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SYNTHESIS AND EVALUATION OF 2-(BIPHENYLMETHYL)GLUTARIC ACID AMIDE DERIVATIVES AS NEUTRAL ENDOPEPTIDASE INHIBITORS.

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Abstract: A series of 2-(biphenylmethyl)glutaric acid amide derivatives were synthesized and evaluated for NEP inhibitory activity. The mode of inhibitor-enzyme interactions of the most potent compound **3a**, with a thiazolylacetic acid group at the P_2' position, was evaluated by a computer analysis. Copyright © 1996 Elsevier Science Ltd

Neutral endopeptidase (NEP, EC 3.4.24.11) degrades atrial natriuretic peptide (ANP) having diuretic, natriuretic and vasodilating activities. Hence, inhibition of the enzyme is likely to be of clinical benefit in the treatment of the cardiovascular diseases.[1]

In the previous paper, we synthesized 2-(biphenylmethyl)-glutaric acid amide derivatives **1** with potent NEP inhibitory activity, demonstrating that a biphenylmethyl moiety at the P_1' position confers high potency in the series of glutaric acid amide derivatives (Figure 1).[2] To obtain further information for the structure requirements for NEP inhibitors, we replaced the P_2' substituent of **1** by a series of amino-heterocycles which might have hydrogen bond interactions with the enzyme. A computer analysis proposes the mode of inhibitor-enzyme interactions of the most potent compound obtained here.

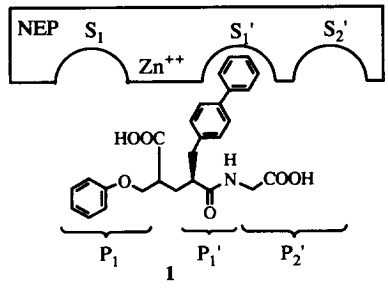

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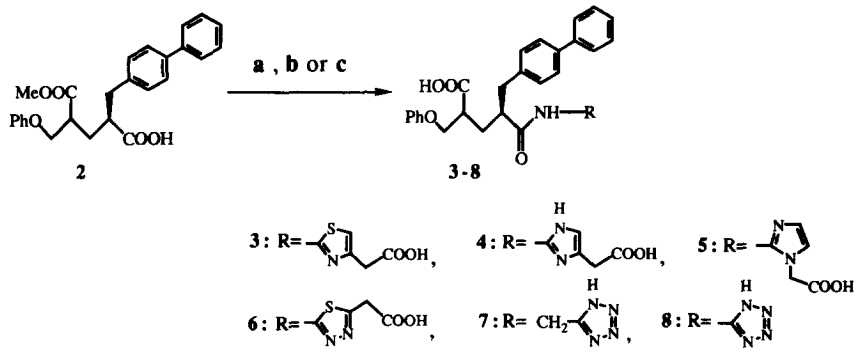
Figure 1.

Chemistry

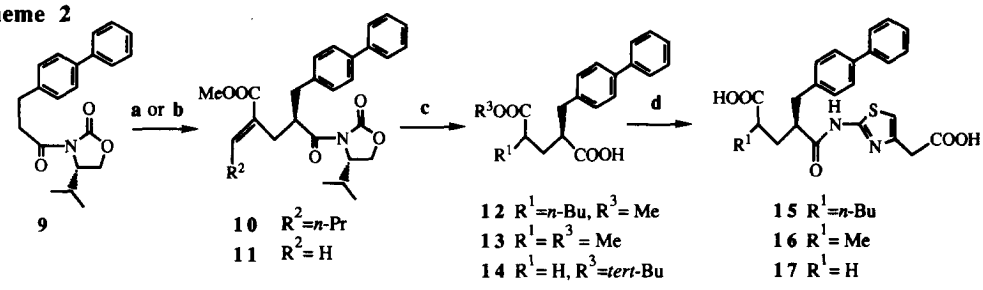
Glutaric acid amide derivatives **3-8** were synthesized by standard coupling reaction of pentanedioic acid mono esters **2**[2] with a variety of amino-heterocycles and successive hydrolysis of the ester group (Scheme 1). Compounds **15-17** were obtained from oxazolidinone **9** through mono acids **12-14** which were prepared by use of the methods reported previously (Scheme 2).[2-4] The acids **12** and **13** were synthesized from **10** and **11**, which were prepared by palladium-catalyzed reaction[3] of lithium enolate of **9** with methyl 3-acetoxy-2-methylenehexanoate and by alkylation[4] of **9** with methyl 2-(bromomethyl)acrylate, respectively.

Scheme 1



Reagents: **a**: i. benzyl (2-aminothiazol-4-yl)acetate, methyl (2-amino-1,3,4-thiadiazol-5-yl)acetate, benzyl (2-aminoimidazol-4-yl)acetate, or *tert*-butyl (2-aminoimidazol-1-yl)acetate, HOBt, $EtN{=}C{=}N(CH_2)_3NMe_2$ (EDCI), *N*-methylmorpholine. ii. $AlCl_3$ Me_2S. **b**: i. [1-(*p*-methoxyphenylmethyl)tetrazol-5-yl]methylamine, HOBt, EDCI, *N*-methylmorpholine. ii. CF_3COOH. iii. $AlCl_3$, Me_2S. **c**: i. 5-aminotetrazole, HOBt, EDCI, *N*-methylmorpholine. ii. $AlCl_3$, Me_2S.

Scheme 2



Reagents: **a**: i. LDA. ii. methyl 3-acetoxy-2-methylenehexanoate, $Pd(Ph_3P)_4$. **b**: i. LDA. ii. methyl 2-(bromomethyl)-acrylate. **c**: i. $PhCH_2OH$, *n*-BuLi. ii. H_2, Pd-C. **d**: i. benzyl (2-aminothiazol-4-yl)acetate, HOBt, EDCI, *N*-methylmorpholine ii. $AlCl_3$, Me_2S.

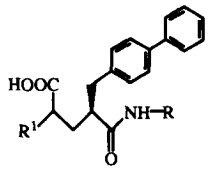
Docking study

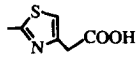
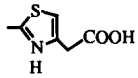
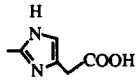
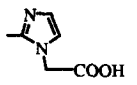
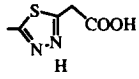
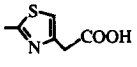
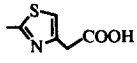
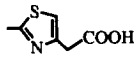
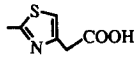
The docking study was performed as follows. Sequence alignment between NEP and thermolysin was performed with the FASTA program[5] and subsequent manual refinement. The three-dimensional model of the active site of NEP was constructed based on the coordinate data of thermolysin-inhibitor complexes in the Brookhaven Protein Data Bank[6] and energy-minimized using QUANTA and CHARMm programs (Biosym/MSI). The three-dimensional model of compound **3a** was constructed based on standard bond angles and lengths and then energy-minimized using the above programs. The resulting conformers were docked into the NEP model using QUANTA on an IRIS computer (Silicon Graphics Incorporation) to give a plausible model of NEP-inhibitor interactions.

Results and discussion

According to the method of Florentin *et al.*, NEP inhibitory activity was determined by measurement of IC_{50} values for the NEP-catalyzed hydrolysis of the synthetic substrate, dansyl-*D*-Ala-Gly-Phe(*p*-NO_2)-Gly (DAGNPG).[7]

Table 1. *In Vitro* NEP-Inhibitory Activity of P_2'-Modified Derivatives



compd	R	R^1	conf.[a]	formula[b] (analysis)	IC_{50}(nM)[c]
1a	CH_2COOH	$PhOCH_2$	S (isomer A)		3.2 ± 0.5[d]
1b	CH_2COOH	$PhOCH_2$	R (isomer B)		5.9 ± 2.6[e]
3a		$PhOCH_2$	(isomer A)	$C_{30}H_{28}N_2O_6S$ (C,H,N)	0.69 ± 0.44
3b		$PhOCH_2$	(isomer B)	$C_{30}H_{28}N_2O_6S$ (C,H,N)	3.3 ± 0.1
4		$PhOCH_2$	SR	$C_{30}H_{29}N_3O_6 \cdot HCl \cdot 1/2H_2O$ (C,H,N,Cl)	218 ± 111
5		$PhOCH_2$	SR	$C_{30}H_{29}N_3O_6 \cdot 1/2H_2O$ (C,H,N)	763 ± 188
6		$PhOCH_2$	SR	$C_{29}H_{27}N_3O_6S$ (C,H,N)	92 ± 33
7		$PhOCH_2$	SR	$C_{27}H_{27}N_5O_4$ (C,H,N)	19 ± 5.7
8		$PhOCH_2$	SR	$C_{26}H_{25}N_5O_4$ (C,H,N)	7.4 ± 3.7
15a		*n*-Bu	(isomer A)	$C_{27}H_{30}N_2O_5S$ (C,H,N)	10 ± 1.8
15b		*n*-Bu	(isomer B)	$C_{27}H_{30}N_2O_5S$ (C,H,N)	9.8 ± 1.2
16		Me	SR	$C_{24}H_{24}N_2O_5S \cdot 1/2H_2O$ (C,H,N)	58 ± 4.0
17		H		$C_{23}H_{22}N_2O_5S$ (C,H,N)	122 ± 60
UK-69578[f]					65 ± 15[g]
thiorphan[f]					7.9 ± 1.6[h]

a) The polar diastereoisomer was shown as isomer B and the less polar diastereoisomer as isomer A in silica gel column chromatography or ODS column HPLC. The absolute configurations are not determined. b) All compounds show ^{1}H-NMR data consistent with the assigned structures. Analytical results are within ±0.4 % of the calculated value. c) The values are the mean ±SEM of three independent experiments except for **1a** and **1b**. d) n=8. e) n=6. f) ref.9. g) n=19. h) n=17.

Thiazolylacetic acid derivative **3a** showed five-times more potent activity than **1a** (Table 1). Replacement of the thiazole ring of **3a** by an imidazole or a thiadiazole moiety resulted in a remarkable decrease in potency. Tetrazol-5-ylmethyl derivative **7**, a bioisosteric compound of **1a**, showed decreased potency. However, tetrazole derivative **8** had comparable activity to **1a**.

In the series of thiazolylacetic acid derivatives **3** and **15-17**, the P_1 substituents displayed increased potency in the order of their hydrophobicity ($PhOCH_2$ > *n*-Bu > Me > H).

A docking study of the most potent compound **3a** with a three-dimensional model of the active site of NEP suggested that the guanidium group of Arg^{747} of NEP had hydrogen bonding interactions with both nitrogen and oxygen atoms of the thiazolylacetic acid moiety (Figure 2). With respect to the oxygen atom, there also might be an alternative interaction with Arg^{102}, which is proposed in the study of other NEP inhibitors.[8] These interactions would contribute to high potency of **3a**. In contrast, the basic imidazole ring of **4** or **5** is expected to be protonated and thereby decreases binding affinity between NEP and inhibitors.

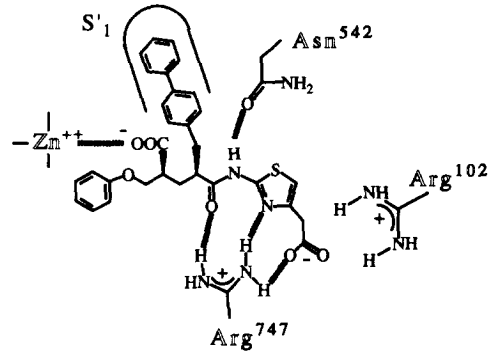


Figure 2. Interaction mode of compound **3a** in the active site of NEP.

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