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POTENT INHIBITORS OF NEUTRAL ENDOPEPTIDASE. 2-BIPHENYL-METHYLGLUTARIC ACID AMIDE DERIVATIVES

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Abstract: A series of glutaric acid amide derivatives were synthesized and tested for NEP inhibitory activity. Compounds **14a**, **14b**, **16a** and **22**, with a biphenylmethyl group at P_1' position, showed potent inhibitory activity.

Atrial natriuretic peptide (ANP), a 28 amino-acid peptide, has diuretic, natriuretic and vasodilating activities which contribute to the regulation of body fluids, electrolytes and vascular tones.[1] Although secretion of ANP is increased in hypertension and congestive heart failure, the peptide is cleaved and inactivated by neutral endopeptidase (NEP, EC 3.4.24.11).[1,2] Therefore, inhibition of the enzyme is likely to be a promising approach to the treatment of the cardiovascular diseases.

A number of studies have described potent NEP inhibitors and have evaluated the mode of inhibitor-enzyme interaction (Figure 1).[3a] A prototype of the inhibitors has a 2-benzylglutaric acid amide skeleton.[3b] Most recently, Ksandar *et al.* have reported 2-biphenylmethylglutaric acid amide derivatives as potent inhibitors,[3c] while the structure-activity relationships (SARs) have not yet been elucidated. We assumed that the hydrophobicity of substituent R^2 should affect activity, since NEP recognizes Phe8 of ANP and cleaves the peptide bond on the amino-terminal side of the residue,[1] suggesting the importance of a hydrophobic benzyl moiety of Phe for the substrate-enzyme interaction. In this study, we synthesized a series of glutaric acid amide derivatives to evaluate the SARs of R^2 and the effects of the substituents R and R^1 on potency.

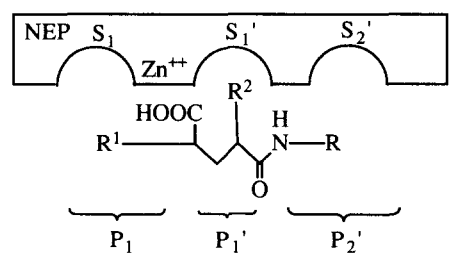
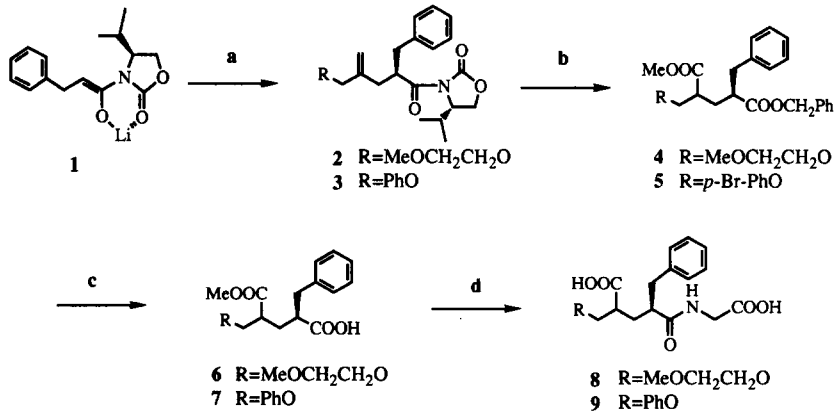


Figure 1. Glutaric acid amide derivative

Chemistry

Reaction of lithium enolate **1** of *N*-(phenylpropanoyl)oxazolidinone with 2-[(2-methoxyethoxy)methyl]-2-propenyl iodide proceeded diastereoselectively to give **2**, which was converted in successive steps to a mixture of diastereomers **6** by alcoholysis, hydroboration, oxidation, sodium bromite oxidation, esterification and debenzylation. Compound **6** was condensed with benzyl glycinate and treated with a combination of aluminum chloride and dimethyl sulfide to give benzyl derivative **8** (Scheme 1). Compound **9** was similarly prepared: however, oxidation reaction of the phenoxy derivative with sodium bromite and subsequent treatment with diazomethane gave 4-bromophenoxy derivative **5**, which could be debrominated by catalytic hydrogenation.

Scheme 1



1

2 R=$MeOCH_2CH_2O$
3 R=PhO

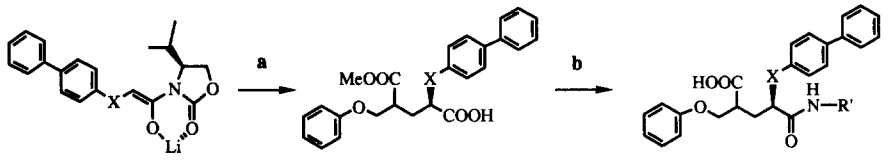
4 R=$MeOCH_2CH_2O$
5 R=*p*-Br-PhO

6 R=$MeOCH_2CH_2O$
7 R=PhO

8 R=$MeOCH_2CH_2O$
9 R=PhO

Reagents: a: 2-(phenoxymethyl)-2-propenyl iodide or 2-[(2-methoxyethoxy)methyl]-2-propenyliodide, THF. **b:** i. $PhCH_2OH$, *n*-BuLi, THF. ii. 9-BBN, THF. iii. H_2O_2, NaOH. iv. $NaBrO_2$, 4-(benzoyloxy)-2,2,6,6-tetramethylpiperidine-1-oxyl, MeCN, 5% $NaHCO_3$. v. CH_2N_2, Et_2O. **c:** H_2, Pd-C, AcOEt. **d:** i. Gly-OCH_2Ph, 1-hydroxybenzotriazole (HOBt), $EtN{=}C{=}N(CH_2)_3NMe_2$ (EDCI), *N*-methylmorpholine, CH_2Cl_2. ii. $AlCl_3$, Me_2S, CH_2Cl_2.

Scheme 2



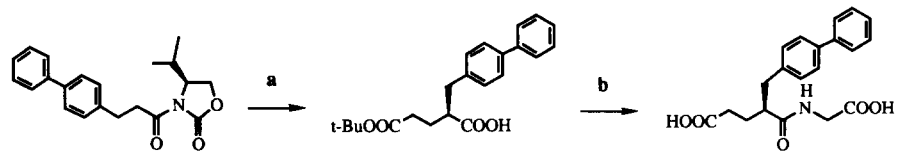
10 $X=CH_2$
11 X=O

12 $X=CH_2$
13 X=O

14-18 $X=CH_2$
19 X=O

Reagents: a: i. methyl 2-(diphenoxymethyl)acrylate, THF. ii. $PhCH_2OH$, *n*-BuLi, THF. iii. H_2, Pd-C, AcOEt. **b:** i. R'-NH_2, HOBt, EDCI, *N*-methylmorpholine, CH_2Cl_2. ii. $AlCl_3$, Me_2S, CH_2Cl_2.

Scheme 3



20 **21** **22**

Reagents: a: i. *tert*-butyl acrylate, $TiCl_4$, diisopropylethylamine, CH_2Cl_2. ii. $PhCH_2OH$, *n*-BuLi, THF. iii. H_2, Pd-C. **b:** i. Gly-OCH_2Ph, HOBt, EDCI, *N*-methylmorpholine, CH_2Cl_2. ii. $AlCl_3$, Me_2S, CH_2Cl_2.

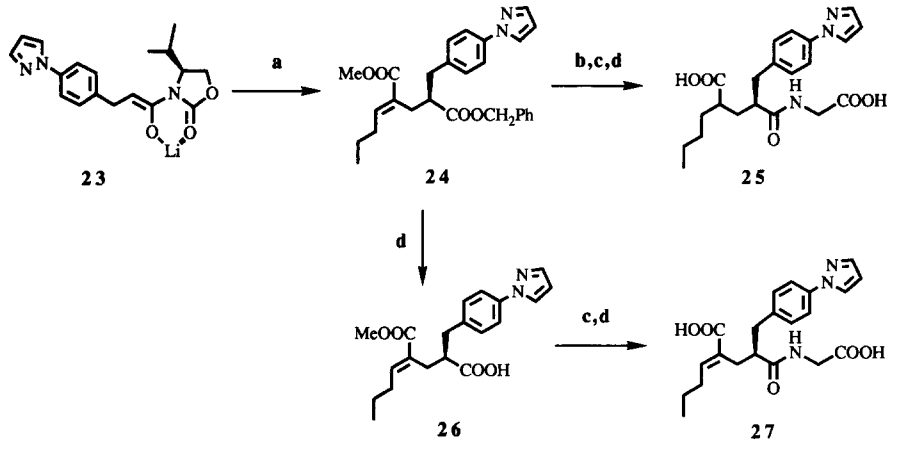
For the synthesis of biphenylmethyl derivatives **14-19** (Tables 1 and 2), an alternative method was employed: addition-elimination reaction[4] of **10** and **11** with methyl 2-(diphenoxymethyl)acrylate and subsequent alcoholysis and catalytic hydrogenation gave **12** and **13**, respectively, which were condensed with a series of amino acid esters and subsequently hydrolyzed to give **14-19** (Scheme 2).

Compound **22**, which was unsubstituted at the P_1 position, was synthesized from 2-(4-biphenylmethyl)-glutaric acid ester **21**, which was obtained by Michael addition reaction of titanium enolate[5] of **20** with *tert*-butyl acrylate and successive reactions (Scheme 3).

Palladium-catalyzed reaction[6] of lithium enolate **23** with methyl 3-acetoxy-2-methylenehexanoate was employed to prepare intermediate **24**, which was converted into **25** and **27** (Scheme 4).

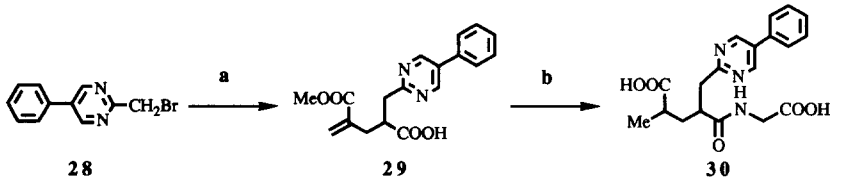
Phenylpyrimidinylmethyl bromide **28** was converted in successive steps to **30**, which was a mixture of four diastereomers (Scheme 5).

Scheme 4



Reagents: a: i. Methyl 3-acetoxy-2-methylenehexanoate, $Pd(Ph_3P)_4$, THF. ii. $PhCH_2OH$, *n*-BuLi, THF. **b**: i. H_2, Pd-C, MeOH. **c**: Gly-OCH_2Ph, HOBt, EDCI, *N*-methylmorpholine, CH_2Cl_2. **d**: $AlCl_3$, Me_2S, CH_2Cl_2.

Scheme 5



Reagents: a: i. Dibenzyl malonate, NaH, THF. ii. methyl 2-(bromomethyl)acrylate, NaH, THF. iii. $AlCl_3$, Me_2S, CH_2Cl_2. iv. heat. **b**: i. Gly-OCH_2Ph, HOBt, EDCI, *N*-methylmorpholine, CH_2Cl_2. ii. H_2, Pd-C, MeOH. iii. NaOH, MeOH.

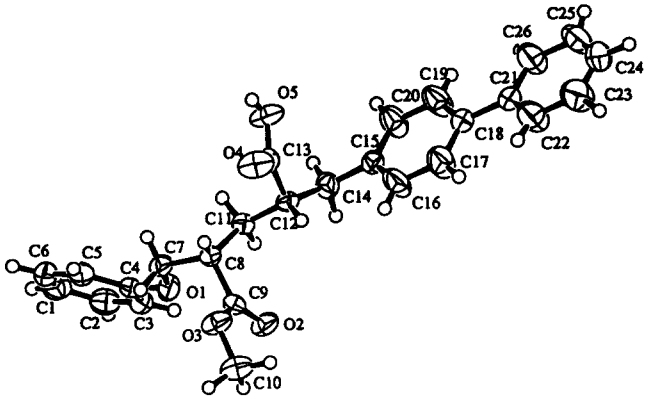


Figure 2. X-ray crystal structure showing absolute configuration of compound **12a**

Diastereomers **8**, **9**, **14-16** and **18** prepared above could be separated by HPLC to give the corresponding chiral compounds, but not diastereomers **17**, **19**, **25** and **30** under similar conditions. The absolute configuration of the most potent compound **14a** was determined as follows: (2*S*,4*S*)-2-(*p*-biphenylmethyl)-4-methoxycarbonyl-5-phenoxypentanoic acid **12a** was separated from a mixture of diastereomers **12** by chiral HPLC,[7] confirmed by X-ray crystal analysis, and then converted to **14a** (Figure 2).

Results and discussion

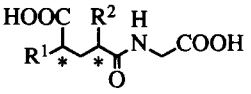
The NEP inhibitory activity of these glutaric acid amide derivatives was examined by measuring the NEP-catalyzed hydrolysis of the synthetic substrate dansyl-*D*-Ala-Gly-Phe(*p*-NO2)-Gly (DAGNPG) according to the method of Florentin *et al.*[8] UK-69578 and thiorphan,[3a] well-known NEP inhibitors, were used as controls in each round of IC_{50} determination.

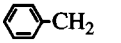
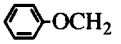
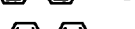
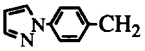
Glutaric acid amide **14a** with a biphenylmethyl group showed potent activity, while benzyl derivative **9b** resulted in a 130-fold decrease in potency (Table 1). This result confirmed the conclusion of Ksander *et al.* There was a two-fold difference in potency between the two isomers **14a** and **14b**, indicating that the absolute configuration of the carboxyl moiety was not critical for potency. Compound **22** was as potent as **14a**, suggesting that substituent R^1 is not essential. Introduction of an oxygen atom to the biphenylmethyl group resulted in the loss of activity (compound **19**). Pyrazolylphenylmethyl derivatives **25** and **27** and phenylpyrimidinylmethyl derivative **30** also decreased in potency. Thus, NEP is sensitive to the electronegative effect or hydrophilic property of hetero atoms of the substituent R^2, suggesting that the S_1'

pocket is highly hydrophobic. With respect to substituent R^3, Gly, Ala, β-Ala residues did not largely influence activity, while Met resulted in decreased potency and Pro no activity (Table 2). The results of Pro derivatives **18a** and **18b** suggest that the amide proton is crucial for NEP inhibition.

In conclusion, we have synthesized a series of glutaric acid amide derivatives and found **14a**, **14b**, **16a** and **22**, with a biphenylmethyl group at the P_1' position, as potent NEP inhibitors.

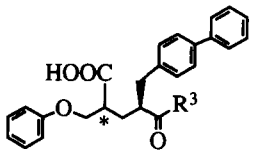
Table 1. *In Vitro* NEP-inhibitory Activity of P_1'-modified Derivatives



compd	R^2	R^1	conf.[a]	formula[b] (analysis)	IC_{50} (nM)[c]
8a		$MeOCH_2CH_2OCH_2$	isomer A	$C_{18}H_{25}NO_7 \cdot 1/4H_2O$ (C,H,N)	>1000[d]
8b		$MeOCH_2CH_2OCH_2$	isomer B	$C_{18}H_{25}NO_7$[e]	945[d]
9a			isomer A	$C_{21}H_{23}NO_6$ (C,H,N)	>1000[d]
9b			isomer B	$C_{21}H_{23}NO_6 \cdot H_2O$ (C,H,N)	407 ± 74[f]
14a			*S,S*	$C_{27}H_{27}NO_6$ (C,H,N)	3.2 ± 0.5[g]
14b			*R,S*	$C_{27}H_{27}NO_6$ (C,H,N)	5.9 ± 2.6[h]
19			*SR,S*	$C_{26}H_{25}NO_7 \cdot 1/2H_2O$ (C,H,N)	>1000[d]
22		H	*S*	$C_{27}H_{27}NO_6$ (C,H,N)	2.3[d]
25		*n*-Bu	*SR,S*	$C_{21}H_{27}N_3O_5 \cdot 1/2H_2O$ (C,H,N)	366 ± 224[f]
27		*n*-PrCH=	*S*	$C_{21}H_{25}N_3O_5$ (C,H,N)	>1000[d]
30		Me	*SR,SR*	$C_{19}H_{21}N_3O_5 \cdot 1/2H_2O$ (C,H,N)	>1000[d]
	UK-69578				65 ± 15[i]
	thiorphan				7.9 ± 1.6[j]

a) The absolute configuration of the R^2 substituent was (*S*)-configuration except for **30**. 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. b) All compounds show ^{1}H-NMR data consistent with the assigned structures. Analytical results are within ±0.4% of the calculated value. c) Values are shown as the mean ±SEM except for **8a**, **8b**, **9a**, **19**, **22**, **27** and **30**. d) n=2. e) This compound was analyzed by Mass spectrometry. f) n=3, g) n=8. h) n=6. i) n=19. j) n=17.

Table 2. *In Vitro* NEP-inhibitory Activity of Biphenylmethyl Derivatives



compd	R^3	conf.[a]	formula[b] (analysis)	IC_{50} (nM)[c]
14a	Gly-OH	*S* (isomer A)		3.2 ± 0.5
14b	Gly-OH	*R* (isomer B)		5.9 ± 2.6
15a	β-Ala-OH	isomer A	$C_{28}H_{29}NO_6$ (C,H,N)	8.7 ± 4.8
15b	β-Ala-OH	isomer B	$C_{28}H_{29}NO_6$ (C,H,N)	13 ± 6.2
16a	Ala-OH	isomer A	$C_{28}H_{29}NO_6 \cdot 1/2H_2O$ (C,H,N)	2.8 ± 0.1
16	Ala-OH	*SR*	$C_{28}H_{29}NO_6 \cdot 1/2H_2O$ (C,H,N)	20 ± 0.5
17	Met-OH	*SR*	$C_{30}H_{31}N_2O_6S \cdot 1/2H_2O$ (C,H,N)	101 ± 13
18a	Pro-OH	isomer A	$C_{30}H_{32}N_2O_6 \cdot 1/2H_2O$ (C,H,N)	>1000[d]
18b	Pro-OH	isomer B	$C_{30}H_{32}N_2O_6$ (C,H,N)	>1000[d]

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 except for **14a** and **14b**. 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 **14a**, **14b**, **18a** and **18b**. d) n=2.

Acknowledgment: We thank Ken-ichi Yamazaki for X-ray crystal analysis.

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7 Separation of diastereoisomers was performed using a Chiral Pak AS column (2 cm, 30 cm) with 3.5% EtOH in hexane for elution at a flow rate of 5 mL per min.

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