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NON-PEPTIDE NEUROPEPTIDE Y ANTAGONISTS DERIVED FROM THE HISTAMINE H_2 AGONIST ARPROMIDINE: ROLE OF THE GUANIDINE GROUP+

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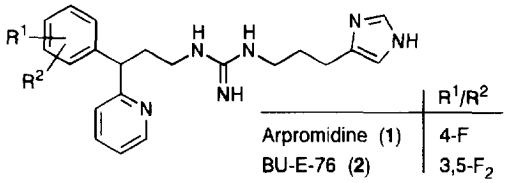
Abstract. Arpromidine analogs in which the guanidino group was replaced or substituted were investigated in functional studies for NPY Y_1 and histamine H_1 antagonism as well as for H_2 agonism (HEL cells, guinea pig ileum and atrium). A basic guanidine or amidine system proved to be important for both Y_1 and H_2 receptor activity. Additional guanidine substitution (Me, cHex, Ph) is compatible with Y_1 antagonism but not with high H_2 agonist potency.

Neuropeptide Y (NPY), a 36 amino acid peptide first described in 1982[1], is widely distributed in the central and peripheral nervous system and known to be involved in numerous biological processes[2]. In the periphery, for example, NPY is a cotransmitter in the sympathetic nervous system and acts as a potent vasoconstrictor via stimulation of postsynaptic Y_1 receptors[3]. Further evaluation of the physiological role of NPY requires potent and selective antagonists. In particular, non-peptides could be interesting as both pharmacological tools and potential drugs, e. g., Y_1 antagonists as antihypertensives. Aside from two very recent reports on BIBP 3226[4] and SR 120819A[5], two peptoides reported to display high Y_1 receptor affinity and selectivity, promising leads for the development of non-peptide NPY antagonists have not been published up to now. One group of the previously described, but weakly active non-peptidic NPY antagonists comprises benextramine, an irreversible α-adrenoceptor blocker, and some analogs[6,7]. Michel and Motulsky[8] have previously reported NPY-antagonism with Y_1 selectivity for BU-E-76 (He 90482), a very potent histamine H_2 agonist closely related to arpromidine[9] and developed as an inotropic vasodilator. But similar to benextramine, BU-E-76 and its close analogs are also only weakly active (pA_2 4.43 for

Tyr[1]-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg[33]-Gln-Arg[35]-Tyr[36]-NH_2

amino acid sequence of porcine Neuropeptide Y



	R^1/R^2
Arpromidine (1)	4-F
BU-E-76 (2)	3,5-F_2

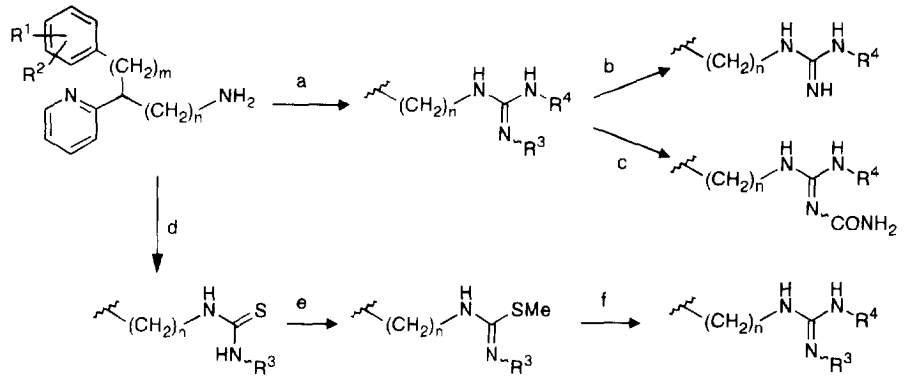
+ Dedicated to Prof. Dr. Dr. W. Schunack, Berlin, on the occasion of his 60th birthday

inhibition of NPY-stimulated Ca^{2+} mobilization in HEL cells)[8]. The imidazolylpropylguanidine moiety is essential for H_2-agonistic activity of arpromidine and related compounds. It seems to play a crucial role for Y_1 receptor affinity, too. Molecular modeling studies[10] comparing conformations of arpromidine derivatives with possible spatial arrangements of NPY residues relevant to Y_1 agonistic activity[11] have suggested that the guanidino group and the imidazole ring mimic the guanidino groups of Arg^{33} and/or Arg^{35} in the NPY molecule. The present study was performed in order to investigate the impact of structural modifications in the strongly basic guanidine moiety of arpromidine-derived compounds on both Y_1 antagonism and H_2 agonism in more detail. For example, the guanidine substructure was substituted with diverse groups (COOR, CN, $CONH_2$, Me, cHex, Ph) or replaced by related basic (amidine or amine) or non-basic functional groups (amide). Additionally, the substitution pattern at the phenyl ring and the chain length were varied.

Chemistry

The arpromidine-type N,N'-disubstituted guanidines were preferentially prepared according to a previously described procedure[9] by aminolysis of appropriate N-acylated or related diphenyl carbonimidates. The central building blocks were allowed to react consecutively with the pheniramine-like primary amines[12] and 3-(1*H*-imidazol-4-yl)propanamine[13] followed by removal of the protecting group by acid hydrolysis (Scheme 1). The trisubstitued guanidines with R = Me, cHex or Ph were synthesized by addition of the amines to isothiocyanates followed by S-methylation and aminolysis of the methylisothiourea by analogy with the method described[14,15].

Scheme 1

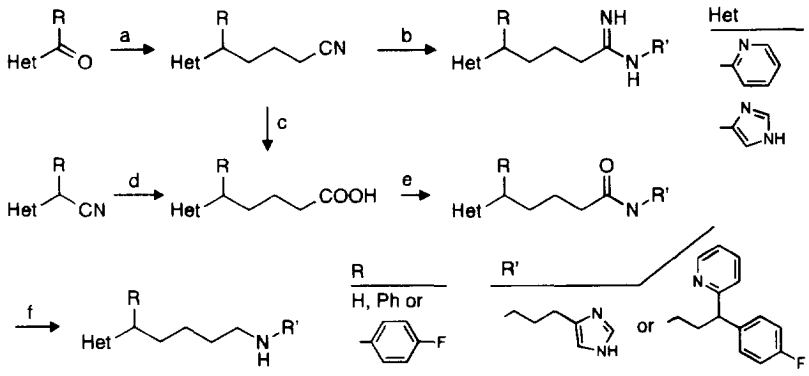


m = 0 or 1; n = 2 or 3; R^1/R^2 = H, F, Cl, or Br; R^4 = H or $(CH_2)_3$(4-imidazolyl)
a: 1) $(PhO)_2C{=}N\text{-}R^3$ (R^3 = COPh, CN, COOEt, BOC), r.t., 2): $H_2N\text{-}R^4$, Δ; **b**: dil. HCl, Δ (R^3 = COPh, COOEt, BOC); **c**: conc. HCl, r.t. (R^3 = CN); **d**: $S{=}C{=}N\text{-}R^3$, Δ (R^3 = Me, cHex, Ph); **e**: MeI, r.t.; **f**: $H_2N\text{-}R^4$, Δ.

The compounds with replaced guanidino group (Scheme 2) were synthesized starting from appropriate ketones by WITTIG reaction with cyanopropyltriphenylphosphonium bromide. The crude alkenes (Z/E mixture) were directly hydrogenated over Pd-C (10 %) catalyst in EtOH/HCl. The nitriles were either hydrolyzed with

dilute HCl affording the corresponding carboxylic acids or converted into an amidine via preparation of the methyl imidate which was treated with the appropriate primary amine. The carboxylic acids were also available via C-alkylation of a phenylpyridylacetonitrile with ω-bromobutyric acid ethyl ester with subsequent removal of the nitrile functional group and ester cleavage in H_2SO_4 (75%). Transformation to the amide using 1,1'-carbonyldiimidazole as coupling reagent was followed by addition of imidazolepropanamine. Reduction with $LiAlH_4$ in THF afforded the amine.

Scheme 2



a: 1) $Ph_3P^+(CH_2)_3CN\ Br^-$, KOtBu, r.t., 2) H_2 / 10 % Pd-C; **b**: 1) MeOH / HCl, 2) H_2N-R' **c**: 20 % HCl, Δ; **d**: 1) NaH, $Br(CH_2)_3COOEt$, Δ, 2) 75 % H_2SO_4, Δ; **e**: 1) CDI, 2) H_2N-R'; **d**: $LiAlH_4$ / Et_2O, Δ.

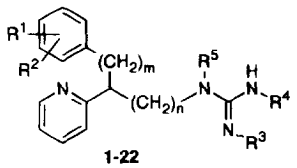
Pharmacology

Using well-introduced methods, all compounds were investigated for their activity at histamine H_2 receptors (isolated spontaneously beating guinea pig right atrium[16]) and Y_1 receptors (human erythroleukaemia (HEL) cells[17]), respectively (Table 1). Furthermore, histamine H_1 receptor antagonist activity was determined (isolated guinea pig ileum[18]) since arpromidine-type guanidines are well known to be active at this receptor subtype, too. The screening for Y_1 antagonistic activity in HEL cells was performed according to Motulsky and Michel[17] by measuring the inhibition of NPY (10 nM) stimulated submaximal increase in $[Ca^{2+}]_i$ (FURA-assay).

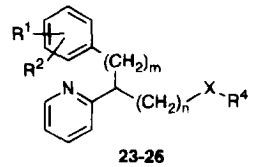
Structure-Activity Relationships

Generally, changes lowering basicity at the position of the central guanidine reduce Y_1 antagonistic as well as histamine H_2 agonistic activity. Introduction of basic groups like amines and amidines leads to compounds which still are moderate Y_1 antagonists, while the simple amide is no longer active at this receptor. Substitution at the central guanidino group is only favorable if a pronounced increase in lipophilicity and bulk is achieved by substituents like cyclohexyl or phenyl. Possibly by additional hydrophobic interaction, these groups appear to counterbalance the drop in activity observed for smaller and for polar substituents.

Table 1. Structures and pharmacological activities of imidazolylpropyl guanidines and structural analogs[a]



1-22



23-26

	R4
1, 2, 4-22	$(CH_2)_3$-imidazole-NH
3	H

	R5
1-3, 5-22	H
4	Me

no.	R^1/R^2	m	n	R^3 (1-22) or X (23-26)	Y_1 antagonism[b] % inhibition of NPY-induced increase in $[Ca^{2+}]_i$ at antagonist concentration 100 µM	10 µM[b]	H_1 antagonism (guinea pig ileum) pK_B[c] ± 0.2	H_2 agonism (guinea pig atrium) pD_2 ± 0.2 (i.a.)[d] or H_2 *antagonism*, pK_B[e]
1	4-F	0	2	H	75 ± 7	18 ± 4	7.65	8.01
2	3,5-diF	0	2	H	62 ± 8[f]		6.56	8.05
3	4-F	0	2	H	inactive		6.44[g]	inactive
4	4-F	0	2	H	42 ± 6	n. d.	5.96	5.40 (0.8)
5	4-F	0	2	COOEt	4 ± 1	2 ± 2	5.30[g]	inactive
6	4-F	0	2	CN	13 ± 1	inactive	n. d.	n. d.
7	4-F	0	2	$CONH_2$	62 ± 2	2 ± 1	n. d.	n. d.
8	4-F	0	2	Me	13 ± 2	n. d.	5.08	5.65
9	H	0	2	H	69 ± 3	2 ± 10	7.01	7.39
10	H	0	3	H	84 ± 4	8 ± 9	6.06	6.61
11	H	0	3	cHex	53 ± 5	6 ± 4	5.56[g]	4.49 (0.25)
12	4-Me	1	2	H	n. d.	23	6.40	5.81
13	4-Me	1	2	cHex	75 ± 5	24 ± 6	5.40	*5.93*
14	4-Br	0	2	H	85 ± 1	27 ± 10	6.92	7.48
15	4-Br	0	2	Me	38 ± 7	inactive	5.71	5.75 (0.9)
16	4-Br	0	2	cHex	82 ± 0	36 ± 9	5.42	4.69 (0.65)
17	4-Br	0	2	Ph	76 ± 4	24 ± 4	5.81[g]	*5.14*
18	3,4-diCl	0	2	H	93 ± 1[h]	81 ± 4[h]	7.10	8.19
19	3,4-diCl	0	3	H	78 ± 3	31 ± 4	7.46	6.67
20	3,4-diCl	0	3	Me	41 ± 5	n. d.	6.16	5.58
21	3,4-diCl	0	3	cHex	87 ± 2	30 ± 3	5.76[g]	4.65 (0.4)
22	3,4-diCl	0	3	Ph	78 ± 3	48 ± 4	5.50[g]	*4.97*
23	H	0	2	C(=NH)NH	45 ± 13	n.d.	6.45	6.50
24	4-F	0	2	C(=O)NH	n.d.	inactive	3.97	< 3
25	4-F	0	2	CH_2NH	24 ±	n.d.	6.43	5.17 (0.5)
26	4-F	0	2	$NH(HN=)CCH_2$	27 ± 2	5 ± 1	6.56	6.47 (0.6)

a: data for H_2 agonism and H_1 antagonism of compounds **1-4**, **8-10**, **14**, **18**, **19** see ref. [9], standard agonist: histamine; **b**: % inhibition of NPY (10 nM) induced increase of $[Ca^{2+}]_i$, mean of at least 3-5 independent experiments; antagonist concentrations used: 100 and/or 10 µM; **c**: pK_B (antagonist activities; inhibition of histamine-stimulated contraction) calculated from the expression pK_B = -log [antagonist] + log (concentration ratio -1)[19]; antagonist concentrations used: 10^{-7}-10^{-4} M; SEM within ± 0.2; pK_B of pheniramine = 7.8; **d**: agonist activity (positive chronotropism) expressed as pD_2 (i. a.: intrinsic activity = 1 unless otherwise indicated in parentheses), SEM within ± 0.2; reference agonist: histamine, pD_2 = 6.0, i. a. = 1; **e**: values given in italics represent pK_B (antagonist activities), all antagonists (concentrations used: 10^{-7}-10^{-4} M) induced a dose-dependent depression of histamine's concentration response curve, SEM within ± 0.2; **f**: pA_2 = 4.43; **g**: dose-dependent depression of histamine's concentration response curve; **h**: pK_B = 6.25.

Surprisingly, the guanidine carboxamide **7** still shows a remarkable activity at Y_1 receptors. According to previous investigations[20], the pK_a value of the amidinourea-moiety should not be substantially lower than 7 so that a sufficient degree of basicity is preserved. Alternatively, in this position the amide might mimic a backbone element of NPY leading to another binding mode where no positive charge is needed.

The imidazolylpropylguanidine moiety is well known to be optimal for H_2 receptor stimulation[21]. The activity of arpromidine analogs at H_2 receptors decreases with any further substitution at the guanidine system. The imidazolylpropylamidines **23** and **26** are about 10 times less potent than the corresponding guanidine **9** with **26** acting as a partial agonist. With the amine **25**, the positive chronotropic response is further reduced. Beyond all quantitative differences, the structure-activity relationships of H_2 agonism as well as H_1 and Y_1 antagonism are similar with respect to the significance of a markedly basic central link.

About the influence of substitution patterns in the pheniramine moiety (R^1/R^2), Table 1 presents only some preliminary information. Comparing compounds **1**, **9**, **12**, **14**, and **18** with an approximate ranking of H < 4-F < 4-Me (m=1) < 4-Br < 3,4-di-Cl, it appears that Y_1 antagonistic activity increases with more hydrophobic phenyl or benzyl groups. Additionally, the chain length between the guanidino group and the arylic part of the molecule must be considered. Whereas prolongation of the chain from two to three C atoms slightly enhances activity in the case of the unsubstituted phenyl derivatives **9** and **10**, a marked decrease has been observed for the 3,4-di-Cl analogs **18** and **19**.

Although the imidazolylpropylguanidine moiety is a necessary structural feature and more hydrophobic substituents at the pyridylalkyl chain are favorable, a possible Y_1 antagonistic pharmacophore remains hidden since also the best analogs are only moderately active. The high flexibility of the structures leads to many conformations equivalent in energy, so that only a small part of the population may contain a sufficient arrangement of pharmacophoric elements. Forthcoming papers will deal with a further optimization of the pheniramine moiety and with its replacement by mepyramine-like structures.

Conclusion

In conclusion, the structure-activity relationships of guanidines derived from arpromidine may be useful for further investigations in the field of non-peptide Y_1 antagonists. The imidazolylpropylguanidine moiety and its combination with pheniramine-like substructures bearing phenyl or benzyl groups with hydrophobic para substituents already fulfills some basic conditions for the design of an Y_1 antagonistic pharmacophore. The dependence of Y_1 antagonism on the structure differ from that of H_1-antagonistic and H_2-agonistic activity, which are both reduced by increasing length of the pyridylalkyl chain, by an exchange of phenyl against benzyl, and by additional substituents R^3 at the guanidine group.

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14. A mixture of 3-(4-fluorophenyl)-3-(2-pyridyl)propanamine (1.22 g, 5.3 mmol) and MeSCN (0.39 g, 5.3 mmol) in EtOH (30 mL) is refluxed for 1 h. After evaporation amorphous N-[3-(4-fluorophenyl)-3-(2-pyridyl)propyl]-N'-methylthiourea (**27**) can be obtained in analytical purity. ^{1}H NMR ($CDCl_3$): δ 2.25 (m, 1H, CHC*H*H), 2.59 (m, 1H, CHCH*H*), 3.12 (s, 3H, NHCH_3), 3.36 (m, 2H, NHCH_2), 4.11-4.16 (m, 1H, C*H*), 6.46 (s, 1H, N*H*CH$_3$, exchangeable), 6.95-7.22 (m, 6H, 4 Ph-*H*, Py-5-*H*, Py-3-*H*), 7.58-7.64 (m, 1H, Py-4-*H*), 8.56 (d, J = 4.8 Hz, 1H, Py-6-*H*); EI-MS *m/z* (relative intensity): 303 (2, M^+), 200 (22), 187 (100), 186 (38). Anal. ($C_{16}H_{18}N_3FS$) C, H, N. Then, thiourea **27** is redissolved in EtOH (30 mL) and MeI (0.80 g, 5.6 mmol) is added. The mixture is stirred at ambient temperature for 36 h and then evaporated to dryness to give N-[3-(4-fluorophenyl)-3-(2-pyridyl)propyl]-N',S-dimethylisothiuronium iodide (**28**) as an amorphous solid (yield: 2.27 g, 96 %), which is pure enough for further reaction. ^{1}H NMR ($CDCl_3$): 2.42-2.53 (m, 2H, CHCH_2), 2.92 (s, 3H, SCH_3), 3.29 (s, 3H, NHCH_3), 3.42 (m, 2H, NHCH_2), 4.18-4.21 (m, 1H, C*H*), 6.94-7.16 (m, 5H, 4 Ph-*H*, Py-5-*H*), 7.31 (m, 1H, Py-3-*H*), 7.68-7.73 (dd, J = 7.0 Hz / 7.4 Hz, 1H, Py-4-*H*), 8.52 (d, J = 4.4 Hz, 1H, Py-6-*H*), 9.04 (br, 0.5H) and 10.56 (br, 0.5H, N*H*, exchangeable); EI-MS *m/z* (relative intensity): 200 (18), 186 (100), 185 (32), 142 (63). Anal. ($C_{17}H_{20}N_3FS{\cdot}HI$) C, H, N.
15. Isothiourea **28**[13] (1.34 g, 3.0 mmol) is dissolved in MeCN/pyridine 3:1 (10 mL), 3-(1*H*-imidazol-4-yl)propanamine (0.39 g, 3.0 mmol) is added and the mixture is refluxed for 4 h (control by TLC on silica gel, CH_3Cl/MeOH 10:1, NH_3 atmosphere). After removal of the solvent in vacuo, the crude compound **8** is purified chromatographically (Chromatotron™ 7924, glass rotors with 4 mm of silica gel PF_{254} containing gypsum, eluent: CH_2Cl_2/MeOH, 95:5, NH_3 atmosphere) to yield 0.58 g (37 %) of pure compound **8**·HI as an amorphous solid. ^{1}H NMR ($CDCl_3$): δ 1.99 (m, 2H, ImCH$_2$CH_2), 2.30 (m, 1H, CHC*H*H), 2.52 (m, 1H, CHCH*H*), 2.55-2.64 (m, 2H, ImCH_2), 3.12 (s, 3H, NHCH_3), 3.55 (m, 4H, NHCH_2), 4.16-4.19 (m, 1H, C*H*), 6.81 (s, 1H, Im-5-*H*), 6.92-6.98 (m, 1H, Py-5-*H*), 7.07-7.11 (m, 2H, Ph-3,5-*H*),7.22-7.27 (m, 3H, Ph-2,6-*H*, Py-3-*H*), 7.59-7.67 (m, 2H, Py-4-*H*, Im-5-*H*), 8.52 (d, J = 4.2 Hz, 1H, Py-6-*H*); $^+$FAB-MS *m/z* (relative intensity): 395 (46, $[M+H]^+$), 214 (100, $[FPhCH(Py)C_2H_4]^+$), 109 (71, $[ImC_3H_6]^+$); IR (KBr) ν (cm^{-1}): 1626 (C=N), 3221 (N-H); Anal. ($C_{22}H_{27}N_6F{\cdot}HI{\cdot}H_2O$), C, H, N.
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