide strongly binding inhibitors of PAM. The "methylene equivalent" compounds may be more hydrolytically stable than the corresponding glycolate esters and may advantageously therefore have longer term effects in some applications. Correspondingly, the glycloate esters may advantageously provide short term activity in appropriate applications due to more rapid hydrolysis.

In one aspect, the present invention provides a compound of Formula (I)



wherein R is a biomolecular residue, or derivative thereof, of a *C*-terminal amide biomolecule which is activated by the action of PAM; and

X is O or CH_2;

or a salt, or prodrug thereof.

In one embodiment, the present invention provides a compound of Formula (I) wherein R is a peptide chain residue, or derivative thereof, of a *C*-terminal amide peptide which is activated by the action of PAM; and

X is O or CH_2;

or a salt, or prodrug thereof.

The invention also provides a method for inhibiting PAM comprising the step of contacting PAM with an inhibiting effective amount of a compound of Formula (I).

Inhibition of PAM may occur *in vivo* such as in a mammalian system, or *in vitro,* for example as used in a laboratory assay for determining inhibitory activity of a compound.

In yet another aspect, the invention provides a composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt or prodrug thereof, together with at least

one pharmaceutically acceptable carrier or adjuvant.

Still another aspect of the invention relates to a method of treating a disease or condition in which the activity of PAM is implicated, comprising the step of administering a compound of Formula (I), or a pharmaceutically acceptable salt, derivative or prodrug thereof, to a subject in need of said treatment.

The present invention further relates to the use of a compound of Formula (I), or a pharmaceutically acceptable salt, or prodrug thereof, in the manufacture of a medicament for the treatment of a disease or condition in which the activity of PAM is implicated.

In particular, the invention relates to diseases or conditions which involve the undesirable or inappropriate formation of a *C*-terminal amide peptide or other biomolecule by virtue of the action of PAM on a glycine extended compound.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated in part on the previously unrecognised instability of the α-glycolate radical and the surprising finding that the glycolate ester analogue has a binding affinity for PAM comparable to that of the biological glycine substrate.

Without intending to limit the invention by theory, the present invention may provide potential inhibitors which not only essentially retain the binding affinity of the natural amido-glycine substrate but whose corresponding α-radical is too unstable to be formed in the PAM hydroxylation step as described above, ie by action of PHM, in Scheme 1. It is believed that glycolate ester (or methylene equivalent) analogues of glycine extended biomolecules may provide selective inhibitors of the PAM enzyme which acts on the glycine extended biomolecule to provide the *C*-terminal amido biomolecule. In particular, inhibitors which are based on a natural biomolecule may advantageously show selectivity for one or more types of PAM and may therefore act as selective inhibitors for the formation of the corresponding biomolecule.

As used herein, a "PAM substrate" refers to a glycine extended precursor molecule, R-CO-NH-CH_2-CO_2H, which is oxidatively cleaved by PAM to afford the corresponding *C*-terminal amide biomolecule R-$CONH_2$.

A "*C*-terminal amide biomolecule" or "biomolecule" refers to a compound R-$CONH_2$ which is biologically active and includes peptide, lipid and bile acid amides. Particular biomolecules of interest are those whose activity are implicated in a disease or condition in a subject, eg the disease or condition is characterised by excessive, undesirable or inappropriate activity of the biomolecule. The "biomolecular residue" is the non-terminal amido portion (R-) of the biomolecule.

The compounds of Formula (I) are glycolate ester or methylene analogues of the glycine extended biomolecules which are oxidatively cleaved by PAM to provide the *C*-terminal amide biomolecule. As used herein, a biomolecule which is activated by the action of PAM is the compound R-$CONH_2$ resulting from the PAM-mediated oxidative cleavage of the corresponding glycine extended precursor. Thus R is the biomolecular residue of an amido compound R-C(O)-NH_2 which is activated or released by action of PAM on the corresponding glycine extended precursor (PAM substrate).

In one embodiment of the invention, the compounds of Formula (I) are preferably glycolate ester or methylene analogues of the glycine extended peptides which are oxidatively cleaved by PAM to provide the biological *C*-terminal amido peptides. As used herein, a peptide which is activated by PAM is the *C*-terminal amide peptide resulting from the oxidative cleavage of the corresponding *C*-terminal glycine extended precursor. Thus, R is the peptide chain residue of any *C*-terminal amide peptide R-C(O)-NH_2 which is activated or released by action of PAM on the corresponding glycine extended precursor.

C-Terminal and primary amides play extensive and wide-ranging roles in the functioning of an animal, particularly mammalian systems, and as noted above, are implicated in many disease states or conditions. Thus, prevention of their activation by action of PAM on the corresponding glycine extended precursor may have therapeutic benefits.

A preferred class of biomolecules contemplated by the present invention are peptides, such as peptide hormones. As referred to herein, the term "peptide" includes naturally occurring peptides and other amino acid sequences comprising amino acids, including those selected from conventional or common amino acids Ala, Asn, Cys, Gln, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, Asp, Glu, Arg, His and Lys, non-conventional amino acids and modifications and variations (such as homo- or non-natural forms) thereof. Preferred peptides are naturally occurring peptides. Preferably, the peptide, (and accordingly R-) is at least 3 amino acid residues in length.

A number of non-conventional amino acids and their abbreviations are listed below:

α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr

	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
	D-threonine	Dthr	L-norleucine	Nle
5	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
	D-valine	Dval	α-methyl- -aminobutyrate	Mgabu
	D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
	D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
10	D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
	D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
	D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
15	D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
	D-α-methylleucine	Dmleu	α-napthylalanine	Anap
	D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
	D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
20	D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
25	D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
30	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg

	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
5	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
10	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
15	γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
	L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L-α-methylalanine	Mala
	L-α-methylarginine	Marg	L-α-methylasparagine	Masn
20	L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
	L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
	L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
	L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
	L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
25	L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
	L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
	L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
	L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
	L-α-methylserine	Mser	L-α-methylthreonine	Mthr
30	L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
	L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe

carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc	pyroglutamyl	PyroGlu

The following non-exhaustive list of peptides includes examples of those which have bioactivity and are contemplated by the present invention (the cited documents incorporated herein by reference).

1) Neuropeptide Y controls appetite and blood pressure as well as pain processing and response in the spine. In particular, it is implicated in obesity and gastrointestinal problems associated with diabetes.[8]

2) Bombesin regulates bile secretion and hepatic proliferation and is implicated in prostate cancer, obesity and tumour growth (breast, prostatic and pancreatic).[9]

3) Substance P is thought to induce asthma through stimulation of the neurokinan receptor.[10]

4) Gastrin is a gastrointestinal hormone and neurotransmitter and is believed to be involved in tumour growth (breast, prostatic and pancreatic) and in gastrointestinal adenocarcinoma.[9b,9e,11]

5) Oxytoxin is involved in the female reproductive system and is implicated in osteoporosis.[9b,12]

6) α-Melanocyte stimulating hormone is implicated in Rheumatoid arthritis and control of food uptake (anorexia nervosa and obesity).[9b,13]

7) Pancreastatin is found in the pancreas, inhibits insulin secretion and is thus implicated in Type 2 diabetes.[9b,14]

8) Enkephalin is an opioid peptide implicated in prolonged stress, affective and behaviour disorders and cardiovascular and gastric diseases.[15]

9) Galanin is implicated in neuropathic and inflammatory pain and Alzheimer's disease.[16]

10) Pituitary Adenylate Cyclase is involved in asthma, bronchioconstriction, chronic obstructive pulmonary disease and other inflammatory airway diseases.[17]

11) Neuromedin B acts on the central nervous system and is implicated in obesity and spontaneous feeding.[9b,18]

12) Calcitonin gene related peptide is a vasorelaxant and is involved in systemic vasodilation and microvascular leakage during mesenteric reperfusion.[19]

13) Cholecystokinin is involved in inflammation and axotomy.[16a,20]

14) Neurokinin **A** is involved in the modulation of keratinocyte nerve growth factor and may be useful in the treatment of cutaneous nerves during inflammation and wound healing.[9b,21]

15) Amylin is expressed in β-cells and is co-secreted with insulin in response to nutrient stimuli. It is implicated in human insulinomas and Type II diabetes.[9b,22]

16) Vasopressin is implicated in aggressive behaviour and is an anti-diuretic and vasoconstrictor.[23]

17) Secretin is implicated in autism.[9b,24]

18) Growth Hormone Releasing hormone regulates the release of growth hormone from the anterior pituitary lobe and is implicated in cancers and carcinomas.[9b,25]

Thus in preferred embodiments of the invention, R of Formula (I) is the peptide chain residue of one of the above-mentioned peptides, however it is to be understood that the invention extends to all *C*-amidated peptides or other biomolecules whose activity, by virtue of the action of PAM on the corresponding *C*-terminal glycine precursor, is involved or implicated in a pathological condition.

Other suitable examples of biomolecules released by action of PAM on the corresponding glycine extended precursor substrate are fatty acid and bile acid primary amides. These may include those mentioned in References 3 and 4, the contents of which are incorporated herein by reference. Thus, examples of compounds of Formula (I) may include, but are not limited to, the glycolate ester or methylene equivalent analogues of deoxycholylglycine, cholylglycine, chendeoxycholylglycine, 3-sulfolithocholylglycine, oleamide, palmitamide, ecruamide, palmitoleamide, elaidamide, and linoleamide.

Furthermore, *C*-terminal amides are essential to biological functioning of insects[26] and cnidarians.[27] Thus compounds of Formula (I) where the R group corresponds to the peptide chain residue of an insecticidal peptide hormone may provide effective insecticides. One example is Allostatin which is a juvenile insect hormone.[9b,28]

The compounds of Formula (I) may be prepared in accordance with the generalised Scheme 3 of Example 2. Thus for example, where the compound of Formula (I) is an analogue of a peptide, the desired starting peptide R-CO_2H may be prepared according to standard procedures in the art for peptide synthesis. Reaction with benzyl bromoacetate and subsequent cleavage of the benzyl group affords the compound of Formula (I). Where appropriate, alternative protecting groups to the benzyl group may be used. Suitable alternative protecting groups, are described in *Protective Groups in Organic Synthesis*, T.W. Greene and P.G.M. Wutz, 3rd Edition, Wiley Interscience.

The compounds of Formula (I) may be useful in therapeutic applications for the treatment of a disease or condition where the activity of PAM is implicated, in particular in the *C*-terminal amide formation of a peptide or other biomolecule whose undesirable or

inappropriate formation and action is implicated in a disease or condition. Thus, by inhibiting the activity of PAM, excessive, undesirable or inappropriate formation of the *C*-terminal amide peptide from the corresponding glycine extended precursor may be prevented or reduced.

Diseases and conditions contemplated by the present invention include, but are not limited to, those discussed together with the noted peptides above. Thus, for example, a compound of Formula (I) wherein R corresponds to the peptide chain residue of Substance P may be useful in the treatment of a disease or condition in which Substance P is implicated, eg asthma.

In therapeutic applications, the compounds of the invention, or a pharmaceutically acceptable salt, or prodrug thereof, are administered to a subject in an amount sufficient to inhibit the action of PAM on the glycine extended precursor peptide.

The subject may be human or other animal such as a mammal. Non-human subjects include, but are not limited to primates, livestock animals (eg sheep, cows, horses, pigs, goats), domestic animals (eg dogs, cats), birds and laboratory test animals (eg mice, rats, rabbits, guinea pigs).

An amount sufficient or effective to inhibit the action of PAM relates to an amount of compound which when administered according to a desired dosing regime inhibits the action of PAM and provides the desired therapeutic effect. Inhibition refers to the reduction, slowing down, halting, delaying or otherwise preventing the oxidative cleavage amidation by PAM on a glycine extended precursor.

The compounds of the invention are administered to the subject in a treatment effective amount. As used herein, a treatment effective amount attains the desired therapeutic effect and is intended to include an amount which at least partially attains the desired effect, or delays the onset of, or inhibits the progression of, or halts or reverses altogether the onset or progression of the particular disease or condition being treated.

As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired inhibitory or therapeutic activity. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. Suitable dosages lie within the range of about 0.1 mg per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 μg to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician and may depend on the particular condition being treated, the severity of the condition as well as the general age, health and weight of the subject.

The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition. The formulation of such compositions is well known to those skilled in the art. The composition may contain any suitable carriers, diluents or excipients. These include all conventional solvents, dispersion media, fillers, solid carriers, coatings, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those routinely employed for the administration of peptide compounds and may include those suitable for oral, rectal, pulmonary, nasal, topical (including buccal and sublingual), vaginal or parental (including subcutaneous, intramuscular, intravenous and

intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g inert diluent, preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or

sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Compositions suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal patches may also be used to administer the compounds of the invention.

Compositions for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter, glycerin, gelatin or polyethylene glycol.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

The compounds of the invention may also be presented for use in veterinary compositions. These may be prepared by any suitable means known in the art. Examples of such compositions include those adapted for:

(a) oral administration, external application (eg drenches including aqueous and non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pellets for admixture with feedstuffs, pastes for application to the tongue ;

(b) parenteral administration, eg subcutaneous, intramuscular or intravenous injection as a sterile solution or suspension

(c) topical application eg creams, ointments, gels, lotions etc.

The term "salt, or prodrug" includes any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly, for example, by photolytic, chemical or *in vivo* enzymatic or hydrolytic degradation) a compound as described herein. However, it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the

invention since these may be useful in the preparation of pharmaceutically acceptable salts. Any compound that is a prodrug of a compound of formula (I) is within the scope and spirit of the invention. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted in vivo to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester, such as an acetate, or where a free amino group is converted into an amide. Procedures for acylating the compounds of the invention are well known in the art and may include treatment of the compound with an appropriate carboxylic acid, anhydride or chloride in the presence of a suitable catalyst or base. Other suitable prodrugs may include esters of free carboxylic acid groups. Glycolate esters, such as alkyl or benzyl esters, may provide useful inhibitors of PAM for short term applications or other temporal applications. The preparation of suitable prodrugs is further described in "Design of Prodrugs", H. Bundgaard, *Elsevier*, 1985. Other suitable prodrugs may include those which incorporate a photolytically cleavable group as described below.

Suitable pharmaceutically acceptable salts include, but are not limited to salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benezenesulphonic, salicyclic sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

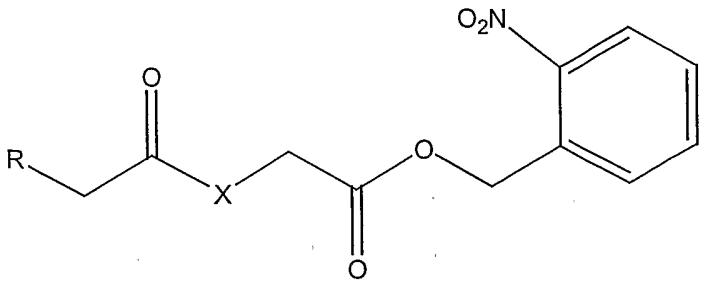
Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium. In particular, the present invention includes within its scope cationic salts eg sodium or potassium salts, or alkyl esters (eg methyl, ethyl).

Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

The compounds of the invention may be in crystalline form either as the free compounds or as solvates (e.g. hydrates) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

Derivatives of biomolecular residues include variations and modifications of the residue such that the (preferably selective) PAM inhibitory activity is essentially retained. Derivatives of a peptide chain residue include any substitutions, deletions, insertions or other variations in a peptide chain such that the (preferably selective) PAM inhibitory activity is essentially retained. Such variations of one or more amino acids may include the insertion, deletion or substitution of one or more conventional, non-conventional or modified amino acids. Preferably the peptide chain residue (R) is at least 3 amino acid residues in length.

Administration of a compound of Formula (I) as a pharmaceutically acceptable salt, derivative or prodrug may assist in controlling the rate and location of release of the inhibitor. In addition to those described above, a suitable prodrug of a compound of Formula (I) could incorporate a photolytically cleavable group. Thus, by masking the glycolate moiety of the inhibitor with a photolabile group, the masked inhibitor could be delivered to a specific target area and irradiated to release the inhibitor. In addition, the rate of release may be controlled by control of the intensity of radiation employed. Thus, targeted delivery and/or controlled release of the inhibitor may be possible. Suitable photolabile groups would be known to the skilled person.[29] One example thereof is the 2-nitrobenzyl group. Thus, coupling of an appropriate 2-nitrobenzyl compound with the terminal carboxylic acid of the compound of Formula (I) may be effected to provide the masked product illustrated below.



The invention will now be described with reference to the following Examples which are included for the purpose of illustration and are not intended to limit the generality hereinbefore described.

EXAMPLES

General

NMR spectra were recorded in $CDCl_3$ or CD_3OD, on a Varian Gemini 300 or a VRX 500S spectrometer. Electron impact (EI) mass spectra were obtained using a VG Autospec double focussing trisector mass spectrometer operating at 70 eV. Optical rotations were recorded on a Perkin Elmer polarimeter. Microanalyses were performed on a Carlo-Erba 1106 autoanalyser, by the Research School of Chemistry Microanalytical Service.

An Osram Ultra-Vitalux (240 V, 300 W, E27) sunlamp was used as the light source, to initiate radical reactions, at a distance of 5–10 cm from the reaction vessel. Chromatography was performed with Merck Kieselgel 60 (230-400 mesh ATSM). High performance liquid chromatography (HPLC) was carried out on a Waters Alliance 2690 Chromatography Module, with a Waters 996 Photodiode Array Detector and a Waters Fraction Collector II, in conjunction with a Compaq Deskpro Data Station running Waters Millennium 32 chromatography management software, and either a Waters Symmetry Prep™ C18 5 μm column (4.6 x 250 mm) for analytical separations, or a YMC-Pack ODS-AQ 5 μm column (10 x 250 mm) for preparative separations.

Solvents were generally used as purchased. CCl_4 was purified by fractional distillation over 4Å sieves. Benzyl bromoacetate, iodomethane, and decanoic acid, were obtained from Aldrich Chem. Co. *N*-acetyl-L-phenylalanyl-L-phenylalanine, *N*-acety-DL-phenylalanine and BOP were obtained from Auspep. 2-[*N*-Morpholino]-ethanesulfonic acid (MES), ascorbic acid, glycine t-butyl ester and Tween 20 were obtained from Sigma Chem. Co. Recombinant peptidylglycine α-amidating enzyme (PAM) was obtained from Wako Pure Chemical Industries. *N*-Benzoylglycine methyl ester (**1a**) and *N*-benzoylvaline were prepared according to standard procedures. [30-32]

Example 1

Radical formation of a glycolate ester vs an amide

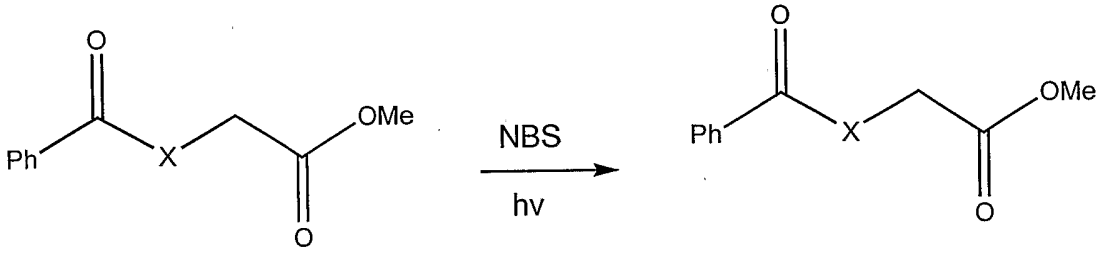
An indirect method for determining the relative stability of an α-glycine radical and an α-glycolate radical is by examining the rate at which bromination at the α-position occurs. Thus, in radical brominations using N-bromosuccinimide (NBS) a bromine radical abstracts an α-hydrogen from the substrate to afford a substrate radical intermediate which reacts with bromine to form the α-bromo compound. The transition state for the homolysis of the α-C-H bond is close to the radical intermediate and thus, the rate of radical bromination reflects the stability of the α-radical.

The respective brominations of N-benzoylglycine methyl ester (1a) and the analogous glycolate ester (1b) were examined as a mixture of the two as follows:

Methyl-O-benzoylglycolate (1b). Benzoic acid (50 mg, 0.41 mmol) was dissolved in acetone (30ml) and treated with methyl bromoactetate (0.060 mL, 0.63 mmol) and di-potassium carbonate (100 mg, 0.73 mmol). The solution was refluxed overnight, then purified by column chromatography to give the title compound (40 mg, 50%). Anal. calcd: C, 61.85; H, 5.19: Found: C, 61.84; H, 5.22. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.8 (s, 3H), δ 4.9 (s, 2H), δ 7.45 (m, 2H), δ 7.6 (m, 1H), δ 8.1 (m, 2H).

Treatment of methyl hippurate with NBS. *N*-Bromo succinimide (16.6 mg, 0.09 mmol) was added to a solution of methyl hippurate (1a) (20 mg, 0.10 mmol) in CCl_4 (5 mL), and the mixture was heated at reflux for 0.3 h while being irradiated with a sunlamp. The reaction mixture was cooled and filtered, and the filtrate evaporated to give a solid residue. The crude solid was taken up in $CDCl_3$ for analysis. ^1H NMR (300 MHz, $CDCl_3$), δ 3.95 (s, 3H), δ 6.7 (d, 1H), δ 7.5 (m, 2H), δ 7.6 (m, 1H), δ 7.8 (m, 2H).

Treatment of methyl hippurate (1a) and methyl O*-benzoylglycolate (1b) with NBS.* *N*-bromo succinimide (8.3 mg, 0.046 mmol) was added to a solution of methyl *O*-benzoylglycolate (1b) (10 mg, 0.05 mmol) and methyl hippurate (1b) (10 mg, 0.05 mmol) in CCl_4 (5 mL). The reaction mixture was cooled and filtered, and the filtrate evaporated to give a solid residue. The crude solid was taken up in $CDCl_3$ for analysis. ^1H NMR revealed a combination of the spectra obtained for α-bromo hippurate methyl ester and methyl *O*-benzoylglycolate. The resulting determined relative rates of reaction are depicted in Table 1.



(1a) X = NH

(1b) X = O

Table 1

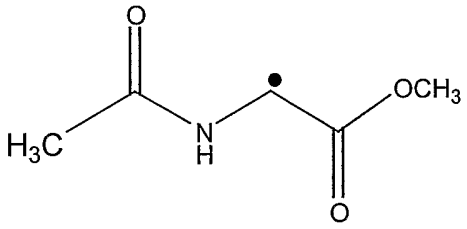
Compound	k_{rel}
1a	1.0
1b	0

Thus, only the amide was reactive, indicating the relative instability of the α-glycolate radical.

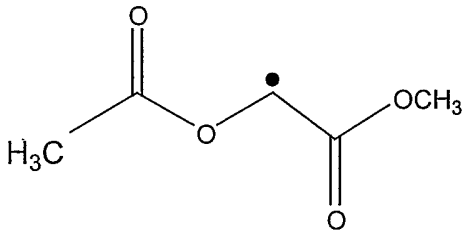
In addition, the stability of a radical may be assessed by theoretical calculations of the Radical Stabilization Energy (RSE). Radical Stabilization Energies were calculated by the isodemic method[34], which relates the stability of the radical in question to that of the methyl radical, with the more stable radical having the more positive value.

The calculated RSEs for acetylglycine methyl ester radical (2a), acetylglycolate methyl ester radical (2b) and propionic acid radical (2c) are shown in Table 2. Calculations were performed according to RMP2/6-311+G(2df,p)//UB3-LYP/6-31G(d). It is noted that the RSE values for 2b and 2c are comparable.

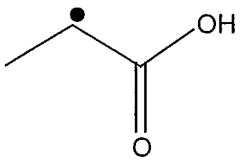
Table 2



(2a)



(2b)



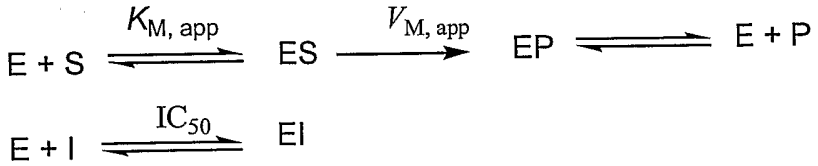
(2c)

Radical	RSE (kJ/mol)
2a	82.2
2b	44.7
2c	42.2

Example 2

Comparative PAM binding studies

The interaction between a substrate and enzyme and an inhibitor and enzyme can be illustrated as below in Scheme 2.



where E = enzyme, S = substrate, I = inhibitor and P = product

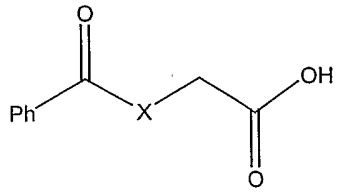
Scheme 2

$K_{M, app}$ indicates the ease of binding of a substrate to an enzyme – lower numbers correspond to a greater extent of binding at low concentrations,

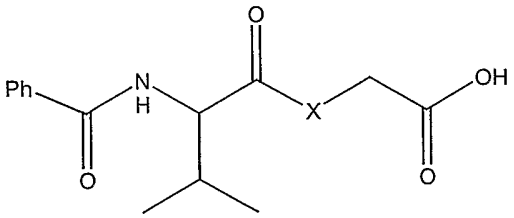
$V_{M, app}$ is the rate at which the enzyme converts bound substrate to product,

IC_{50} is the concentration required to decrease the enzyme activity by 50% and indicates the ease of binding of an inhibitor to an enzyme - lower numbers correspond to a greater extent of binding at low concentrations and therefore a more potent inhibitor. An inhibitor is not processed by an enzyme and therefore has a $V_{M, app}$ of zero.

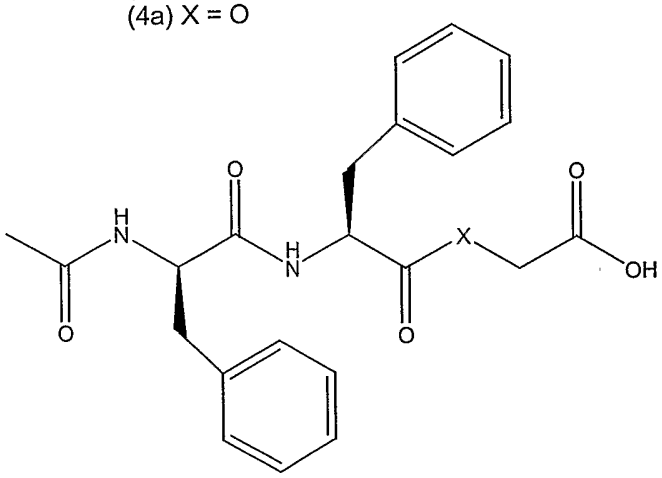
Four model substrate (glycine) (3a-d)\ inhibitor (glycolate) (4a-d) systems were evaluated for their binding affinity to PAM. Methods for the preparation thereof are described below. The comparative binding results are depicted in Table 3 which follows the methodology.



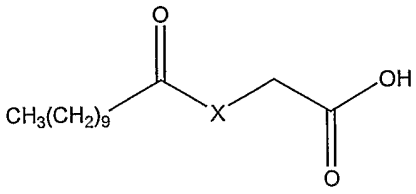
(3a) X = NH
(4a) X = O



(3b) X = NH
(4b) X = O



(3c) X = NH
(4c) X = O



(3d) X = NH
(4d) X = O

N-Benzoyl (3a), N-Acetyl-phenylalanyl-phenylalanyl (3c) and decanoyl glycine (3d) were synthesised by carbodiimide coupling of glycine methyl ester and the respective carboxylic acids. Saponification of the methyl ester provided (3a), (3c) and (3d).

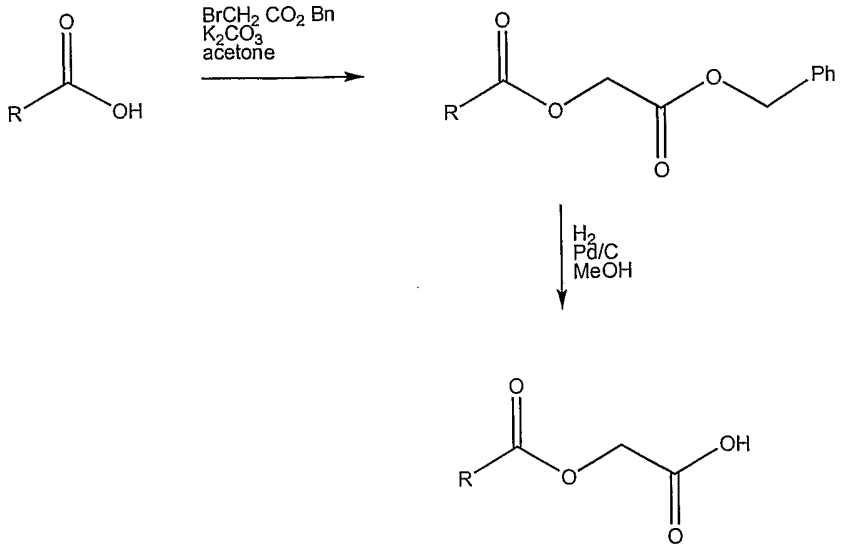
Synthesis of 3b

N-*Benzoylvalylglycine t-butyl ester.* Glycine t-butyl ester (485 mg, 2.26 mmol), BOP (1.28 g, 2.9 mmol) and DIPEA (1.0 mL, 4.52 mmol) were added to a solution of *N*-benzoylvaline (640 mg, 2.9 mmol) in CH_2Cl_2 (100 mL. The solution was allowed to stir overnight before being evaporated. The residue was taken up in ethyl acetate (50 mL) and washed with saturated sodium bicarbonate (3 × 50 mL) and hydrochloric acid (1M,

50 mL). The organic layer was then dried and evaporated to give a solid residue which was recrystallisd from ethyl acetate/hexane to give the title compound (672mg, 89%). Mp 172 °C. $[\alpha]_D$ –1.25° (c = 0.001, EtOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (d, J = 1.8 Hz, 3H), 1.04 (d, J = 2.1 Hz, 3H), 1.46 (s, 9H), 2.24 (m, 1H), 3.85 (dd, J = 4.8, 4.8 Hz, 2H), 4.04 (dd, J = 5.7, 5.4 Hz, 2H), 4.55 (dd, J = 6.6, 6.9 Hz, 1H), 6.65 (s, 1H), 6.89 (d, J = 8.7 Hz, 1H), 7.39 (m, 3H), 7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5, 19.5, 28.3, 31.7, 42.3, 58.9, 82.6, 127.3, 128.8, 132.0, 134.3, 167.6, 168.7, 171.5. HRMS (EI) calcd for $C_{18}H_{26}N_2O_4$ [MH^+] *m/zI* 334.1893, found 334.1892. Anal. Calcd for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.48; H, 8.02; N, 8.22.

N-Benzoylvalylglycine (3b). Triethylsilane *N*-Bz-Valyl-Glycine t-Bu Ester (29 mg, 0.086 mmol), was dissolved in dichloromethane (20 ml), to which tri-ethyl silane (45 µl) and trifluoroacetic acid (0.85 ml) was added. The reaction was left overnight and then dried by rotary evaporation and (high vacuum) to give the title compound (25mg, 88%): m.p 180 °C: $[\alpha]_D$ –32.21°: ^{1}H NMR (CD_3OD), δ 1.0 (m, 6H), 2.2 (m, 1H), 4.0 (q, 2H), 4.5 (d, 1H), 7.4 (m, 3H), 7.8 (m, 2H).

Compounds (4a)-(4d) were synthesised according to the generalised Scheme 3. Details are provided below.



Scheme 3

Synthesis of 4a

Benzyl O-benzoylglycolate. Benzoic acid (5.37 g, 44 mmol) was added to a suspension of potassium carbonate (6.07 g, 44 mmol) in acetone (100 mL). Benzyl bromoacetate (6.9 mL, 44 mmol) was then added and the resulting suspension was refluxed overnight. After cooling, the solution was filtered and the filtrate evaporated to give the title compound (10.77 g, 90.7 %) as a colourless solid. Mp 54°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.86 (s, 3H), 5.20 (s, 3H), 7.32 (m, 5H), 7.41 (m, 2H), 7.54 (m, 1H), 8.07 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 167.4, 165.6, 134.9, 133.2, 129.7, 128.9, 128.4, 128.3, 128.2, 128.1, 66.9, 61.0 ppm. MS (EI): *m/z* = 270 ($M^{+\bullet}$,5), 211 (12), 163 (12), 148 (65), 120 (20), 105 (100), 91 (85), 77 (60). HRMS (EI) calcd for $C_{16}H_{14}O_4$ [$M^{+\bullet}$] *m/z* 270.0892, found 270.0892. Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10, H, 5.22. Found: C, 71.05, H, 5.27.

O-Benzoylglycolic Acid (4a). Palladium on carbon (10%, 2 g) was placed in a dry flask, which was then purged with nitrogen gas and evacuated three times, followed by purging with hydrogen gas and evacuating three times. The reaction vessel was kept under an atmosphere of hydrogen gas. Benzyl *O*-benzoylglycolate (2 g, 7.4 mmol) in methanol (60 mL) was added and the resulting suspension was stirred overnight at room temperature. The suspension was filtered through a plug of celite, and evaporated to give the title compound (1.3 g, 98%) as a white solid. A portion (1.1 g) of this material was recrystallised from chloroform to give colourless crystals (0.47 g) for use in assays. Mp 110°C. ^{1}H NMR (300 MHz, CD_3OD) δ 4.83 (s, 3H), 7.48 (m, 2H), 7.60 (m, 1H), 8.07 (d, 2H, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 167.4, 134.2, 130.7, 130.5, 129.3, 62.78. MS (EI): *m/z* = 180 ($M^{+\bullet}$,24), 148 (10), 136 (15), 105 (100), 91 (18), 77 (60). HRMS (EI) calcd for $C_9H_8O_4$ [$M^{+\bullet}$] *m/z* 180.0423, found 180.0424. Anal. Calcd for $C_{16}H_{14}O_4$: C, 60.00, H, 4.48. Found C, 59.74 , H, 4.52.

Synthesis of 4b

Benzyl-O-(N-*benzoylvalyl)-glycolate.* *N*-Benzoylvaline (360 mg, 1.62 mmol) was dissolved in acetone (100 mL) to which potassium carbonate (134 mg, 1.62 mmol) and benzyl bromoacetate (0.25 mL, 1.62 mmol) were added. The resulting suspension was then heated to reflux overnight, allowed to cool and then filtered. The filtrate was then evaporated to dryness and the crude white solid recrystallised from hot ethyl acetate to afford the title compound (540 mg, 90%) as a colourless solid. m.p 92 °C. $[\alpha]_D$ –22.9 °C (*c* = 0.001, CH_3CH_2OH). ^1H NMR (300 MHz, $CDCl_3$): 1.04 (d, 1H, *J* = 6.9), 1.07 (d, 1H, *J* = 6.9), 2.34-2.45 (m, 1H), 4.64 (d, 1H, *J*= 15.9), 4.86 (d, 1H, *J* = 15.9), 4.91 (dd, 1H, *J* = 3.9), 5.21 (s, 2H), 6.60 (d, 1H, *J* = 8.7), 7.33-7.55 (m, 8H), 7.79-7.82 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 171.3, 167.8, 135.6, 131.6, 128.4, 128.3, 128.2, 128.2, 127.4, 66.9, 61.2, 58.8, 30.7, 18.7, 17.9. HRMS calcd for $C_{21}H_{23}NO_5$ [MH^+] 369.1576, found 369.1574. Anal. Calcd for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.38; H, 6.60; N, 3.67.

O-*(N-Benzoylvalyl)-glycolic acid (4b).* Benzyl-O-(N-benzoylvalyl)-glycolate (500mg, 1.35mmole) was dissolved in tetrahydrofuran (100ml) to which activated palladium on carbon (100mg) was added. The reaction was placed under an atmosphere of nitrogen and stirred for 12 hours. Filtration followed by rotary evaporation of the solution gave white solid which was purified by HPLC to give the title compound (360mg, 1.29 mmol) in 95% crude yield. M.p: 100°C. ^1H NMR (300 MHz, CD_3OD): 1.1 (m, 6H), 2.4 (m, 1H), 4.5 (q, 2H), 4.72 (d, 1H), 7.45 (m, 3H), 7.9 (m, 2H). ^{13}C NMR (300 MHz, CD_3OD): 171.29, 169.10, 168.42 134.12, 131.67 128.33, 127.42, 60.98, 58.78, 51.60, 30.71, 18.66, 17.96. HRMS calcd. for $C_{14}H_{17}NO_5$ (MH^+) 279.110673, found 279.110476.

Synthesis of 4c

Benzyl-O-(N-acetyl-phenylalanyl-phenylalanyl)-glycolate Benzyl bromoacetate (0.46 mL, 0.14 mmol) was added to a suspension of *N*–acetylphenylalanyl-phenyalanine (500 mg, 0.14 mmol) and potassium carbonate (111 mg, 86.7 mmol) in acetone (100 mL). The reaction mixture was stirred at reflux for 24 h, then cooled and filtered. The filtrate was evaporated to give a white solid which was recrystallised from ethylacetate/hexane to give the title compound (600 mg, 84%) as a white powder. Mp 169 °C. $[\alpha_D]$ -27.12° (c = 0.001, CH_3CH_2OH). ^1H NMR (300 MHz, CD_3OD): δ 1.82 (s, 3H), 2.75 (dd, 1H, J = 9.3 Hz), 2.94 (dd, 1H, J = 9.3 Hz), 3.05 (dd, 1H, J = 5.7Hz), 3.22 (dd, 1H, J = 5.1 Hz), 4.60 (dd, 1H, J = 5.7 Hz), 4.68 (d, 1H, J = 15.9 Hz), 4.75 (d, 1H, J = 15.9 Hz), 4.76 (dd, 1H, J = 5.1 Hz), 5.20 (s, 2H), 7.15-7.38 (m, 15H). ^{13}C NMR (300 MHz, CD_3OD): 174.41, 173.76,172.95, 169.87, 139.30, 138.85, 137.74, 131.23, 131.12, 130.48, 130.35, 130.33, 130.30, 130.22, 128.70, 125.51, 68.95, 63.15, 56.53, 55.57, 39.64, 39.06, 23.19. Anal. Calcd: C, 69.31; H, 6.02; N, 5.57. Found: C, 69.24; H, 6.43; N, 5.49. MS (ESI): *m/z* 503.2 $[MH]^+$, 525.1 $[MNa]^+$, 541.1 $[MK]^+$. HRMS (EI) calcd. for $C_{29}H_{30}N_2O_6$ $[MH]^+$: 502.210, found 502.211.

O-*(N-Acetyl-phenylalanyl-phenylalanyl)-glycolic acid (4c).* Benzyl-*O*-(*N*-acetyl-phenylalanyl-phenylalanyl)-glycolate (300 mg, 0.60 mmol) was added to a suspension of activated palladium on carbon (10%, 30 mg) in tetrahydrofuran (50 mL) under a hydrogen atmosphere. The reaction mixture was stirred overnight, filtered through a plug of celite, and evaporated to give a white solid which was crystallised from EtOH/hexane to give the title compound (203mg, 82%) as a white powder: Mp 152 °C. $[\alpha_D]$ –21.71°. ^1H NMR (300 MHz, CD_3OD): δ 1.82 (s, 3H), 2.77 (dd, J = 9.3 Hz, 1H), 2.99 (dd, J = 9.0 Hz, 1H), 3.07

(dd, J = 5.4 Hz, 1H), 3.27 (dd, J = 4.8 Hz, 1H), 3.30 (dd, J = 1.8 Hz, 1H), 4.60 (d, J = 15.9 Hz, 1H), 4.59-4.64 (m, 1H), 4.64 (d, J = 16.2 Hz, 1H), 4.74-4.82 (m, 1H), 7.14-7.28 (m, 10H), 8.43 (d, J = 8.1 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.2, 37.1, 37.6, 53.7, 54.54, 61.0, 126.5, 126.7, 128.2, 128.3, 129.1, 129.3, 136.9, 137.3, 170.9, 171.8, 172.3.

Synthesis of 4d

Benzyl-O-decanoylglycolate. Decanoic acid (200mg, 1.16mmol) was dissolved in acetone (50ml) to which di-potassium carbonate (156.88mg, 1.16mmole) and 2-bromo benzylacetate (0.14ml, 1.16mmole) were added. The solution was refluxed for 12 hours, filtered and evaporated to dryness by rotary evaporation. The oil was then purified by column chromatography (ethyl acetate/hexane) to give the decanoic-glycolate ester (196mg, 0.612 mmol) as a clear oil in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$): 0.90 (t, J = 6.9 Hz, 3H), 1.28 (s, 12H), 1.65 (m, 2H), 2.42 (t, J = 7.5 Hz, 2H), 4.65 (s, 2H), 5.19 (s, 2H), 7.35 (m, 5H). ^{13}C NMR (300 MHz, $CDCl_3$): 173.3, 168.0, 135.3, 128.9, 128.9, 128.9, 128.8, 128.6, 67.4, 60.9, 34.2, 32.3, 29.8, 29.7, 29.7, 29.5, 25.2, 23.1, 14.6. HRMS calcd. for $C_{19}H_{28}O_4$ (MH^+) 320.198760, found 320.198945.

O-Decanoylglycolic acid (4d). Benzyl-*O*-decanoylglycolate (780 mg, 2.44 mmol) was dissolved in methanol (50 mL), to which activated palladium on carbon (10%, 156 mg) was added. The reaction was placed under an atmosphere of hydrogen and stirred overnight at room temperature. The suspension was filtered through a plug of celite, and evaporated to give a white solid which was crystallised from hot ethyl acetate/hexane to give the title compound (450mg, 80%). Mp 54 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.90 (t, 3H, J = 6.9 Hz), 1.30 (s, 12H), 1.56-1.67 (m, 2H), 2.41 (t, 2H, J = 7.5 Hz), 4.59 (s, 2H). ^{13}C NMR (300 MHz, CD_3OD): 173.50, 60.40, 33.55, 32.01, 23.53, 29.39, 29.38, 29.08, 24.87, 22.71, 13.44. MS (EI): *m/z* 231.0. HRMS (EI) calcd for $C_{12}H_{22}O_4$ [MH^+]: 231.1596, found 231.1599. Anal. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.21; H, 9.81.

Assays for PAM substrates (glycines) and inhibitors (glycolates) were performed using a Modified Literature Procedure.[35]

Assay Procedure.

Assays for PAM inhibition were performed with 3 mM ascorbic acid, 2 μM copper sulfate, 100 μg/mL bovine liver catalase, 0.01% Tween 20, 1% ethanol with 0.1 mM substrate (Tyr-Val-Gly), and varying concentrations of inhibitors in 100 mM MES buffer at pH 6.6. Assays were initiated by the addition of the appropriate amount of enzyme to a final volume of 60 μL, then incubated at 37 °C for one hour, after which the assay was quenched by the addition of 30 μL of 1M NaOH, then neutralized with 30 μL of 1M HCl, a 20 μL aliquot was then taken and diluted to 110 μL with water. The diluted solution was then injected directly onto RP-HPLC, and the ratio of substrate (*D*-Tyr-*L*-Val-Gly) to product (*D*-Tyr-*L*-Val-NH_2) determined by integration, to give the amount processed.

Double reciprocal plots were used to determine $K_{M,\,app}$ and $V_{M,\,app}$ values. Dixon plots were used to determine K_I values.

The results are depicted in Table 3

Table 3

Compound	$K_{M,app}$ (mM)	$V_{M,app}$ (μmol min^{-1} mg^{-1})[a]	IC_{50} (mM)
3a (Bz-Gly)	1.3[3]	6.5[3]	
4a (Bz-Glc)	-	-	0.25
3b (Bz-Val-Gly)	0.03		
4b (Bz-Val-Glc)	-	-	0.5
3c (Ac-Phe-Phe-Gly)	0.0012[34]	3.3[34]	
4c (Ac-Phe-Phe-Glc)	-	-	0.5
3d (Dec-Gly)	0.1[3]	12.6[3]	
4d (Dec-Glc)	-	-	0.04

[a] $V_{m,app}$ varies from different sources, and depends on enzyme purity, as such this value is only comparable from the same publication and enzyme source. Sources – [3]-rat, -[34] – bovine

These results indicate that the binding affinity of the glycolate analogues to PAM are comparable to that of the glycine substrates.

Example 3

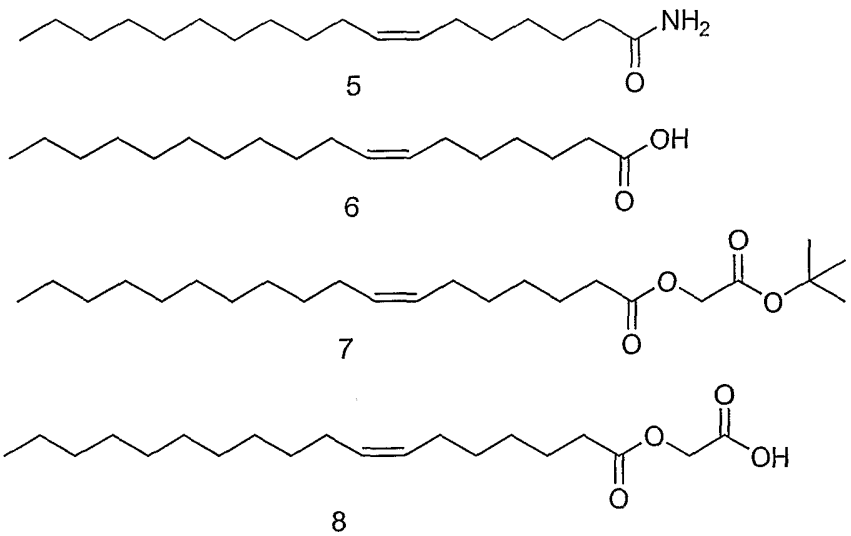
Preparation of glycolate ester analogues of biomolecules.

Oleic acid was obtained from Aldrich Chemical Co. TRH free acid was obtained from Sigma Chemical Co.

3-I *Glycolate analogue of oleamide.*

Oleamide (5) is a hormone involved in sleep regulation, and has been shown to be produced in the brains of sleep deprived cats, and to induce sleep.[4a, b] The glycolate analogue (8) of the glycine extended prohormone of oleamide has been synthesised by the previously described methodology, with a minor protection group modification. As a

result of the double bond in oleic acid, the benzyl ester could not be used, so the *t*-butyl ester protecting group was used instead, and deprotection effected by treatment with TFA (6→ 7→ 8)



t-*Butyl*-O-*oleoylglycolate (7)*

Oleic acid (6) (0.56 mL, 1.76 mmol) was dissolved in acetone (10 mL), and potassium carbonate (242 mg, 1.76 mmol) and *t*-butyl bromoacetate (1.76 μL) was added and the resulting solution refluxed overnight. After cooling, the reaction solution was filtered and evaporated to give the title compound as a colourless oil (0.44 g, 63%).

^{1}H NMR ($CDCl_3$) δ 0.82 (t, J = 6 Hz, 3H), 1.15-1.35 (m, 20H), 1.40 (s, 9H), 1.60 (m, 2H), 1.94 (m, 4H), 2.33 (t, J = 7.5 Hz, 2H), 4.42 (s, 2H), 5.27 (m, 2H).

^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 24.6, 27.0, 27.1, 27.6, 27.8, 28.9, 29.0, 29.2, 29.4, 29.5, 29.6, 37.8, 33.7, 60.8, 82.0, 129.6, 129.8, 166.8, 172.8.

ESI m/z = 419.3 (MNa^+).

O-*Oleoylglycolic Acid (8)*

t-Butyl-*O*-oleoylglycolate *(7)* (121.4 mg, 0.31 mmol), was dissolved in CH_2Cl_1 (2 mL), and triethylsilane (98 μL, 0.61 mmol) added, followed by trifluoroacetic acid (236 μL, 3.1 mmol), and the resulting solution stirred overnight at room temperature, then evaporated to dryness to give the title compound (103 mg, 98.5%) as a waxy, white solid.

^{1}H NMR (CD_3OD) δ 0.89 (t, J = 6.5 Hz, 3H), 1.20-1.45 (m, 20H), 1.64 (m, 2H), 2.02 (m,

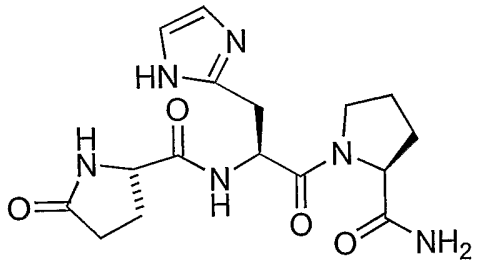
4H), 2.39 (t, J = 6.5 Hz, 2H), 4.58 (s, 2H), 5.39 (apparent t, J = 6 Hz, 2H).

^{13}C NMR (CD_3OD) δ 14.5, 23.8, 25.9,, 28.2, 30.1, 30.2, 30.3, 30.4, 30.5, 30.7, 30.8, 30.9, 33.1, 34.6, 61.4, 130.8, 130.9, 171.3, 174.6.

ESI m/z = 363.4 (MNa^+), 341.4 (MH^+).

3-II *Glycolate ester analogue of Thyrotropin Releasing Hormone*

Thyrotropin releasing hormone (TRH) (9) is a hormone produced by the hypothalamus, and, as its name suggests, stimulates the release of thyrotropin, as well as having effects on a variety of other hormones. A glycolate ester inhibitor based on the structure of TRH is the ester 12, and this has been derived from the free acid 10 analogue of the natural hormone 9.



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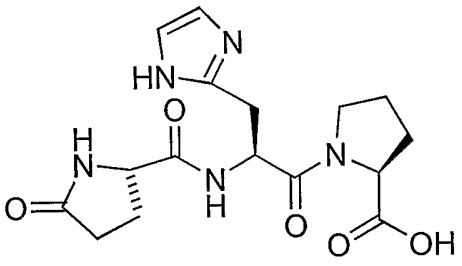

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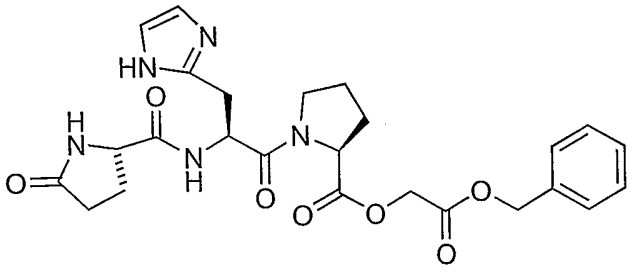

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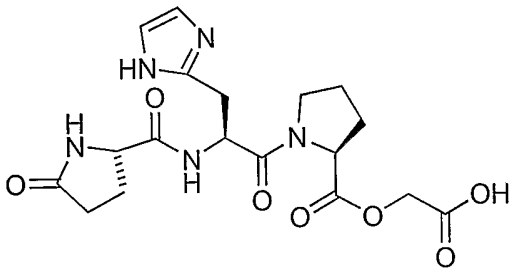


12

Benzyl-O-(pyroglutamyl-histidinyl-prolyl)-glycolate (11)

The tripeptide 10 (2.9 mg, 83% purity, 6.9 μmol) was dissolved in DMF (2 mL), and added to potassium carbonate (0.96 mg, 6.9 μmol), followed by benzyl bromoacetate (1.09 μL in

DMF (100 μL), 6.9 μmol), and the resulting solution heated at 80°C overnight. Evaporation gave a residue containing the title compound. ESI *m/z* = 512.0 (MH^+).

O-*(pyroglutamyl-histidinyl-prolyl)-glycolic acid (12).*

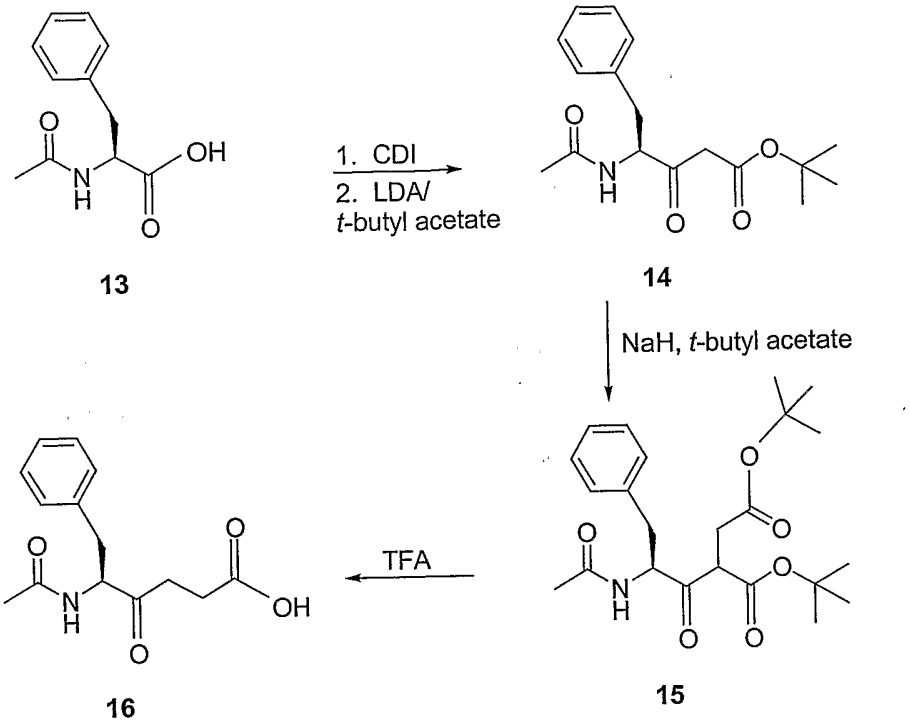
The residue containing benzyl-*O*-(pyroglutamyl-histidinyl-prolyl)-glycolate (11) from the above reaction was added to a suspension of activated palladium on carbon (10%, 4 mg) in tetrahydrofuran (3 x 2 mL) under a hydrogen atmosphere. The resulting suspension was stirred overnight at room temperature. The reaction mixture was filtered through a plug of celite, and evaporated to give a residue containing the title compound. ESI *m/z* = 421.9 (MH^+).

Example 4

Preparation of methylene analogues

4-I

A suitable procedure for the preparation of methylene analogues is illustrated below and is based on a minor modification of a procedure described by Hoffman and Tao[36]. The illustrated procedure may in particular be readily adapted to any peptide sequence either by direct reaction with the free acid of the desired peptide or more preferably, through coupling the modified penultimate amino acid to the *C*-terminal end of a growing chain in a peptide synthesis.



Malonate (14).

(S)-*N*-acetylphenylalanine (13) (0.5 g, 2.4 mmol) was added to THF (10 mL). As the solution was being stirred, carbonyl di-imidazole (CDI) (0.41 g, 2.54 mmol) was added and the solution was left to stir at room temperature under a nitrogen atmosphere for 1 hr. Meanwhile a solution of lithium *t*-butylacetate was prepared from butyllithium (1.6 M, 6 mL, 9.6 mmol), diisopropylamine (2 mL, 14.4 mol), and *t*-butylacetate (1.29 mL, 9.6 mmol) at –78 °C under N_2 atmosphere. The imidazole solution was added dropwise to the pale yellow solution of lithium enolate and left to stir at –78 °C for 40 mins before the reaction was quenched with HCl (25 mL, 1 M) and extracted with ethyl acetate (3 × 20 mL). The organic extracts were combined, washed with brine (25 mL) and dried before being passed through a short pad of silica, followed by chromatography to give the title compound as an orange oil (0.58 g, 1.9 mmol, 79%).

^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.95 (s, 3H0, 3.02 (dd, 1H, J = 6.5, 14.5 Hz), 3.16 (s, 2H), 4.92 (dd, 1H, J = 6.5, 14 Hz), 6.12 (d, 1H, J = 7 Hz), 7.15-7.32 (m, 5H).

ESI m/z = 328.2 (MNa^+), 344.2 (MK^+).

Diester (15).

The ester (14) (0.58 g, 1.9 mmol) was dissolved in dry THF (5 ml) and added dropwise to a stirred suspension of NaH (0.04 g of 60% in mineral oil, 1 mmol) in dry THF (15 ml) at 0 °C under N_2 atmosphere. The mixture was stirred for 10 mins giving a grey suspension to which a solution of *t*-butylbromoacetate (0.31 mL, 1.92 mmol) in dichloromethane (5 mL) was added dropwise. The resulting mixture was stirred at room temperature for 24 h before being quenched with HCl (25 mL, 1 M) and then extracted with ethyl acetate (3 × 25 mL). The organic extracts were combined and washed with brine (50 mL), then dried and evaporated to give a pale yellow oil which was purified by flash chromatography to give a mixture of the diasteroisomers of the title compound (0.13 g, 0.3 mmol, 49.5%).

^{1}H NMR (300MHz, $CDCl_3$) δ, 1.35 and 1.38 (s and s, 4.5H and 4.5H), 1.39 and 1.42 (s and s, 4.5H and 4.5H), 1.90 and 1.91 (s and s, 1.5H and 1.5H), 2.70-3.24 (m, 4H), 4.00 and 4.07 (t and t, J = 6.9 and 6.9 Hz, 0.5H and 0.5H), 5.03 and 5.025 (m and m, 0.5H and 0.5H), 6.21 and 6.38 (d and d, J = 8.1 and 8.1 Hz, 0.5H and 0.5H), 7.11-7.29 (m, 5H).

ESI m/z = 442 (MNa^+).

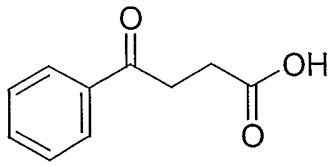
γ-Keto acid (16).

The diester (15) (0.13 g, 0.31 mmol) was dissolved in dichloromethane (30 mL) and treated with TFA (0.6 mL) along with a drop of triethylsilane and refluxed for 16 hours. The reaction was monitored by T.L.C and evaporated upon the disappearance of starting material. Purification by HPLC eluting with acetonitrile and 1% trifluoroacetic acid in water gave the title compound (40mg, 50%) as a white solid.

^{1}H NMR (300Mhz, CD_3OD) δ 1.91 (s, 3H), 2.52 (t, J = 6 Hz, 2H), 2.73-2.85 (m, 5H), 3.15 (d, J = 5.2 Hz, 1H), 3.20 (d, J = 5.2 Hz), 4.66 (dd, J = 4.2 Hz, 1H), 7.19-7.30 (m, 5H).

ESI m/z = 263.9 (M^+), 286.1 (MNa^+).

4-II Methylene analogue of Hippuric acid.



4-Phenyl-4-oxobutanoic acid was synthesized as reported by Mahmoodi[37], and is also available commercially.

5

REFERENCES

1. Bolkenius, F.N.; Ganzhorn, A. J., *Gen. Pharmac.* (1998), **31**(5), 655–659.

2. Prigge, S.T.; Kolhekar, A.S.; Eipper, B.A.; Mains, R.E.; Amzel, L.M., *Science,* (1997), **278**(5341), 1300–1305.

3. Wilcox, B.J.; Ritenour-Rodgers, K.J.; Asser, A.S.; Baumgart, L.E.; Baumgart, M. A.; Boger, D.L.; DeBlassio, J.L.; deLong, M.A.; Glufke, U.; Henz, M.E.; King, L., III; Merkler, K.A.; Patterson, J.E.; Robleski, J.J.; Vederas, J.C.; Merkler, D.J., *Biochemistry,* (1999), **38**, 3235–3245.

4. (a) Cravatt. B.F., Prospero-Garcia, O., Suizdak, G., Gilula, N.B., Henriksen, S.J., Boger, D.L., Lerner, R.A., *Science*, (1995), **268**, 1506.

   (b) Cravatt, B.F.; Boger, D.L.; Lerner, R.A., *J. Am. Chem., Soc.*, (1996), **118,** 580.

5. King III, L.; Barnes, S.; Glufke, U.; Henz, M.E.; Kirk, M.; Merkler, K.A.; Vederas, J.C.; Wilcox, B. J.; Merkler, D.J., *Arch., Biochem. Biophys.*, (2000), **374**, 107.

6. Merkler, D.J.; Glufke, U.; Ritenour-Rodgers, K.J.; Baumgart, L.E.; DeBlasio, J.L.; Merkler, K.A.; Vederas, J.C.; *J. Am. Chem. Soc.*, (1999), **121**, 4904.

7. DeBlasio, J.L.; deLong, M.A.; Glufke, U.; Kulathila, R.; Merkler, K.A.; Vederas, J.C.; Merkler, D.J.; *Arch., Biochem. Biophys*, (2000), **383**, 46.

8. (a) Holmgren, S.; Jensen, J., *Brain Res. Bull.,* (2001), **55**(6), 723–735.

   (b) Marx, R.; El Meskini, R.; Johns, D.C.; Mains, R.E., *J. Neurosci.,* (1999), **19**(19), 8300–8311.

   (c) Spångéus, A.; Suhr, O.; El-Salhy, M., *Histol. Histopathol.,* (2000), **15**, 739–744.

(d) Yaksh, T. L.; Hua, X.-Y.; Kalcheva, I.; Nozaki-Taguchi, N.; Marsala, M., *Proc. Natl. Acad. Sci. U.S.A.,* (1999), **96**(14), 7680–7686.

9. (a) Yoneda, M.; Watanobe, H.; Terano, A., *J. Gastroenterol.,* (2001), **36**(6), 361–367.

(b) Merkler, D. J., *Enzyme Microb. Technol,.* (1994), **16**, 450–456.

(c) Salido, M.; Vilches, J.; López, A.; Roomans, G. M., *Cancer,* (2002), **94**(2), 368–377.

(d) Yamada, K.; Wada, E.; Wada, K., *Ann. Med.,* (2000), **32**(8), 519–529.

(e) Schally, A. V.; Comaru-Schally, A. M.; Nagy, A.; Kovacs, M.; Szepeshazi, K.; Plonowski, A.; Varga, J. L.; Halmos, G., *Front. Neuroendocrinol.,* (2001), **22**, 248–291.

10. (a) DeVane, C.L., *Pharmacotherapy,* (2001), **21**(9), 1061–1069.

(b) Jeng, A.Y.; Fujimoto, R.A.; Chou, M.; Tan, J.; Erion, M.D., *J. Biol. Chem.,* (1997), **272**(23), 14666–14671.

(c) Lundberg, J.M., *Can. J. Physiol. Pharmacol.,* (1995), **73**(7), 908–914.

11. Watson, S.A.; Gilliam, A.D.; Smith, A.M., *Drug. Future,* (2001), **26**(9), 865–872.

12. López, F.J., *Curr. Opin. Chem. Biol.,* (2000), **4**(4), 383–393.

13. (a) Catania, A.; Gerloni, V.; Procaccia, S.; Airaghi, L.; Manfredi, M.G.; Lomater, C.; Grossi, L.; Lipton, J.M., *NeuroImmunoModulation,* (1994), **1**(5), 321–328.

(b) Vrinten, D.H.; Kalkman, C.J.; Adan, R.A.H.; Gispen, W.H., *Eur. J. Pharmacol.,* (2001), **429**(1–3), 61–69.

(c) Adan, R.A.H.; Vink, T., *Euro. Neuropsychopharmacol.,* (2001), **11**(6), 483–490.

14. Sánchez-Margalet, V., *Diabetologia,* (1999), **42**(3), 317–325.

15. Drolet, G.; Dumont, É.C.; Gosselin, I.; Kinkead, R.; Laforest, S.; Trottier, J.-F., *Prog. Neuro-Psychopharmacol. Biol. Psychiat.,* (2001), **25**(4), 729–741.

16. (a) Wiesenfeld-Hallin, Z.; Xu, X.-J., *Eur. J. Pharmacol.* (2001), **429**(1-3), 49–59.

(b) Nilsson, C. L.; Brinkmalm, A.; Minthon, L.; Blennow, K.; Ekman, R., *Peptides,* (2001), **22**(12), 2105–2112.

17. (a) Kinhult, J.; Uddman, R.; Laan, M.; Lindén, A.; Cardell, L.-O., *Peptides,* (2001), **22**(12), 2151–2154.

(b) Hansel, D.E.; May, V.; Eipper, B.A.; Ronnett, G.V., *J. Neurosci.,* (2001), **21**(13), 4625–4636.

18. Yamada, K.; Wada, E.; Yamano, M.; Sun, Y.-J.; Ohara-Imaizumi, M.; Nagamatsu, S.; Wada, K., *Brain Res.,* (2002), **942**(1,2), 71–78.

19. (a) Madeddu, P.; Emanueli, C.; Bonaria Salis, M.; Franca Milia, A.; Stacca, T.; Carta, L.; Pinna, A.; Deiana, M.; Gaspa, L., *Peptides,* (2001), **22**(6), 915–922.

(b) Guembe, L.; Villaro, A.C.; Treston, A.M., *J. Histochem. Cytochem.*, (1999), **47**(5), 623–636.

20. Tozawa, K.; Arakawa, E.; Chikuma, T.; Ohhashi, Y.; Yajima, R.; Takeda, K.; Shinozaki, H.; Kato, T., *J. Neurochem.,* (1990), **55**(3), 745–749.

21. Burbach, G. J.; Kim, K. H.; Zivony, A. S.; Kim, A.; Aranda, J.; Wright, S.; Naik, S.M.; Caughman, S.W.; Ansel, J.C.; Armstrong, C.A., *J. Invest. Dermatol.,* (2001), **117**(5), 1075–1082.

22. Gebre-Medhin, S.; Olofsson, C.; Mulder, H., *Diabetologia,* (2000), **43**(6), 687–95.

23. (a) Russell, J.A., *J. Neuroendocrinol.,* (2002), **14**(1), 1–3.

(b) Grindstaff, R. R.; Cunningham, J. T., *Exp. Neurol.*, (2001), **171**(2), 219–226.

(c) Du, J.; Keegan, B. P.; North, W. G., *Cancer Lett.*, (2001), **165**(2), 211–218.

24. (a) Herzig, K.-H., *Regul. Pept.*, (1998), **73**(2), 89–94.

(b) Hunsinger, D.M.; Nguyen, T.; Zebraski, S.E.; Raffa, R.B., *Life Sci.* (2000), **67**(14), 1667–1682.

25. Schally, A. V.; Varga, J. L., *TEM,* (1999), **10**(10), 383–391.

26. (a) Konopinska, D; Rosinski, G.; Sabotka, W., *Int. J. Protein Res.*, (1992), **39**, 1.

(b) Schoofs, L.; Veelaert, D.; Vanden Broeck, V.; DeLoof, A., *Peptides*, (1997), **18**, 145.

27. (a) Hauser, F.; Williamson, M.; Grimmlhuijzen, C.J.P., *Biochem. Biophys. Res. Commun.*, (1997), **241**, 509.

(b) Williamson, M.; Hauser, F.; Grimmlhuijzen, C.J.P., *Biochem. Biophys. Res. Commun.*, (2000), **277**, 7.

28. Meyering-Vos, M.; Wu, X.; Huang, J.; Jindra, M.; Hoffmann, K. H.; Sehnal, F., *Mol. Cell. Endocrinol.*, (2001), **184**(1–2), 103–114.

29. Bochet, C.G.; *J. Chem. Soc., Perkin Trans.*, (2002), **1,** 125-142.

30. Easton, C.J.; Peters S.C. *Aust J. Chem.*, 1994, **47,** 859-868.

31 Carpino, L.A.; Chao, H.G.; Howshad, F.; Shroff, H.J., *J. Org Chem.,* 1988, **53**, 6139-6144.

32. Burgess, V.A.; Easton, C.J., *Spectrose, Lett.*, 1991, **24**, 1059-1070.

33. Ping, D.; Mounier, C.E.; May, S.W., *J. Biol Chem.,* 1995, **270**, 29250-29255.

34. (a) Parkinson, C.J., Mayer, P.M.; Radom, L., *J. Chem. Soc., Perkin Trans.*, (1999), **2**, 2305.

(b) Parkinson, C.J., Mayer, P.M.; Radom, L., *Theor. Chem. Acc.*, (1999), **102**, 92.

35. (a) Katopodis, A.G.; May, S.W., *Biochem.*, (1990), **29**, 4541.

(b) Casara, P.; Ganzhorn, A.; Philppo, C.; Chanal, M.-C.; Danzin, C., *Bioorg. Med. Chem., Lett.*, (1996), **6**, 393.

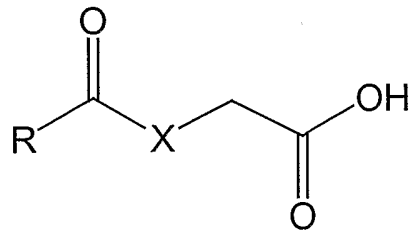
(c) Ogonowski, A.A.; May, S.W.; Moore, A.B.; Barrett, L.T.; O'Bryant, C.L.; Pollock, S.H., *J. Pharmacol. Exp. Ther.*, (1997), **280**, 846.

36 Hoffaman, R.V.; Tao, J. H., Tetrahedron 1997, **53**, 7119-7126.

37. Mahmoodi, N.O.; Jazayri, M., *Synthetic Communications*, 2001, **31**, 1467-1475.

CLAIMS

1. A compound of Formula (I):



wherein R is a biomolecular residue, or derivative thereof, of a *C*-terminal amide biomolecule which is activated by the action of PAM; and

X is O or CH_2;

or a salt, or prodrug thereof.

2. A compound according to claim 1 wherein X is O.

3. A compound according to claim 1 wherein X is CH_2.

4. A compound according to claim 1 wherein the biomolecule is a peptide, a fatty acid amide or bile acid amide.

5. A compound according to claim 2 wherein the biomolecule is a peptide.

6. A compound according to claim 5 wherein the peptide is selected from the group consisting of: Neuropeptide Y, Bombesin, Substance P, Gastrin, Oxytoxin, α-Melanocyte stimulating hormone, Pancreastatin, Enkephalin, Galanin, Pituitary Adenylate Cyclase, Neuromedin B, Calcitonin gene related peptide, Cholecystokinin, Neurokinin, Amylin, Vasopressin, Secretin, Growth Hormone Releasing hormone and Thyrotropin Releasing Hormone.

7. A compound according to claim 4 wherein the biomolecule is selected from the

group consisting of: deoxycholylglycine, cholylglycine, chendeoxycholylglycine, 3-sulfolithocholylglycine, oleamide, palmitamide, ecruamide, palmitoleamide, elaidamide, and linoleamide.

8. A prodrug of a compound according to claim 1 which incorporates a photolytically cleavable group.

9. A compound according to claim 1 wherein R is a biomolecular residue of a *C*-terminal amide biomolecule which is activated by the action of PAM

10. A method for inhibiting the action of PAM comprising the step of contacting PAM with an inhibitory effective amount of a compound of Formula (I), or a salt or prodrug thereof, as defined in claim 1.

11. A composition comprising a compound of Formula (I), or a salt or prodrug thereof, as defined in claim 1, together with at least one pharmaceutically acceptable carrier or adjuvant.

12. A method of treating a disease or condition in which the activity of PAM is implicated, comprising the step of administering a compound of Formula (I), or a salt or prodrug thereof, as defined in claim 1, to a subject in need of said treatment.

13. The use of a compound of Formula (I), or a salt or prodrug thereof, as defined in claim 1, in the manufacture of a medicament for the treatment of a disease or condition in which the activity of PAM is implicated.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00905

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07C 59/06, 63/06, 229/12, 229/14, 233/48, 59/205; C07K 5/065, 5/117; A61K 31/23, 31/231, 31/235, 38/05, 38/06, 31/225; A61P 29/00, 5/02, 25/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATA BASES BELOW

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Files Medline, CA: key words peptidylglycine, inhibitor, analog?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. PING *et al.*, "Reaction versus subsite stereospecificity of peptidylglycine α-monooxygenase and peptidylglycolate lyase, the two enzymes involved in peptide amidation," J. Biol. Chem., 270(49), 1995, pp. 29250-29255. See whole document.	1-13
X	A. B. MOORE *et al.*, "Kinetic and inhibition studies on substrate channelling in the bifunctional enzyme catalysing C-terminal amidation," Biochem. J., 341, 1999, pp. 33-40. See whole document.	1-13
X	A. G. KATOPODIS *et al.*, "Novel substrates and inhibitors of peptidylglycine α-amidating monooxygenase," Biochemistry, 29, 1990, pp. 4541-4548. See whole document.	1-13

☐ Further documents are listed in the continuation of Box C ☐ See patent family 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 application or patent 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

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2003	13 AUG 2003
Name and mailing address of the ISA/AU	Authorized officer
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