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Structure–Activity Relationships of Neuropeptide Y Y_1 Receptor Antagonists Related to BIBP 3226†

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Abstract—Analogues of BIBP 3226, (*R*)-N^{α}-diphenylacetyl-*N*-(4-hydroxybenzyl)argininamide, were synthesized and investigated for Y_1 antagonism (Ca^{2+}-assay, HEL cells) and binding on Y_1, Y_2 and Y_5 receptors. Replacing the benzylamino by a tetrahydrobenzazepinyl group preserves most of the Y_1 activity. Combination with a N^{G}-phenylpropyl arginine and a N^{α}-*p*-biphenylylacetyl moiety shifted the NPY receptor selectivity towards Y_5.

Previously, ω-guanidino- and ω-aminoalkanamides,[1] structurally derived from arpromidine-like histamine H_2 receptor agonists, were reported as novel neuropeptide Y (NPY) Y_1 antagonists. Except for the backbone, these compounds resemble BIBP 3226 (**1**),[2] an argininamide with both high NPY Y_1 receptor affinity and selectivity, with respect to the nature and arrangement of the 'terminal' diaryl, guanidine, and hydroxyphenyl groups. Hybrid compounds were synthesized combining the argininamide backbone of BIBP 3226 or partial structures derived from the C-terminal dipeptide of NPY with structural elements of arpromidine- and amide-type NPY antagonists.[3] Based on these investigations and supported by molecular modeling studies we have suggested that the binding sites of NPY Y_1 antagonists with one or two basic groups are not identical. In order to get more information about the interaction of argininamides with the human Y_1 receptor we further varied the *N*- and the C-terminus as well as the terminal basic group of argininamides. Additional to the functional assay for Y_1 antagonism (inhibition of the NPY-stimulated increase in $[Ca^{2+}]_i$ in HEL cells), radioligand binding studies with Y_1, Y_2, and Y_5 receptors were performed for selected compounds.

Chemistry

The argininamides were synthesized according to a previously described procedure[3] either from (*R*)-N^{α}-Boc-N^{G}-nitroarginine and (*R*)-N^{α}-*Z*-N^{δ}-Boc-ornithine or via batchwise solid-phase synthesis using Rinke amide resin and (*R*)-N^{α}-*F*moc-N^{G}-Pmc-Arg-OH as arginine building block. (*R*)-N^{α}-Boc-N^{G}-nitroarginine was activated with *N,N'*-carbonyldiimidazole (CDI) and coupled with the pertinent primary and secondary amines (Scheme 1). After cleavage of the N^{α}-Boc protecting group with trifluoroacetic acid (TFA), the substituted (*R*)-N^{G}-nitroargininamides were acylated with arylalkanoic acid *N*-hydroxysuccinimidyl esters. The title compounds (Table 1) were obtained after hydrogenation using Pd-C (10%) as catalyst in acetic acid.[4] The phenylpropyl substituted argininamides **31**, **32** (Table 1) were prepared using (*R*)-N^{α}-*Z*-N^{δ}-Boc-ornithine as building block. After amidation with a secondary amine, the *Z* group was cleaved by hydrogenation over Pd-C (10%) catalyst and the obtained N^{δ}-Boc-ornithinamides were N^{α}-acylated with *p*-biphenylylacetic acid *N*-hydroxysuccinimidyl ester. The Boc group was cleaved with TFA and the ornithinamides were guanylated with *S*-methyl-*N*-(3-phenylpropyl)isothiourea according to standard procedures.[5] Argininamides with a C-terminal carboxamide group (**14**–**16**) were prepared by batchwise solid-phase synthesis starting from N^{α}-*F*moc-protected phenylglycine or *p*-hydroxyphenylglycine anchored to Rinke amide resin. The *F*moc group was removed with 30% piperidine in anhydrous DMF. For the amide preparation (*R*)-N^{α}-*F*moc-N^{G}-Pmc-arginine and diphenylacetic acid were allowed to react with the solid-phase bound amine component using 2-[(1*H*)-benzotriazol-1-yl]-1,1,3,3-tetramethyluronium-tetrafluoroborate (TBTU) and diisopropylethylamine (DIPEA) for the coupling process. Deprotection of Arg(Pmc) and cleavage of the products from the polymeric support

†Dedicated to Professor Dr. Dr. Dr.h.c. W. Schunack, Berlin, on the occasion of his 65th birthday.

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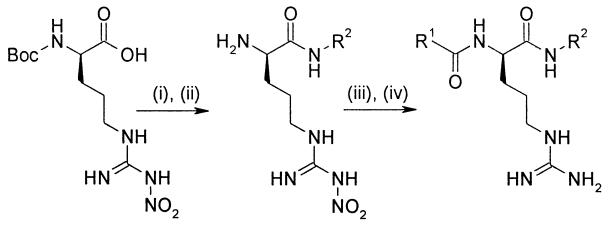


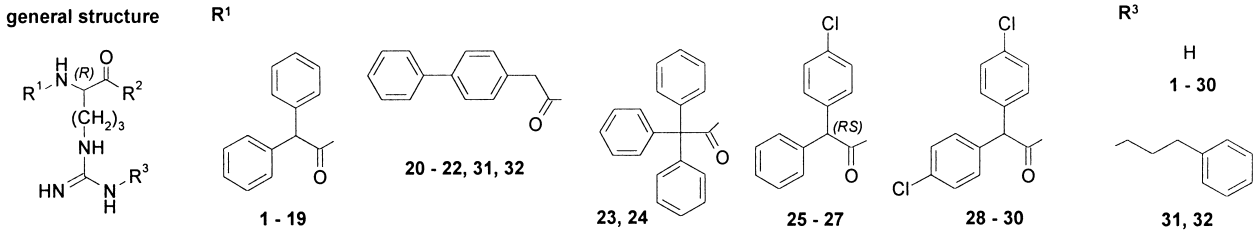
Scheme 1. (i) CDI, primary or secondary amines, THF, rt; (ii) TFA, rt; (iii) arylalkanoyl-*N*-hydroxysuccinimidyl ester (acyl group cf. compds **1**–**30**, R^1 = Ph_2CH-, Ph-C_6H_4-CH_2-, Ph_3C-, *p*-Cl-C_6H_4-CH-Ph, (p-Cl-C_6H_4)$_2$CH-), DIPEA, THF, 40 °C; (iv) H_2/Pd-C (10%), 5 bar, HOAc, rt.

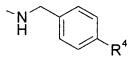
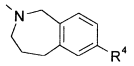
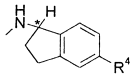
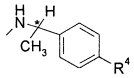
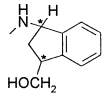
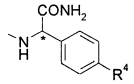
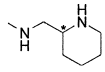
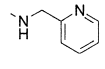
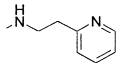
was carried out with 95% TFA. The arginylphenylglycinamides **14**, **15** and the arginyl-*p*-hydroxyphenylglycinamide **16** were purified by preparative reversed-phase HPLC.

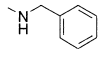
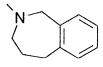
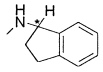
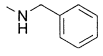
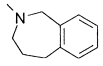
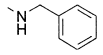
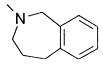
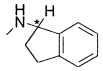
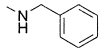
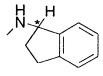
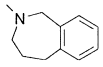
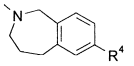
Pharmacology

The compounds were screened for their Y_1-antagonistic activity in human erythroleukemia (HEL) cells by measuring the inhibition of porcine NPY (10 nM) stimulated increase in the intracellular Ca^{2+} concentration in a FURA-assay[1] (for structures and biological data see

Table 1. Structures and NPY Y_1 antagonistic activities of argininamides in HEL cells (calcium assay)[a,1]



No.	R^2	R^4	R^2 config.	Y_1-antag.[a] IC_{50} [μM]
1[b]		OH	—	0.017
2[c,d]		H	—	0.11
3[c]		H	—	2.20
4[c]		OH	—	0.28
5[c]		H	*R*	21
6[c]		H	*S*	1.4
7		OH	*RS*	1.0
8		H	*R*	0.20
9		H	*S*	2.5
10		OH	*RS*	0.06
11			*R*	22.3
12			*S*	0.9
13			1*R*, 3*S*	inact.
14		H	*R*	4.6
15		H	*S*	7.5
16		OH	*R*	8.1
17			*RS*	inact.
18				3.75
19				11.5

No.	R^2	R^4	R^2 config.	Y_1-antag.[a] IC_{50} [μM]
20			—	21.8
21			—	76
22			*S*	inact.
23			—	inact.
24			—	51
25			—	0.081
26			—	1.1
27			*S*	3.0
28			—	0.17
29			*S*	> 10[e]
30			—	1.1
31		H	—	inact.
32		OH	—	11.9

[a]Inhibition of NPY (10 nM) induced increase in $[Ca^{2+}]_i$; antagonist concentrations used: 0.01, 0.025, 0.1, 0.5, 1, 10, 50 and 100 μM depending on activity; compounds designated inactive produced less than 20% inhibition at 100 μM.
[b]BIBP 3226.
[c]Compounds **2**–**6** cf. ref 3.
[d]See also ref 7.
[e]28.7% Inhibition at 10 μM; the compound could not be tested in higher concentrations due to induction of cytolysis.

Table 1). Experiments were performed in triplicate at antagonist concentrations between 0.01 and 100 μM, and IC_{50} values were calculated.[1] The SEM of the percentual inhibition at a single concentration was always less than 10%, resulting in an error of pIC_{50} (-log IC_{50}) of less than 0.35 log units. Additionally, selected substances were investigated in radioligand binding studies on Y_1-(SK-N-MC cells),[3] Y_2- (SMS-KAN cell membranes)[1] and Y_5-receptors (Y_5-transfected HEC-1B cells)[6] by displacement of [^{3}H]propionyl-NPY (1 nM) according to the procedures described elsewhere[1,3,6] in detail (Table 2). The assays were run in triplicate.

Structure–Activity Relationships

In order to investigate in which spatial position a certain bulk is tolerated by the Y_1-receptor, argininamides **3**–**7** with conformationally constrained benzylamide groups were synthesized (**2**–**6**: ref. 3, **2** and other analogues of **1** see also ref. 7. Among these bicyclic compounds[3] the 7-hydroxybenzazepine derivative **4** is the most active one. Introduction of a '*para*-equivalent' OH group in the indane moiety slightly increases Y_1-antagonistic activity (**7** versus **6**; note that **7** is a mixture of diastereomers). Among the diastereomers **8** and **9**, the (*R*,*R*)-configurated *N*-[1-phenylethyl]argininamide **8** is 13 times more active than **9** (*R*,*S*). Introduction of an OH group in the phenylethylamide moiety further increases Y_1-receptor affinity, but **10** (mixture of diastereomers)[8] is 4 times less potent than **1** (BIBP 3226). Representative concentration response curves for NPY in the presence of **10** are shown in Fig. 1.

A hydroxymethyl group (**11**–**13**) results in decreased Y_1 antagonistic activity compared to the parent compounds **2** and **6**. The absolute configuration of R^2 in the more active indane diastereomer **6** (*S*) does not correspond to the eutomers of the methyl and the hydroxymethyl derivatives **9** (*R*) and **12** (*S*), respectively. Thus, overlap of the 'additional' bulk of **6** with that of **9** and **12** on binding is unlikely.

Compounds containing the C-terminal dipeptide Arg^{35}-Tyr^{36}-NH_2 of NPY were nearly inactive.[3] The phenylglycinamide derivatives **14**–**16** are moderate Y_1 antagonists. In contrast to **2**, **6** and **8**, introduction of an OH group decreases Y_1-receptor affinity (**16** versus **14**). It is suggested that the dipeptide Arg-Phg-NH_2 interacts with the Y_1 receptor in a different way than the C-terminus of NPY. Among the pyridylalkyl substituted argininamides **18** and **19**, the shorter homolog is the more active one, whereas the piperidine derivative **17** shows no Y_1 antagonism. Comparing the benzylamide **2** and its pyridine analogue **18**, the reduced activity of the latter may be due to the basicity or an unfavorable charge distribution of the pyridine ring.

Table 2. Radioligand[a] binding data (IC_{50}±SEM [μM]) of selected substances in SK-N-MC cells (Y_1), SMS-KAN cell membranes (Y_2) and HEC-1B cells (Y_5)

Compds	Y_1	Y_2	Y_5
1	0.0059 (±0.001)	> 100	80 (±7)
4	0.67 (±0.1)	247 (±22)	25 (±3)
8	0.15 (±0.03)	177 (±43)	48 (±2)
10	0.019 (±0.003)	575 (±105)	75 (±2)
12	0.184 (±0.009)	540 (±120)	68 (±7)
14	2.4 (±0.2)	> 400	163 (±36)
25	0.17 (±0.01)	97 (±11)	2.6 (±0.3)
27	5.3 (±0.4)	59 (±17)	11.9 (±0.6)
32	22 (±2)	78 (±9)	0.77 (±0.08)

[a]Radioligand: [^{3}H]propionyl-NPY (1 nM); assays were run in triplicate; the procedures are described elsewhere in detail.[1,3,6]

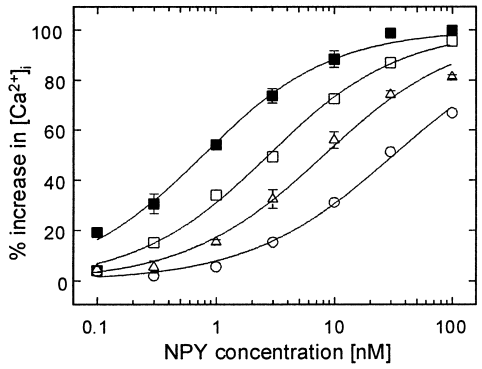


Figure 1. NPY-induced increase in intracellular [Ca^{2+}] (HEL cells). Concentration–response curves of (■) pNPY alone and in presence of 10 nM (□), 25 nM (△) and 100 nM (○) of compound **10**. The rightward shift of the concentration–response curves for NPY in the presence of compound **10** corresponds to a K_B value of 3.01 ± 0.44 nM ($pK_B = 8.52$, slope constrained to 1.0) or a pA_2 value of 8.38 (slope 1.17) determined by Schild plot.

Previous investigations regarding the N-terminus of argininamides have shown, that homologization and vicinal arrangement of the phenyl rings reduce Y_1-receptor antagonistic activity.[3] Incorporation of a *p*-biphenyl-yl-acetyl (**20**–**22**) or a triphenylacetyl group (**23**, **24**) results in inactive or only weakly active compounds. Interestingly, *p*-Cl monosubstitution in the diphenylacetyl moiety may increase Y_1-antagonistic activity (**25** versus **2**, **26** versus **3**), indicating that hydrophobic and/or van-der-Waals interactions might contribute to binding in this position. The effect of *p*,*p*′-di-Cl substitution is varying (**28**–**30**).

Replacement of the diphenylacetyl by a *p*-biphenylylacetyl group together with phenylpropyl substitution of the terminal guanidine (**31**, **32**) decreases Y_1-antagonistic activity (**31** versus **21**) but leads to a change in receptor-subtype selectivity: **32** has 29 times higher affinity to the Y_5-receptor than to the Y_1-receptor (see Table 2). Compound **32**, in some respect resembling a recently published NPY-antagonistic amidine[9] (selectivity data not given in ref. 9), shows submicromolar affinity to the Y_5-receptor.

Radioligand binding data of selected substances on SK-N-MC cells (Y_1), SMS-KAN cell membranes (Y_2) and HEC-1B cells (Y_5) show that the substituted argininamides (except for **32**, see above) preferentially bind to the Y_1 receptor (see Table 2). Surprisingly, increasing hydrophobicity in the diphenylacetyl portion (**25**, **27**) enhances Y_5-affinity. For the nine compounds considered, Y_1 antagonistic activity in the functional test (pIC_{50} = -log IC_{50}) is very highly correlated with pIC_{50} values in the binding assay:

$$pIC_{50(Ca^{2+})} = 0.78(\pm 0.11)pIC_{50(bind.)} + 1.31(\pm 0.70)$$
$$r = 0.94 \quad s = 0.35 \quad F = 53.6$$

Conclusions

The C-terminal 4-hydroxybenzamide moiety of BIBP 3226 (**1**) seems to be optimal for Y_1 antagonism although a certain bulk, which may be also part of a bicyclic structure (e.g., of a benzazepinyl group), is well tolerated. At the N-terminus, compounds with a diphenylacetyl or even more with a (*RS*)-4-chlorodiphenylacetyl group are most active. This suggests to prepare Cl-substituted positional isomers and derivatives with other substituents. Interestingly, a p-biphenylylacetyl moiety as N-terminus in combination with a phenylpropyl substituent at the guanidino group and with a C-terminal hydroxybenzazepine leads to Y_5-receptor affinity in the submicromolar range. Further work is ongoing to investigate whether this structure indeed represents a promising lead for new Y_5 receptor ligands. Possibly, compounds with combined Y_1 and Y_5 antagonism are interesting as potential drugs, as both NPY receptor subtypes appear to be associated with increased food intake.

Acknowledgements

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References and Notes

1. Müller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A. *Arch. Pharm. Pharm. Med. Chem.* **1997**, *330*, 333.
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4. (*R*)-N^{α}-Diphenylacetyl-*N*-[(*RS*)-1-(4-hydroxyphenyl)-ethyl] argininamide (**10**): (*R*)-N^{α}-Diphenylacetyl-N^{G}-nitro-*N*-[(*RS*)-1-(4-hydroxyphenyl)ethyl]argininamide (0.26 g, 0.49 mmol) was dissolved in 60% acetic acid and hydrogenated at room temperature in an autoclave (5 bar, H_2/Pd-C 10%). The catalyst was filtered off, washed with 60% acetic acid and the filtrate was evaporated in vacuo. The crude product was purified chromatographically on silica gel with $CHCl_3$:MeOH (1:1) as eluent. Yield: 0.20 g (0.37 mmol; 62%) hygroscopic yellow solid; $C_{28}H_{32}N_5O_3 \cdot CH_3CO_2H$ (547.65); mp 76–80 °C ($CHCl_3$: MeOH); $[\alpha]_D^{25} = +9.4 \pm 2°$ (c = 0.16 g/100 ml; MeOH); IR (KBr): ν 3408 br (OH, NH), 3061w, 3013w (Ar-H), 2958w, 2883w (CH), 1652s (CO); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (d, J = 6.7Hz, 3H, CH_3), 1.39−71 (m, 4H, CH_2CH_2CH_2), 3.00−17 (m, 2H, CH_2CH_2CH_2), 4.29–4.31 (m, 1H, C*H*), 4.76–4.81 (m, 1H, C*H*CH_3), 5.11 and 5.13 (2s, 0.6/0.4H, Ph_2C*H*), 6.04 (br, 3H, N*H*), 6.66 (d, J = 8.3 Hz, 2H, *Ar*-OH), 7.03 (d, J = 8.3Hz, 2H, *Ar*-OH), 7.21–7.30 (m, 10H, *Ph*), 7.99–8.29 (br, 1H, O*H*), 8.31–8.37 (m, 1H, N*H*CH), 8.40–8.46 (m, 1H, N*H*CH), 8.61–8.68 (m, 1H, N*H*CH_2), 9.32 (br, 1H, N*H*); $^+$FAB–MS m/z (relative intensity): 975 (>1, $[2M+H]^+$), 488 (100, $[M+H]^+$), 167 (62, $[Ph_2CH]^+$).
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8. Compound **10** was among a series of substances presented by our group on a poster at the 5th NPY meeting, Grand Cayman, 1999. At the same meeting Gedda et al. (ASTRA-Hässle, Mölndal, Sweden) presented the (*R,R*)-configurated stereoisomer of **10**, (*R*)-N^{α}-diphenylacetyl-*N*-[(*R*)-1-(4-hydroxyphenyl)ethyl]argininamide (H 409/22), as a potent and selective Y_1-antagonist (Gedda, K.; Berglund, M.-L.; Larefalk, A.; Nilson, A.-K.; Ebstand, J.; Vauquelin, G.; Chkajlani, V. *Abstracts of Papers*, 5th International NPY Meeting, April 17–22, 1999; Grand Cayman; p 25). The reported IC_{50} value of 13.6 nM (radioligand binding, SK-N-MC cells using [^{3}H]propionyl-NPY (0.5 nM)) is in good agreement with our data (19 nM, cf. Table 2).
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