Synthesis and Neuropeptide Y Y_1 Receptor Antagonistic Activity of *N,N*-Disubstituted ω-Guanidino- and ω-Aminoalkanoic Acid Amides☆

Manfred Müller, Sebastian Knieps, Karin Geßele, Stefan Dove, Günther Bernhardt, and Armin Buschauer*

Institute of Pharmacy, University of Regensburg, D-93040 Regensburg, Germany

Key Words: *Neuropeptide Y; Y_1 receptor; NPY antagonists; HEL cells; calcium assay*

Summary

Potent arpromidine-type histamine H_2 receptor agonists such as BU-E-76 (He 90481) were among the first non-peptides reported to display weak neuropeptide Y (NPY) Y_1 receptor antagonist activity. In search of new chemical leads for the development of more potent NPY antagonists, a series of *N,N*-disubstituted ω-guanidino and ω-aminoalkanoic acid amides were synthesized on the basis of structure-activity relationships and molecular modeling studies of arpromidine and related imidazolylpropylguanidines. In one group of compounds the imidazole ring was retained whereas in the second group it was replaced with a phenol group representing a putative mimic of Tyr^{36} in NPY. Although the substitution patterns have not yet been optimized, the title compounds are NPY Y_1 antagonists in human erythroleukemia (HEL) cells (Ca^{2+} assay) achieving pK_B values in the range of 6.3–6.6. For representative new substances tested in the isolated guinea pig right atrium histamine H_2 receptor agonism could not be found. In the *N*-(diphenylalkyl)amide series, compounds with a trimethylene chain were more active Y_1 antagonists than the ethylene homologs. Concerning the spacer in the ω-amino or ω-guanidinoalkanoyl portion, the best activity was found in compounds with a four- or five-membered alkyl chain or a 1,4-cyclohexylene group. Surprisingly, in contrast to the phenol series, in the imidazole series the compounds with a side chain amino group turned out to be considerably more potent than the corresponding strongly basic guanidines. Thus, the structure-activity relationships appear to be different for the diphenylalkylamide NPY antagonists with one or two basic groups.

Introduction

The 36 amino acid peptide neuropeptide Y (NPY)[1] is found in the central and peripheral nervous system and is involved in numerous biological processes[2–5]. In the periphery, NPY is a cotransmitter in the sympathetic nervous system and acts, for instance, as a potent vasoconstrictor via postsynaptic Y_1 receptor stimulation or as an inhibitor of neurotransmitter release owing to presynaptic Y_2 receptor stimulation[4]. Y_1 and Y_2 were the first pharmacologically characterized and cloned NPY receptors. Very recently, additional NPY receptor subtypes have been cloned (Y_4, Y_5, Y_6) (NPY receptor nomenclature cf. ref. [6]). For pharmacological discrimination of the receptors mainly NPY, PYY, analogs and peptide fragments of NPY were used[5]. Apart from numerous peptide ligands, only a few non-peptide antagonists have been described in the literature. Recently, the peptide-like compounds BIBP 3226[7] and SR 120819A[8] were reported to have high Y_1 receptor affinity and selectivity, and quinoline derivatives such as PD 160710[9] were described as novel NPY antagonists (Fig. 1) (for recent reviews ref. [10]).

Previously, the potent histamine H_2 receptor agonist BU-E-76 (He 90481), an analog of arpromidine[11], had been described as one of the first non-peptide NPY Y_1 receptor ligands[12]. The compound is a weakly active but competitive antagonist at Y_1 receptors (pA_2 4.43 in HEL cells)[12]. Structural modifications such as increasing hydrophobicity (e.g.

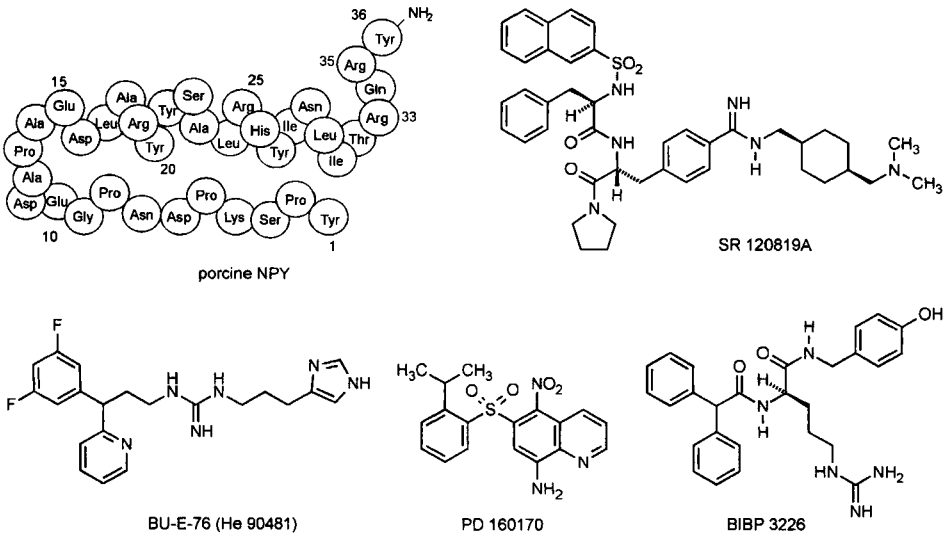


Fig. 1. Structures of porcine neuropeptide Y and neuropeptide Y antagonists

  0365-6233/97/1111/0333 $17.50 +.50/0

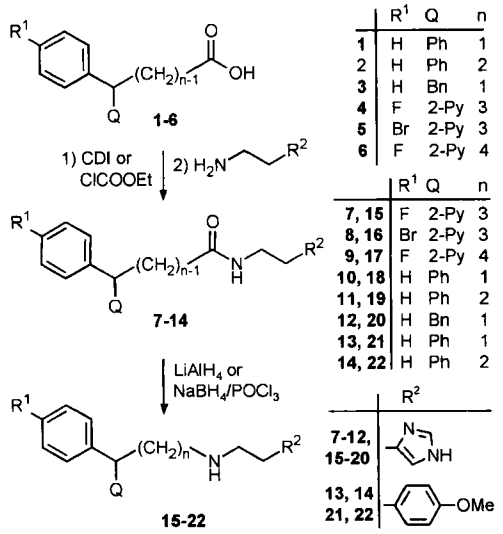
3,4-dichloro substitution at the phenyl ring) and/or replacement of the pheniramine-like phenyl(pyridyl)alkyl portion by a mepyramine-like halogenated benzyl(pyridyl)aminoalkyl partial structure, resulted in an up to 100-fold increase in Y_1 antagonistic activity[13]. The imidazole ring, which is a characteristic of guanidine-type histamine H_2 receptor agonists, is not essential for Y_1 antagonism[14]. However, structure-activity relationships of the arpromidine series and modeling studies indicate the importance of two basic centers, which are supposed to mimic the guanidino groups of Arg^{33} and Arg^{35} of NPY (cf. Fig. 1) in its putatively active conformation[13,15,16]. Based on this hypothesis, we have transferred the guanidino group from the chain into a terminal position resulting in alkanamides with a more arginine-like arrangement of the strongly basic center. In addition, the ring substituents as well as the chain lengths and the nature of the basic group were varied.

As an alternative to the imitation of an Arg residue, it is conceivable that the imidazole ring in arpromidine-like compounds and in corresponding new NPY antagonists mimics a tyrosine residue such as Tyr^{36} in NPY. Therefore, structural analogs bearing a phenol group instead of the imidazole ring were also synthesized in order to test this hypothesis and to compare the structure-activity relationships of both series of compounds.

Chemistry

The secondary amines **15–22** were prepared from diarylalkanoic acids **1–6**, which were first activated with carbonyldiimidazole (CDI) or with ethyl chloroformate and converted to the corresponding *N*-imidazolylalkyl- and *N*-(arylalkyl)amides **7–14**. Subsequently, the amides **7–14** were reduced with either lithium aluminum hydride or sodium borohydride/phosphorus oxychloride (Scheme 1, Table 1).

N-Acylation of the amines **15–22** with different ω-phthalimidoalkanoyl chlorides, prepared from the carboxylic acids **23–30**, and subsequent deprotection of compounds **31–57** by hydrazinolysis gave the *N,N*-disubstituted ω-aminoalkanoic acid amides **58–84** (Scheme 2, Table 2). The ether cleavage of the compounds with a *p*-methoxyphenyl substi-

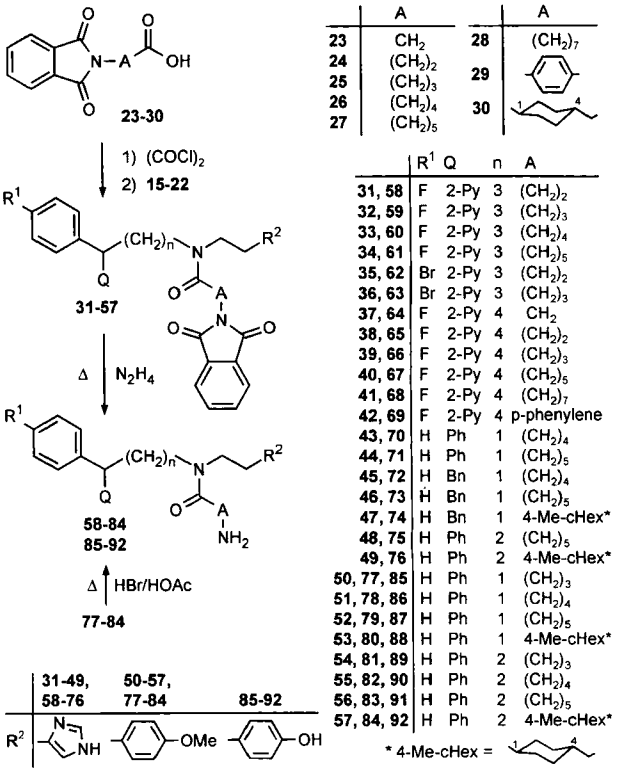


Scheme 1

Table 1. Analytical data of diarylalkanoic acid amides **7–14**, diarylalkanamines **15–22** and characterized phthalimides **31–42**.

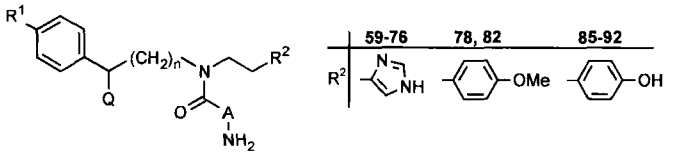
No.	Yield %	mp, °C	Analysis C, H, N[a]	+FAB-MS/[EI-MS] m/z[b] (%)
7	69	oil	$C_{20}H_{21}FN_4O$	352 (43), 242 (100)
8	63	oil	$C_{20}H_{21}BrN_4O$	413 (45), 302 (100)
9	68	oil	$C_{21}H_{23}FN_4O$	366 (30), 242 (100)
10	91	215	$C_{19}H_{19}N_3O$	[305 (19), 82 (100)]
11	85	167	$C_{20}H_{21}N_3O$	[319 (76), 82 (100)]
12	71	oil	$C_{20}H_{21}N_3O$	[319 (25), 82 (100)]
13	63	110	$C_{23}H_{23}NO_2$	[345 (9), 134 (100)]
14	68	100	$C_{24}H_{25}NO_2$	[359 (12), 134 (100)]
15	82	oil	$C_{20}H_{23}FN_4$	339 (9), 228 (100)
16	72	oil	$C_{20}H_{23}BrN_4$	[398 (2), 210 (100)]
17	84	oil	$C_{21}H_{25}FN_4$	353 (13), 105 (100)
18	65	oil	$C_{19}H_{21}N_3$	[291 (12), 67 (100)]
19	80	oil	$C_{20}H_{23}N_3$	[305 (21), 95 (100)]
20	95	oil	$C_{20}H_{23}N_3$	[305 (17), 81 (100)]
21	89	oil	$C_{23}H_{25}NO$	[331 (1), 164 (100)]
22	97	oil	$C_{24}H_{27}NO$	[345 (4), 44 (100)]
31	67	amorphous	$C_{31}H_{30}FN_5O_3$	[540 (15), 160 (100)]
33	63	amorphous	$C_{33}H_{34}FN_5O_3$	[568 (20), 228 (100)]
34	59	amorphous	$C_{34}H_{36}FN_5O_3$	[582 (15), 228 (100)]
35	67	amorphous	$C_{31}H_{30}BrN_5O_3$	[600 (7), 160 (100)]
37	62	amorphous	$C_{32}H_{32}BrN_5O_3$	[614 (4), 210 (100)]
38	70	amorphous	$C_{32}H_{32}FN_5O_3$	[554 (16), 160 (100)]
39	61	amorphous	$C_{33}H_{34}FN_5O_3$	[568 (31), 95 (100)]
40	70	amorphous	$C_{35}H_{38}FN_5O_3$	[596 (61), 160 (100)]
41	51	amorphous	$C_{37}H_{42}FN_5O_3$	[624 (15), 95 (100)]
42	45	amorphous	$C_{36}H_{32}FN_5O_3$	[602 (2), 93 (100)]

[a] Analysis C, H, N within ± 0.4%. [b] +FAB- or EI-MS (EI in square brackets); molecular ion, $[M + H]^+$ (FAB) or $[M^{+\bullet}]$ (EI) and base peak (100%).



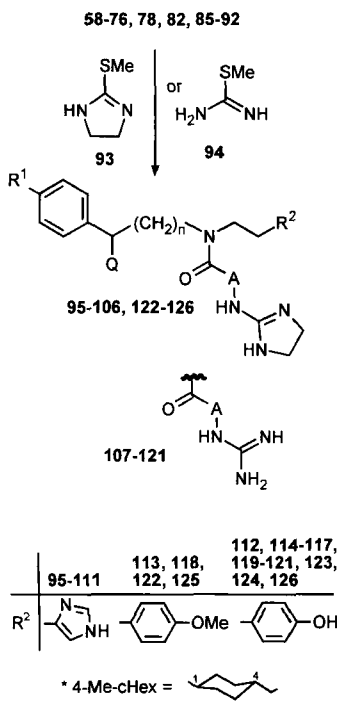
Scheme 2

Table 2. Analytical data and Y_1 receptor antagonistic activity of ω-aminoalkanoic acid amides.



No.	R^1	Q	*n*	A	Mp °C	Yield %	Analysis C, H, N[a]	$^+$FAB-MS *m/z*[b] (%)	Y_1 antagonism[c] IC_{50} [μM]
59	F	2-Py	3	$(CH_2)_3$	–[d]	66	$C_{24}H_{30}FN_5O$	424 (71), 277 (100)	> 10000
60	F	2-Py	3	$(CH_2)_4$	–[d]	54	$C_{25}H_{32}FN_5O$	438 (20), 228 (100)	n. d.
61	F	2-Py	3	$(CH_2)_5$	–[d]	49	$C_{26}H_{34}FN_5O$	452 (12), 228 (100)	n. d.
63	Br	2-Py	3	$(CH_2)_3$	–[d]	72	$C_{24}H_{30}BrN_5O$	484 (4), 210 (100)	6109
64	F	2-Py	4	CH_2	–[d]	88	$C_{23}H_{28}FN_5O$	410 (12), 95 (100)	n. d.
65	F	2-Py	4	$(CH_2)_2$	–[d]	58	$C_{24}H_{30}FN_5O$	424 (16), 95 (100)	n. d.
66	F	2-Py	4	$(CH_2)_3$	–[d]	84	$C_{25}H_{32}FN_5O$	438 (10), 95 (100)	n. d.
70	H	Ph	1	$(CH_2)_4$	–[d]	40	$C_{24}H_{30}N_4O$	391 (100)	20
71	H	Ph	1	$(CH_2)_5$	–[d]	50	$C_{25}H_{32}N_4O$	405 (60), 292 (100)	4
72	H	Bn	1	$(CH_2)_4$	–[d]	57	$C_{25}H_{32}N_4O$	405 (51), 305 (100)	1.7
73[f]	H	Bn	1	$(CH_2)_5$	–[d]	44	$C_{26}H_{34}N_4O$	419 (100)	0.9
74	H	Bn	1	4-Me-cHex[e]	–[d]	45	$C_{28}H_{36}N_4O$	445 (100)	>10000
75	H	Ph	2	$(CH_2)_5$	–[d]	49	$C_{26}H_{34}N_4O$	419 (70), 185 (100)	9
76	H	Ph	2	4-Me-cHex[e]	–[d]	51	$C_{28}H_{36}N_4O$	445 (80), 277 (100)	5
78	H	Ph	1	$(CH_2)_4$	oil	42	$C_{28}H_{34}N_2O_2$	431 (100)	n. d.
82	H	Ph	2	$(CH_2)_4$	oil	62	$C_{29}H_{36}N_2O_2$	445 (100)	n. d.
85	H	Ph	1	$(CH_2)_3$	45	75	$C_{26}H_{30}N_2O_2$	403 (100)	116
86	H	Ph	1	$(CH_2)_4$	–[d]	75	$C_{27}H_{32}N_2O_2$	417 (100)	367
87	H	Ph	1	$(CH_2)_5$	47	66	$C_{28}H_{34}N_2O_2$	431 (100)	860
88	H	Ph	1	4-Me-cHex[e]	50	69	$C_{30}H_{36}N_2O_2$	457 (100)	247
89	H	Ph	2	$(CH_2)_3$	–[d]	70	$C_{27}H_{32}N_2O_2$	417 (100)	80
90	H	Ph	2	$(CH_2)_4$	–[d]	78	$C_{28}H_{34}N_2O_2$	431 (100)	61
91	H	Ph	2	$(CH_2)_5$	–[d]	69	$C_{29}H_{36}N_2O_2$	445 (100)	132
92	H	Ph	2	4-Me-cHex[e]	50	69	$C_{31}H_{38}N_2O_2$	471 (100)	576

[a] Analysis C, H, N within ± 0.4%. [b] $[M + H]^+$ and base peak. [c] Inhibition of NPY (10 nM) induced increase in $[Ca^{2+}]_i$, antagonist concentrations used: 100, 50, 10 and 1 μM; mean of at least 3–5 independent experiments. [d] Hygroscopic amorphous solids. [e] 4-Me-cHex = 4-methylcyclohexane-1,α-diyl. [f] Exemplarily tested for histamine H_1 receptor antagonism (guinea pig ileum) and for H_2 agonism (guinea pig atrium): inactive at both test systems at concentrations ≤10 μM.



	R^1	Q	n	A
95	F	2-Py	3	$(CH_2)_2$
96	F	2-Py	3	$(CH_2)_4$
97	F	2-Py	3	$(CH_2)_5$
98	Br	2-Py	3	$(CH_2)_2$
99	Br	2-Py	3	$(CH_2)_3$
100	F	2-Py	4	CH_2
101	F	2-Py	4	$(CH_2)_2$
102	F	2-Py	4	$(CH_2)_3$
103	F	2-Py	4	$(CH_2)_5$
104	F	2-Py	4	$(CH_2)_7$
105	F	2-Py	4	p-phenylene
106	H	Ph	1	$(CH_2)_4$
107	F	2-Py	3	$(CH_2)_3$
108	H	Ph	1	$(CH_2)_4$
109	H	Ph	1	$(CH_2)_5$
110	H	Bn	1	$(CH_2)_4$
111	H	Bn	1	$(CH_2)_5$
112	H	Ph	1	$(CH_2)_3$
113	H	Ph	1	$(CH_2)_4$
114	H	Ph	1	$(CH_2)_4$
115	H	Ph	1	$(CH_2)_5$
116	H	Ph	1	4-Me-cHex*
117	H	Ph	2	$(CH_2)_3$
118	H	Ph	2	$(CH_2)_4$
119	H	Ph	2	$(CH_2)_4$
120	H	Ph	2	$(CH_2)_5$
121	H	Ph	2	4-Me-cHex*
122	H	Ph	1	$(CH_2)_4$
123	H	Ph	1	$(CH_2)_4$
124	H	Ph	1	4-Me-cHex*
125	H	Ph	2	$(CH_2)_4$
126	H	Ph	2	$(CH_2)_4$

Scheme 3

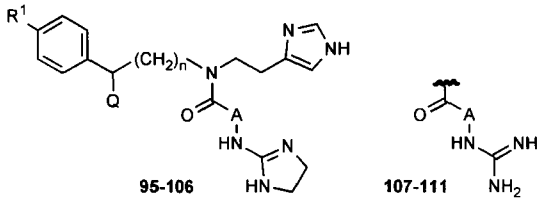
tuent (**77–84**) was carried out with hydrobromic acid leading to the *p*-hydroxyphenylethylamides **85–92**.

The ω-aminoalkanamides **58–76**, **78**, **82** and **85–92** were treated with 2-(methylthio)-2-imidazolinium iodide (**93** • HI) or *S*-methyl isothiuronium iodide (**94** • HI) (Scheme 3). As the resulting guanidines **95–126** (Tables 3, 4) could not be purified by crystallization, isolation and purification were carried out either on a rotatory chromatographic system (Chromatotron™) or, more efficiently, using a reversed phase HPLC and MPLC method developed for the separation of the guanidinium salts (hydroiodides or trifluoroacetates).

Pharmacology

The screening for Y_1 antagonistic activity was performed by measuring the inhibition of NPY-stimulated increase in intracellular $[Ca^{2+}]$ in HEL cells[17]. Some of the compounds were investigated for their activity at histamine H_2 receptors (isolated spontaneously beating guinea pig right atrium)[18] and for histamine H_1 receptor antagonism at the isolated guinea pig ileum[19].

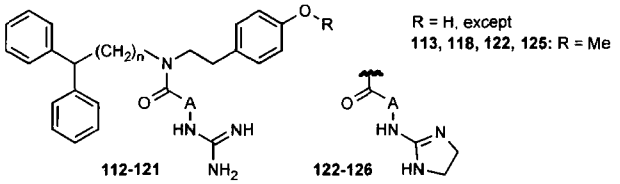
Table 3. Analytical data and Y_1 receptor antagonistic activity of imidazolylethyl substituted ω-guanidinoalkanoic acid amides **95–111**



No.	R^1	Q	*n*	A	Mp °C	Yield %	Analysis C, H, N[a]	$^+$FAB-MS *m/z*[b] (%)	Y_1 antagonism[c] IC_{50} [μM]
95[d]	F	2-Py	3	$(CH_2)_2$	–[e]	22	$C_{26}H_{32}FN_7O \bullet HI \bullet H_2O$	478 (13)	74
96	F	2-Py	3	$(CH_2)_4$	–[e]	32	$C_{28}H_{36}FN_7O \bullet HI \bullet H_2O$	506 (100)	247
97	F	2-Py	3	$(CH_2)_5$	–[e]	29	$C_{29}H_{38}FN_7O \bullet HI \bullet H_2O$	520 (100)	89
98[d]	Br	2-Py	3	$(CH_2)_2$	–[e]	20	$C_{26}H_{32}BrN_7O \bullet HI \bullet H_2O$	538 (7)	53
99	Br	2-Py	3	$(CH_2)_3$	–[e]	20	$C_{27}H_{34}BrN_7O \bullet HI \bullet H_2O$	552 (4), 154 (100)	110
100	F	2-Py	4	CH_2	–[e]	11	$C_{26}H_{32}FN_7O \bullet HI \bullet H_2O$	478 (17), 98 (100)	64
101	F	2-Py	4	$(CH_2)_2$	–[e]	16	$C_{27}H_{34}FN_7O \bullet HI \bullet H_2O$	492 (11), 55 (100)	17
102[d]	F	2-Py	4	$(CH_2)_3$	–[e]	20	$C_{28}H_{36}FN_7O \bullet HI \bullet H_2O$	506 (19), 95 (100)	21
103	F	2-Py	4	$(CH_2)_5$	–[e]	16	$C_{30}H_{40}FN_7O \bullet HI \bullet H_2O$	534 (15), 95 (100)	8
104	F	2-Py	4	$(CH_2)_7$	–[e]	25	$C_{32}H_{44}FN_7O \bullet HI \bullet H_2O$	562 (55), 55 (100)	40
105	F	2-Py	4	*p*-phenylene	–[e]	31	$C_{31}H_{34}FN_7O \bullet H_2O$	540 (<1), 120 (100)	207
106	H	Ph	1	$(CH_2)_4$	40	50	$C_{27}H_{34}N_6O \bullet HI \bullet CF_3COOH$	459 (100)	236
107	F	2-Py	3	$(CH_2)_3$	–[e]	22	$C_{25}H_{32}FN_7O \bullet HI$	466 (18), 228 (100)	519
108	H	Ph	1	$(CH_2)_4$	40	50	$C_{25}H_{32}N_6O \bullet HI \bullet CF_3COOH$	433 (100)	207
109	H	Ph	1	$(CH_2)_5$	43	55	$C_{26}H_{34}N_6O \bullet HI \bullet CF_3COOH$	447 (100)	93
110	H	Bn	1	$(CH_2)_4$	56	48	$C_{26}H_{34}N_6O \bullet HI \bullet CF_3COOH$	447 (100)	144
111	H	Bn	1	$(CH_2)_5$	54	55	$C_{27}H_{36}N_6O \bullet HI \bullet CF_3COOH$	461 (100)	65

[a] Analysis C, H, N within ± 0.4%. [b] $[M + H]^+$ and base peak. [c] Inhibition of NPY (10 nM) induced increase in $[Ca^{2+}]_i$, antagonist concentrations used: 100, 50, 10 and 1 μM; mean of at least 3–5 independent experiments. [d] Exemplarily tested for histamine H_2 receptor agonism (guinea pig atrium): inactive at concentrations ≤ 100 μM. [e] Hygroscopic amorphous solids.

Table 4. Analytical data and Y_1 receptor antagonistic activity of *p*-hydroxy- and *p*-methoxyphenylethyl substituted ω-guanidinoalkanoic acid amides **112–126**



No.	*n*	A	Mp °C	Yield %	Analysis C, H, N[a]	FAB-MS *m/z*[b] (%)	Y_1 antagonism[c] IC_{50} [μM]
112	1	$(CH_2)_3$	43	42	$C_{27}H_{32}N_4O_2 \bullet HI$	445 (100)	59
113	1	$(CH_2)_4$	56	48	$C_{29}H_{36}N_4O_2 \bullet HI$	473 (100)	490
114	1	$(CH_2)_4$	46	58	$C_{28}H_{34}N_4O_2 \bullet HI$	459 (100)	59
115	1	$(CH_2)_5$	49	43	$C_{29}H_{36}N_4O_2 \bullet HI$	473 (100)	37
116	1	4-Me-cHex[d]	89	65	$C_{31}H_{38}N_4O_2 \bullet HI$	499 (100)	30
117	2	$(CH_2)_3$	49	58	$C_{28}H_{34}N_4O_2 \bullet HI$	459 (100)	31
118	2	$(CH_2)_4$	56	48	$C_{30}H_{38}N_4O_2 \bullet HI$	487 (100)	157
119	2	$(CH_2)_4$	46	30	$C_{29}H_{36}N_4O_2 \bullet HI$	473 (100)	25
120	2	$(CH_2)_5$	–[e]	50	$C_{30}H_{38}N_4O_2 \bullet HI$	487 (100)	47
121[f]	2	4-Me-cHex[d]	91	55	$C_{32}H_{40}N_4O_2 \bullet HI$	513 (100)	11
122	1	$(CH_2)_4$	54	55	$C_{31}H_{38}N_4O_2 \bullet HI$	499 (90), 185 (100)	133
123	1	$(CH_2)_4$	62	62	$C_{30}H_{36}N_4O_2 \bullet HI$	485 (100)	99
124	1	4-Me-cHex[d]	92	60	$C_{33}H_{40}N_4O_2 \bullet HI$	525 (100)	30
125	2	$(CH_2)_4$	54	55	$C_{32}H_{40}N_4O_2 \bullet HI$	513 (100)	133
126	2	$(CH_2)_4$	45	40	$C_{31}H_{38}N_4O_2 \bullet HI$	499 (100)	20

[a] Analysis C, H, N within ± 0.4%. [b] $[M + H]^+$ and base peak. [c] Inhibition of NPY (10 nM) induced increase in $[Ca^{2+}]_i$, antagonist concentrations used: 100, 50, 10 and 1 μM; mean of at least 3–5 independent experiments. [d] 4-Me-cHex = 4-methylcyclohexane-1,α-diyl. [e] Hygroscopic amorphous solid. [f] Exemplarily tested for histamine H_1 antagonism (guinea pig ileum): inactive at concentrations ≤ 10 μM.

Results and discussion

Most of the investigated compounds inhibited the NPY-induced Ca^{2+} response in HEL cells (Tables 2–4). For comparison of the substances, Y_1 receptor antagonistic activity is given as IC_{50} values in the presence of 10 nM porcine NPY. Complete concentration response curves of NPY were only constructed in the presence of selected compounds which showed promising antagonist activity in the screening procedure. A representative concentration response curve for NPY in the presence of compound **73** is shown in Fig. 2. The achieved maximum Y_1 receptor antagonist activities expressed as IC_{50} correspond to pK_B values in the range of 6.3–6.7, which is comparable to the results previously found for some chloro-substituted arpromidine analogs[13]. However, in contrast to the latter, the new substances are devoid of histamine H_2 receptor agonistic properties in the guinea pig right atrium (proved for representative compounds).

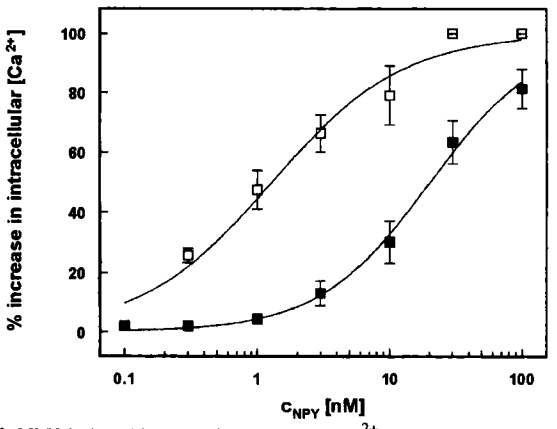


Fig. 2. NPY-induced increase in intracellar $[Ca^{2+}]$. Concentration-response curves of (□) NPY alone and (■) in presence of compound **73** (5 μM). pK_B value for compound **73**: 6.47

The structure-activity relationships for NPY Y_1 antagonism in HEL cells may be summarized as follows:

N-[Phenyl(pyridyl)alkyl]amides **95–105**, **107** *(cf. Table 3)*

Prolongation of the $(CH_2)_n$ chain from 3 to 4 methylene groups enhances activity. A spacer 'A' = $(CH_2)_5$ in combination with a diarylpentyl substituent results in the most active compound **103** within this group. Increasing hydrophobicity in the diarylbutyl portion by replacing F with Br (cf. **96** and **99**) also enhances activity. The guanidines **99** and **107** proved to be more active in the calcium assay than the corresponding amines **59** and **63**. For compounds **95**, **98**, and **102** no H_2 receptor agonistic activity was found.

Diphenylalkylamides **70–76**, **106**, **108–111**, **85–92**, **112–126**

Unlike the aforementioned compounds **95–105**, several substances such as **72**, **73**, or **125** could only be tested at concentrations ≤ 50 μM since they induced a concentration-dependent lysis of the HEL cells. Hemolysis assays confirmed a correlation of this effect with hydrophobicity (data not shown). In most cases, compounds with *N*-(diphenylpropyl)amide partial structure ($n = 2$, or $n = 1$ with Q = benzyl) are more active than their diphenylethyl analogs (Tables 2–4). Activity depends on the length of the spacer A, but this dependence differs between the imidazole and the phenol series. Compounds with *N*-[2-(4-imidazolyl)ethyl]amide partial structure (Tables 2, 3) show highest activity for A = $(CH_2)_5$. In the phenol series, amines with A = $(CH_2)_3$ or $(CH_2)_4$ are more active than with A = $(CH_2)_5$ (compds. **85–92**, Table 2). For the corresponding guanidines (Table 4) no strong influence of A is observed, but a rigid *trans*-4-methylcyclohexane-1,α-diyl spacer is slightly superior to flexible chains.

Imidazole vs. phenol series of diphenylalkylamide NPY antagonists

Although the compounds described in this paper were developed independently from BIBP 3226, there are some obvious structural similarities, in particular considering the phenol series, and possible functional analogies with respect to the putative interaction with the Y_1 receptor. Therefore, the structure-activity-relationships of both series should also be compared to BIBP 3226 and related NPY antagonists. In contrast to analogs of BIBP 3226[20], there is no significant difference in Y_1 receptor antagonist activity between compounds with geminal or vicinal arrangement of the aromatic rings.

Concerning the impact of the basic group, there is a major difference in the rank order of potency comparing compounds of both the imidazole and the phenol series (imidazoles **70–73**, **75**, **76**, **106**, **108–111** vs. phenols **85–92**, **112**, **114–117**, **119–121**, **123**, **124**, **126**). It is known from investigations with receptor mutants that acidic residues in the extracellular loops of the Y_1 receptor are of special importance for NPY binding[21], indicating a key function of the basic groups in the NPY molecule and (possibly) in antagonists, too. Under the assumption that a guanidino function mimics the basic side chain group of an arginine in NPY, an increase in activity should be achieved by conversion of the primary side chain amino group to the corresponding guanidines. This was confirmed for the *N*-imidazolylethyl-*N*-(ω-phenyl-ω-pyridyl)alkylamides (see above) and was also found for the phenol series of diphenylalkylamides (cf. **85–92** in Table 2 and corresponding guanidines in Table 4). By contrast, surprisingly, in the imidazole series, the ω-aminoalkanoic acid diphenylalkylamides **70–76** (except **74**) turned out to be considerably more potent than the corresponding strongly basic guanidines **106**, **108–111** and were among the most potent Y_1 antagonists described in this paper. Thus, in the diphenylalkylamide series the structure-activity relationships appear to be different for those NPY antagonists with one and those with two basic groups, indicating that both partial structures, the phenol and the imidazole group, are not bioisosteric.

Molecular modeling

Molecular modeling studies[13] comparing conformations of arpromidine derivatives with possible spatial arrangements of NPY residues relevant for Y_1 agonistic activity[22] have suggested that the guanidino group and the imidazole ring might mimic the guanidino groups of Arg^{33} and/or Arg^{35} in the NPY molecule. One possible explanation for the remarkable Y_1 receptor affinity of compounds **70–76** (except **74**) is

that the imidazole ring and the amino group also imitate the guanidino groups of these residues. Superpositions of **73** with the putative 3D structure of NPY[22,23] (for example, see Figure 3), derived from the X-ray structure of the highly homologous avian pancreatic polypeptide (APP)[24], indicate that one phenyl group may point to the N-terminus of NPY and, by that way, possibly mimic the aromatic ring of Tyr^1. In this conformation, the spatial position of the second phenyl ring corresponds to the lipophilic residue Ile^{31} and to Tyr^{27}. Activity at the Y_1 receptor might depend on the occupation of the Tyr^1 binding site. Owing to the high flexibility of **73**, a lot of conformations within 5 kcal/mole above the absolute energy minimum can be generated which fulfill the distance constraints between Arg^{33}, Arg^{35}, and Tyr^1. Different superpositions of both stereoisomers of **73** with NPY are possible, e. g. the amino group with Arg^{33} and the imidazole ring with Arg^{35}, or the benzyl instead of the phenyl moiety with Tyr^1. The given example, however, is one of the best fits where, additionally, the volume of **73** falls within the volume of NPY. Compounds **70–72** and **74–76** enable similar arrangements of their basic groups and aromatic rings. Interestingly, with a rigid cyclohexyl chain A, the diphenylpropyl derivative **76** shows Y_1 antagonistic activity, whereas its structural isomer **74**, with a benzylphenylethyl moiety like **73**, is completely inactive. This indicates that indeed some degree of folding is necessary for the fit of the amino group to Arg^{33} or Arg^{35} as suggested by Fig. 3. In the present state of our research, it seems not appropriate to derive a definite model explaining all of these structure-activity relationships. Since the given probable conformation of NPY has not been proven up to now, the present conclusions from molecular modeling are only hypotheses suggesting the design of new derivatives with, e.g., hydroxy-substituted phenyl rings. Molecular modeling also cannot explain the higher activity of the amines **70–73** compared to their analogous guanidines **106, 108–111**. The only difference is additional branching of the guanidino group, which might complicate the folding necessary for binding.

By analogy with BIBP 3226 (IC_{50} = 17 nM in the Ca^{2+} assay), the guanidines of the phenol series **112–126** seem to mimic the C-terminal part of the NPY molecule, i.e. the amino acids Arg^{35} and Tyr^{36}, which are essential for the binding of NPY to the Y_1 receptor[22,25]. Recently, two studies of BIBP 3226 binding at the Y_1 receptor were published[25,26]. In one study[25], Asp^{287} in extracellular loop 3 close to transmembrane domain 6 (TM6) was proposed to interact with the guanidino group of the ligand. Other key residues, including Phe^{173}, Tyr^{211}, Gln^{219}, Asn^{283}, and Phe^{286} were also found to be important for antagonist binding. A superposition of **119** with the putative binding conformation of BIBP 3226[25] (Fig. 4) indicates that the guanidines of the phenol series may interact in a similar way involving several interaction sites with both hydrophobic and polar residues. But Figure 4 also shows that the backbone hetero atoms of BIBP 3226 and **119** are not in the same spatial position. Therefore, the only moderate activity of compounds like **119** should be due to their non-optimal backbones. As suggested by the model in ref. [25], the peptide-like backbone structure of BIBP 3226 appears to be essential for high-affinity binding. Of course, a mode of interaction of compounds **112–126** with the Y_1 receptor completely different from that of BIBP 3226 may not be precluded.

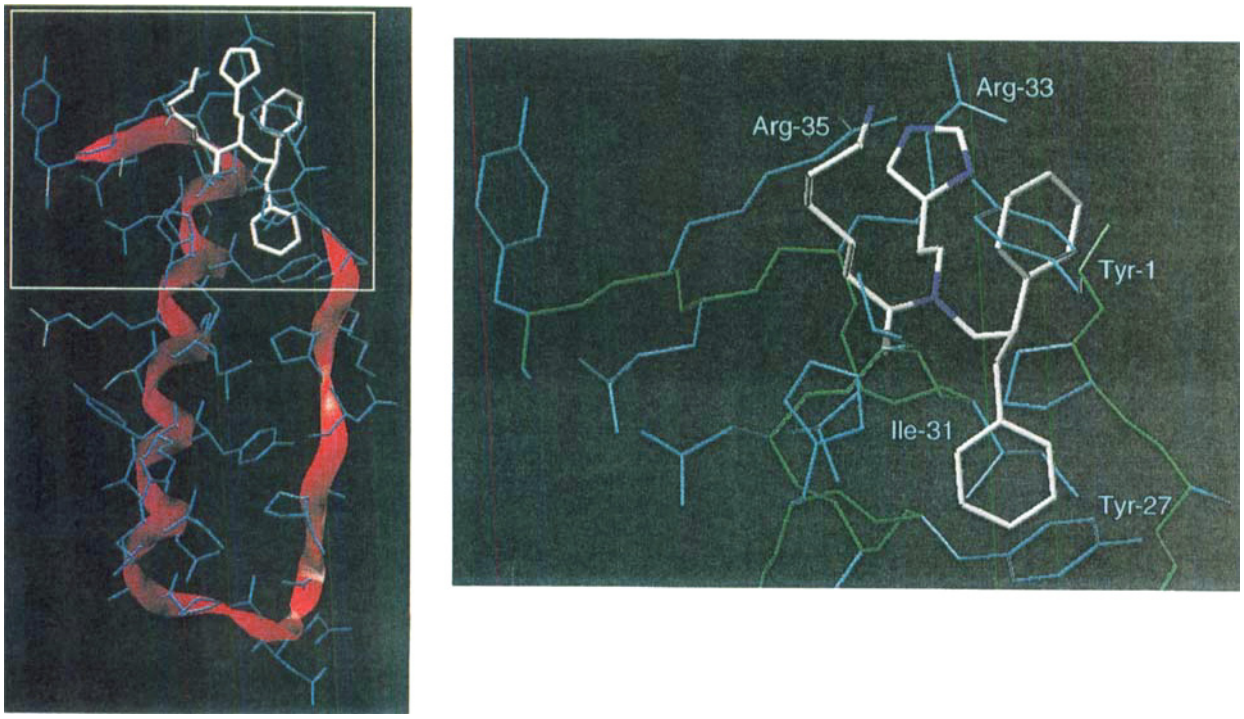


Fig. 3. Exemplary superposition of compound **73** with the putative Y_1-receptor binding conformation of NPY[23,24], comprising fits of the amino group with Arg^{35}, of the imidazole ring with Arg^{33}, and of the phenyl ring with Tyr^1. The folded conformation of **73** represents a local energy minimum about 2 kcal/mole above the energy of a completely extended structure. (**a**). General view. Colors: NPY – light blue, **73** – white. The secondary structure of NPY is shown by a shadowed ribbon (red). (**b**). Zoom of the frame in (a). Colors: NPY backbone – green, NPY side chains – light blue, **73** – white, N atoms – violet.

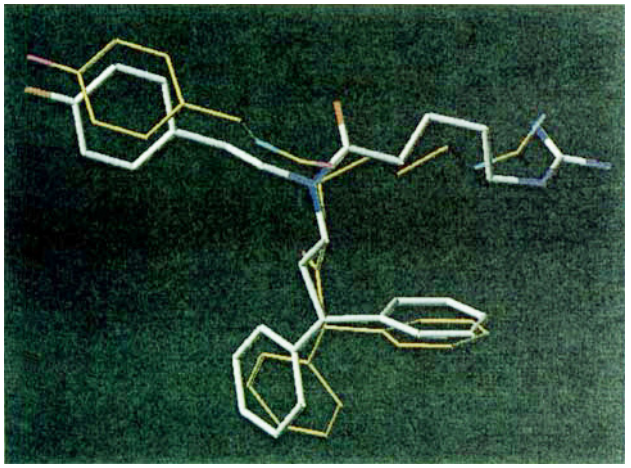


Fig. 4. Superposition of compound **119** with the putative Y_1-receptor binding conformation of BIBP 3226[26], comprising fits of the three phenyl rings and of the guanidino groups. The given conformation of **119** is a local energy minimum 0.5 kcal/mole above the energy of a completely extended structure and about 4 kcal/mole above the absolute minimum (containing some intramolecular van der Waals interactions). Colors of **119**: C – white, N – violet, O – red. Colors of BIBP 3226: C – orange, N – light blue, O – magenta.

Conclusion

In summary, the title compounds may be considered as novel model structures for the development of NPY Y_1 receptor antagonists. Further modifications of the simple parent molecules are required to improve antagonist activity. Analogous structural variations of BIBP 3226, e.g. replacement of phenol by imidazole or guanidine by amine, will be useful to elucidate differences or similarities in the structure-activity relationships of both types of NPY antagonists. The imidazolylethylamides with a side chain amino group are less basic than corresponding guanidines and may be useful with respect to the development of centrally active non-peptide NPY receptor ligands.

Acknowledgement

The authors are grateful to Mrs. E. Schreiber and Mrs. L. Schneider for technical support and to the Fonds der Chemischen Industrie for a grant.

Experimental Part

Chemistry

Melting points (uncorrected) were determined on a Büchi 530 apparatus. Elemental analyses, indicated by elemental symbols, were within ± 0.4% of the theoretical values and were performed at the analytical department of the faculty of chemistry and pharmacy, University of Regensburg. The structures were confirmed by ^{1}H NMR (Bruker WM 250 (250 MHz), TMS as internal standard) and by mass spectroscopy (EI-MS: Varian MAT 112S, FAB: Varian MAT 95 ($^+$FAB-MS, xenon, MeOH/glycerol)). ^{1}H NMR data are given only exemplarily in the experimental section because of the structural resemblance within the groups of compounds synthetized according to general procedures. Chromatographic separations on a preparative scale were carried out on a Chromatotron 8924 (Harrison Research) using glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck) or on a MPLC apparatus (Kronlab) consisting of a Controller Labomat VS 200, UV-Detector Labomatic, a pump MD 80/100, a sampler Roto-Ultra/120, a 2210-2 Channel-Recorder (LKB), a main-column (539 mm × 37 mm) and a pre-column (313 mm × 37 mm), both packed with 20–40 µM LiChrosorb RP 18 (18-30-60). The diarylalkanoic acids **1–3**, required for the preparation of diarylalkanamides **10–14**, were commercially available.

General procedure for the preparation of the diarylalkanoic acids **4–6**

The diarylalkanoic acids **4–6** were prepared by analogy with a procedure described in ref.[27]. In brief, for the synthesis of the intermediates, ω-cyano-ω-phenyl-ω-(2-pyridyl)alkanoic acid esters, the requisite phenyl(pyridyl)-acetonitriles were either treated with acrylic acid ethyl ester and Triton B (40% in MeOH) in dioxane (method A) or were converted to the sodium salt with sodium hydride in DMF, followed by C-alkylation with an appropriate ω-bromoalkanoic acid ethyl ester (method B). The crude intermediates were isolated by extraction with Et_2O or EtOAc. After evaporation of the solvent the ethyl ω-cyanoalkanoates were stirred at 120 °C in 70% sulfuric acid for 5 h. The chilled mixture was rendered alkaline with sodium carbonate and was extracted with Et_2O to remove impurities. The residue was adjusted to pH 6 with acetic acid and extracted with EtOAc. The carboxylic acids **4–6**, obtained in 75–85% yield after evaporation of the solvent, were sufficiently pure for further reaction. Compound **5** was crystallized from EtOAc.

4-(4-Bromophenyl)-4-(2-pyridyl)butanoic acid, **5**: yield 82%, mp 123 °C (EtOAc). Anal. ($C_{15}H_{14}BrNO_2$) C, H, N.– EI-MS: *m/z* (%) = 319 (28) [M^+], 260 (100) [M – COOH]$^+$, 247 (29, [$BrC_6H_4CH_2Py$]$^+$).– ^{1}H NMR ($CDCl_3$): δ = 2.32–2.56 (m, 4H, CH(CHC*H*$_2$C*H*$_2$), 4.28 (t, *J* = 7.4, 1H, PyC*H*), 7.13–7.26 (m, 4H, aromatic), 7.42 (d, *J* = 8.3, 2H, aromatic), 7.61–7.66 (m, 1H, Py-4-H), 8.58 (d, *J* = 4.5, 1H, Py-6-H).

5-(4-Fluorophenyl)-5-(2-pyridyl)pentanoic acid, **6**: yield 75% oil. Anal. ($C_{16}H_{16}FNO_2$) C, H, N.– EI-MS: *m/z* (%) = 273 (39) [M^+], 225 (100) [M – COOH]$^+$, 212 (44) [$FC_6H_4CH_2Py$]$^+$.– ^{1}H NMR ([D$_6$]DMSO): δ = 1.48–1.58 (m, 2H, PyCHCH$_2$C*H*$_2$), 2.19–2.24 (t, *J* = 7.2, 2H, PyCHC*H*$_2$), 2.62–2.66 (m, 2H, CH_2CO), 4.12 (m, 1H, PyC*H*), 7.23–7.29 (dd, *J* = 8.7 and 8.8, 2H, *ortho* to F), 7.37–7.41 (m, 1H, Py-5-H), 7.49–7.53 (m, 3H; 2H *meta* to F and Py-3-H), 7.89 (dd, *J* = 7.5 and 7.5, Py-4-H), 8.62 (d, *J* = 4.1, 1H, Py-6-H), 12.13 (s, 1H, COOH).

General procedure for the preparation of the N-[2-(4-imidazolyl)ethyl]-diarylalkanoic acid amides **7–12**

N,N'-Carbonyldiimidazole (3.3 g, 20 mmol) was added to a solution of the pertinent diarylalkanoic acid **1–6** (20 mmol) in 10 ml of anhydrous THF and allowed to react for 30 min. Subsequently 2.32 g (20 mmol) histamine were added, and the mixture was stirred overnight. After evaporation in vacuo, the remaining solid was triturated with water for 4 h and recrystallized from MeOH. Yield 70–90%.

For analytical data of new compounds see Table 1.

^{1}H NMR ([D$_6$]DMSO) of **7**: δ = 1.91–1.96 (m, 2H, COC*H*$_2$), 2.15–2.22 (m, 1H, CHC*H*H), 2.34–2.41 (m, 1H, CHCH*H*), 2.59 (t, *J* = 7.4 Hz, 2H, ImC*H*$_2$), 2.88 (t, *J* = 6.5, 2H, COCHH), 3.22–3.26 (m, 2H, NHC*H*$_2$), 3.37 (t, *J* = 6.6 Hz, 2H, COC*H*H), 4.04 (t, *J* = 7.6 Hz, 1H, PyC*H*), 6.77 (s, 1H, Im-5-H), 7.06–7.37 (m, 6H, FPh-H, Py-3-H, -5-H), 7.51 (s, 1H, Im-2-H), 7.66–7.71 (dd, *J* = 7.7 Hz, 7.7 Hz, 1H, Py-4-H), 8,52 (d, *J* = 3.8 Hz, 1H, Py-6-H).

General procedure for the preparation of the N-[2-(4-methoxyphenyl)ethyl]-diarylalkanoic acid amides **13** *and* **14**

A solution of the pertinent diphenylalkanoic acid **1** or **2** (10 mmol in 30 ml of anhydrous THF) was stirred and chilled to 0 °C. Triethylamine (1.01 g, 10 mmol) was added, followed by ethyl chloroformate (1.09 g, 10 mmol), and the mixture was stirred for 30 min. Then 1.51 g (10 mmol) 2-(4-methoxyphenyl)ethylamine were added, the ice-bath was removed, and the mixture was allowed to warm to room temperature. Insoluble material was filtered off, the solution was evaporated in vacuo and the remaining solid was stirred in water for about 10 h. The amides **13** and **14** were recrystallized from MeOH. Yield 60-89%, cf. Table 1.

^{1}H NMR ($CDCl_3$) of **13**: δ = 2,49 (t, *J* = 6.8, 2H, $CH_2C\mathit{H}_2$Ar), 2.82 (d, *J* = 7.8, 2H, CHC*H*$_2$CO), 3.32 (q, *J* = 6.7, 2H, NHC*H*$_2$CH$_2$), 3.77 (s, 3H, ArOC*H*$_3$), 4.56 (t, *J* = 7.7, 1H, C*H*CH$_2$CO), 5.34 (br, 1H, N*H*), 6.7–6.95 (AA'BB', 4H, Ar-*H*), 7.1–7.35 (m, 10H, Ph-*H*),

General procedure for the preparation of the [ω-phenyl-ω-(2-pyridyl)alkyl]-[ω-1H-imidazol-4-yl)alkyl]amines **15–17**

A solution of the amides **7–9** (15 mmol in 20 ml of anhydrous THF) was chilled to 0 °C and added to a suspension of 0.86 g (24.4 mmol) $LiAlH_4$ in 25 ml of anhydrous THF. The mixture was refluxed for 3 h and then cooled with a water bath. Subsequently, 20 ml of a saturated aqueous seignette salt solution were slowly dropped to the reaction mixture, 2 ml of a 10% aqueous NaOH solution were added and the formed precipitate was filtered off and washed several times with hot THF. The organic solution was dried over Na_2SO_4 and evaporated in vacuo. The crude oily amines were purified chromatographically (Chromatotron; CH_2Cl_2–MeOH, 90:10, ammonia atmosphere). Yield 72–84%, cf. Table 1.

^{1}H NMR ([D$_6$]DMSO) of **15**: δ = 1.23–1.28 (m, 2H, $CHCH_2CH_2$), 1.98 (m, 1H, $CHCHH$), 2.14 (m, 1H, $CHCHH$), 2.56–2.64 (m, 2H, $ImCH_2$), 3.17–3.38 (m, 4H, $NH(CH_2)_2$), 4.04–4.09 (t, J = 7.8 Hz, 1H, $PyCH$), 6.70 (s, 1H, Im-5-H), 7.05–7.41 (m, 6H, FPh-H, Py-3-H, -5-H), 7.46 (s, 1H, Im-2-H), 7.65–7.70 (dd. J = 6.4 Hz, 6.2 Hz, 1H, Py-4-H), 8.50 (m, 1H, Py-6-H).

General procedure for the preparation of the diphenylalkylamines **18–22**

The amides **10–14** (5 mmol) were dissolved in 20 ml of phosphorus oxychloride at room temperature and the solution was stirred for 5 h. Excess $POCl_3$ was removed in vacuo, the remaining oil was dissolved in 20 ml of glyme, the solution was cooled in an ice-bath and $NaBH_4$ (0.56 g, 5 mmol) was added with vigorous stirring. The reaction mixture was stirred for 12 h at room temperature. Subsequently, under cooling with ice, 10 ml of 10% hydrochloric acid were added dropwise. Glyme was removed by evaporation in vacuo, water was added to adjust the volume to 30 ml, and the mixture was refluxed for 30 min. After extraction with ether to remove starting material and by-products, the aqueous layer was made alkaline with 3.0 g of sodium hydroxide and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated in vacuo affording the oily amines **18–22**. Yield 85–90%, cf. Table 1.

^{1}H NMR ($CDCl_3$) of **22**: δ = 2.2 (q, J = 7.5, 2H, $CHCH_2CH_2$), 2.56 (t, J = 6.9, 2H, $CHCH_2CH_2$), 2.65–2.85 (m, 4H, $NHCH_2CH_2Ar$), 3.78 (s, 3H, $ArOCH_3$), 3.9 (t, J = 7.8, 1H, $CHCH_2$), 6.75–7.1 (AA′BB′, 4H, Ar-H), 7.1–7.3 (m, 10H, Ph-H).

General procedure for the preparation of the ω-phthalimidoalkanoic acids **23–30**

A mixture of the ω-aminoalkanoic acids (10 mmol) and phthalic anhydride (5.18 g, 35 mmol) in 45 ml of glacial acetic acid was heated to reflux for 16 h. The mixture was diluted with 200 ml of water and extracted with $CHCl_3$. The combined organic layers were evaporated in vacuo and **23–30** were recrystallized from 50% acetic acid. **23**: yield 95%, mp 189 °C (ref. [28] 193 °C). Anal. ($C_{10}H_7NO_4$) C, H, N. **24**: yield 96%, mp 157 °C (ref. [29] 151 °C). Anal. ($C_{11}H_9NO_4$) C, H, N. **25**: yield 81%, mp 114 (ref.[30] 117 °C) analysis ($C_{12}H_{11}NO_4$) C, H, N. **26**: yield 92%, mp 113 °C (ref.[31] 117 °C). Anal. ($C_{13}H_{13}NO_4$) C, H, N. 27: yield 89%, mp 98 °C (ref.[32] 107 °C). Anal. ($C_{14}H_{15}NO_4$) C, H, N. **28**: yield 93%, mp 94 °C (ref. [33] 95 °C). Anal. ($C_{16}H_{19}NO_4$) C, H, N. **29**: yield 83%, mp 307 °C (ref. [34] 290 °C). Anal. ($C_{15}H_9NO_4$) C, H, N. 4-(Phthalimidomethyl)cyclohexanecarboxylic acid, **30**: Yield 80%, mp 188 °C. Anal. ($C_{16}H_{17}NO_4$) C, H, N; ^{1}H NMR ($CDCl_3$): δ = 0.95–1.5 (AA′BB′, 4H, cHex-2-H, cHex-6-H), 1.7–2.15 (m, 5H, cHex-3-H, cHex-5-H, $CHCH_2$), 2.27 (m, 1H CHCO), 3.54 (d, J = 6.8, 2H, NCH_2CH), 7.7–7.9 (AA′BB′, 4H), 11.0 (br s, 1H, COOH).

General procedure for the preparation of the ω-phthalimidoalkanoic acid amides **31–57**

The ω-phthalimidoalkanoic acids **23–30** (5 mmol) were dissolved in 20 ml of anhydrous CH_2Cl_2 and chilled to 0 °C. Under nitrogen atmosphere, 2.54 g (20 mmol) of oxalyl chloride were added dropwise, the mixture was warmed to room temperature and stirred for 2 h. After removal of the volatiles in vacuo, a portion (5 mmol) of the pertinent acid chloride was dissolved in anhydrous THF, added to a mixture of the secondary amines **15–22** (5 mmol) and triethylamine (0.51 g, 5 mmol) in anhydrous THF and stirred overnight. Precipitated triethylamine hydrochloride was filtered off, and the solution was evaporated in vacuo. The remaining oily or amorphous amides **31–57** were sufficiently pure for further reactions. Compounds analysed for C, H, N cf. Table 1.

General procedure for the preparation of the ω-aminoalkanoic acid amides **58–84**

Hydrazine hydrate (0.5 g, 10 mmol) was added to a solution of the ω-phthalimidocarboxamides **31–57** (5 mmol) in MeOH. The mixture was heated under reflux for 1 h, then 2 ml of 10% HCl were added, and the mixture was heated under reflux for 30 min. Insoluble material was filtered off and washed with water. The filtrate was concentrated, made alkaline and extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4. The crude amines were purified chromatographically (Chromatotron; MeOH, ammonia atmosphere). Yield 40–80%. Data of compounds analysed for C, H, N cf. Table 2.

^{1}H NMR ($CDCl_3$) of **73**: δ = 1.05-1.6 (m, 6 H, $(CH_2)_3CH_2N$), 1.6–2.1 (2m, 1.2 and 0.8H, $COCH_2CH_2$),), 2.5–2.75 (m, 4H, $CHCH_2N$, CH_2Im), 2.75–3.6 (m, 6.4 H, $PhCH_2CH$, CH_2NH_2, CH_2CH_2Im), 3.78 (quint, J = 5.7, 0.6 H , CH_2CHCH_2), 4.22 (br, 2H, NH_2), 6.61 (s, 1H, Im-5-H), 7.0–7.3 (m, 10H, Ph-H), 7.45 (s, 0.5 H, Im-2-H), 7.49 (s, 0.5 H, Im-2-H)

General procedure for the preparation of the 4-hydroxyphenylethylamides **85–92**

The 4-methoxyphenylethylamides **77–84** (2.5 mmol) were heated under reflux with a 1:1 mixture of 10 ml of glacial acetic acid and 10 ml 48% aqueous HBr. After 1 h the mixture was poured into 50 ml of water, made alkaline with 20% NaOH and extracted with ether. The pH was adjusted to 8–9 by addition of 10% H_2SO_4 and the solution was extracted with $CHCl_3$. The combined organic solutions were dried over Na_2SO_4. The crude compounds **85–92** were purified chromatographically (Chromatotron; MeOH, ammonia atmosphere). Yield 40–80%. Analytical data cf. Table 2.

^{1}H NMR ($CDCl_3$) of **92**: δ = 0.6–1.4 (m, 5H, $CHCH_2NH_2$, cHex-2-H, -6-H), 1.4–1.95 (m, 5H, $CHCO$, cHex-3-H, -5-H), 2.2–2.4 (m, 2H, $CHCH_2CH_2$), 2.47 (d, J = 6.0, 2H, $CHCH_2NH_2$), 2.61 (t, J = 6.4, 1H, CH_2CH_2Ar), 2.69 (t, J = 6.4, 1H, CH_2CH_2Ar), 3.06 (t, J = 6.4, 1H, CH_2CH_2Ar), 3.22 (t, J = 6.4, 1H, CH_2CH_2Ar), 3.36 (t, J = 6.7, 1H, $CHCH_2CH_2$), 3.47 (t, J = 6.7, 1H, $CHCH_2CH_2$), 3.77 (t, J = 7.8, 0.5H, Ph_2CHCH_2), 3.91 (t, J = 7.8, 0.5H, Ph_2CHCH_2), 4.08 (br, 2H, NH_2), 6.7–7.0 (AA′BB′, 4H, Ar-H), 7.1–7.35 (m, 10H, Ph-H).

General procedure for the preparation of the ω-[3,4-dihydroimidazol-2-yl)amino]alkanoic acid amides **95–105**

A solution of an amine **58, 60–69** (0.75 mmol) and 2-(methylthio)-3,4-dihydroimidazole hydroiodide (**93**·HI) (0.19 g, 0.75 mmol) in 5 ml of pyridine–EtOH (4:1) was heated under reflux for 13 h. After evaporation in vacuo, the remaining oil was purified chromatographically (Chromatotron; CH_2Cl_2–MeOH, 95:5 to 80:20, ammonia atmosphere). Yield 10–30% of the corresponding guanidine hydroiodide. Analytical data cf. Table 3.

^{1}H NMR ([D$_6$]DMSO) of **103**: δ = 1.15–1.23 (m, 4H, $CHCH_2(CH_2)_2$), 1.48 (m, 6H, $COCH_2(CH_2)_3$), 1.99 (m, 1H, $CHCHH$), 2.20 (m, 3H, $COCH_2$, $CHCHH$), 2.77 (m, 2H, $ImCH_2$), 3.16 (m, 4H, $CH(CH_2)_3CH_2$, $CO(CH_2)_4CH_2$), 3.45 (m, 2H, $ImCH_2CH_2$), 3.58 (s, 4H, $N(CH_2)_2N$), 4.09 (m, 1H, $PyCH$), 6.92 (br, 1H, NH), 7.09–7.47 (m, 9H, FPh-H, Py-3-H, -5-H, Im-2-H, -5-H, NH), 7.69 (m, 1H, Py-4-H), 8.15 (s, 1H, $=NH^+$), 8.50 (m, 1H, Py-6-H).

General procedure for the preparation of the ω-guanidinoalkanoic acid amides **106–126**

A solution of an amine **59, 70–76, 78, 82, 85–92** (0.75 mmol) and 2-(methylthio)-3,4-dihydroimidazole hydroiodide (**93**•HI) (0.19 g, 0.75 mmol) or S-methylisothiourea hydroiodide[36] (**94**•HI) (0.16 g, 0.75 mmol) in 5 ml of EtOH was heated under reflux for 3 d. After evaporation in vacuo, the remaining oil was purified chromatographically (MPLC; MeOH–0.1% CF_3COOH, gradient 80:20 to 45:55, 13–14 bar). Yield 30-65% of the corresponding guanidine hydroiodide or trifluoroacetate as an oil or an amorphous solid. Analytical data cf. Table 4.

^{1}H NMR ($CDCl_3$) of **121**: δ = 0.55–0.95 (m, 2H, cHex-2-H, -6-H), 1.15–1.8 (m, 6H, cHex-2-H, -6-H, cHex-3-H, -5-H), 1.8–2.0 (m, 1H, $CHCO$), 2.05–2.3 (m, 2H, $CHCH_2CH_2$), 2.55–2.7 (m, 2H, CH_2CH_2Ar), 2.85–2.95 (m,

2H, $CHCH_2NH$), 3.09 (br, 1H, $CHCH_2CH_2$), 3.23 (br, 1H, $CHCH_2CH_2$), 3.47 (m, 2H, CH_2CH_2Ar), 3.81 (br, 0.5H, Ph_2CHCH_2), 3.92 (br, 0.5H, Ph_2CHCH_2), 6.55–6.95 (AA'BB', 4H, Ar-*H*), 7.15–7.3 (m, 13H, Ph-*H*, N*H*), 7.44 (s, 1H, N*H*), 9.20 (s, 1H, N*H*).

Pharmacology

The title compounds were used as hydrohalides, trifluoroacetates or free bases (cf. Tables 2–4) for all pharmacological experiments.

NPY Y_1 receptor antagonist activity in human erythroleukemia (HEL) cells

Cell culture. The human HEL erythroleukemia cell line was kindly provided by Dr. M. C. Michel, Universitätsklinikum Essen. Cell line banking and quality control were performed according to the "seed stock concept" [37]. Cells were maintained as suspension cultures in antibiotic-free RPMI 1640 medium (Sigma, Deisenhofen, GER) containing 0.3 g/l L-glutamine, 2 g/l $NaHCO_3$ and 5% fetal calf serum (Biowhittaker, Vervier, B) using 75-cm^2-culture flasks (Falcon Plastics 3023) in a water saturated atmosphere (95% air/5% CO_2) at 37 °C. The cells were passaged weekly by 1:10 dilution with fresh culture medium.

Cells were routinely monitored for, and shown to be free of, *Mycoplasma* contamination with the Mycoplasma Detection Kit provided by Boehringer, Mannheim, GER.

Preparation of the cells. The day before testing, 25 ml of a plateau-phase culture were transferred to a 175-cm^2 flask (Nunclon, 178883, Nunc, Wiesbaden, GER) containing 125 ml of fresh culture medium. After 24 h, the suspension (2–4 × 10^5 cells per ml), was centrifuged for 10 min at 200 g and room temperature. After resuspension in 10 ml of loading buffer (25 mM HEPES (Sigma, Deisenhofen, GER), 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose), pH 7.4, cell number was determined with a hemocytometer (Neubauer, improved), and the cells were adjusted to a density of 1.3 × 10^6 per ml by addition of an appropriate volume of loading buffer.

Loading cells with Ca^{2+} indicator Fura-2/AM. To three volumes of the prepared cell suspension, one volume of loading dispersion [38] was added, before the cells were incubated in the dark at room temperature for 30 min. The loading dispersion was freshly made by mixing 10 ml of loading buffer, containing 2% bovine serum albumin (BSA), with 50 µl of Pluronic-F-127 (Molecular Probes, Eugene, Oregon, USA) (20% in DMSO), and 40 µl of Fura-2/AM (Molecular Probes, Eugene, Oregon, USA) (1 mM in anhydrous DMSO).

Cells were centrifuged (200 g, 7 min), resuspended in fresh loading buffer and allowed to stand for another 30 min at room temperature in the dark. After two washing/centrifugation cycles (loading buffer, 200 g, 7 min) and adjustment of the cell number to a value of 10^6/ml, cells were incubated for at least 15 min at 20 °C in the dark.

Fluorimetric determination of intracellular $[Ca^{2+}]$. 1 ml-aliquots of loading buffer were filled into disposable acrylic cuvettes (Sarstedt, No. 67.755, Nümbrecht, GER), which were thermostatted at 30 °C in an incubator hood (Infors AG, Bottmingen, CH). Immediately after addition of 1 ml of the Fura-2/AM loaded cell suspension and a magnetic stirrer, the cuvette was placed into the thermostated (25 °C) stirred cell holder of a LS 50 B Luminescence Spectrometer (Perkin Elmer, Überlingen, GER), equipped with a fast filter accessory. Fluorescence signals were registered (instrument settings: excitation 340/380 nm, emission 510 nm, slits 10 nm, resolution 0.1, stirrer low) after addition of 10 µl of 20 µM porcine NPY (Bachem Biochemica GmbH, Heidelberg, GER), dissolved in 10 mM HCl, supplemented with 0.1% BSA, for 300 s.

Calculation of Ca^{2+} concentrations. Calcium concentrations were calculated from dual wavelength fluorescence intensities according to the Grynkiewicz equation [39]:

$$\left[Ca^{2+}\right] = K_d \cdot \frac{(R - R_{min})}{(R_{max} - R)} \cdot SFB \qquad (1)$$

where K_d (224 nM [38]) is the dissociation constant of the Fura-2-Ca^{2+}-complex, R is the experimental fluorescence ratio value (F_{340}/F_{380}), R_{min} and R_{max} are the fluorescence value ratios (F_{340}/F_{380}) under Ca^{2+}-free and Ca^{2+}-saturation conditions, respectively, and SFB is the ratio of fluorescence intensities for Ca^{2+}-bound/Ca^{2+}-free indicator, measured at 380 nm. R_{min}, R_{max} and SFB were determined by calibration experiments, performed in every test series.

To measure R_{max}, 10 µl of an aqueous solution of 2% digitonin (Sigma, Deisenhofen, GER), were pipetted into the cuvette, wheras R_{min} was determined after subsequent addition of 50 µl of a 0.6 M EGTA solution (in 1 M Tris/HCl, pH 8.7).

Screening of compounds for NPY Y_1 receptor antagonist activity. The cells were pre-incubated with 10 µl of the putative antagonists (dissolved either in 50% ethanol or DMSO) for 1 min, prior to the stimulation with porcine NPY at a final concentration of 10 nM. The Ca^{2+} signal in every fifth cuvette was taken as the non-inhibited reference (100%), i.e. the cells were only exposed to the respective solvent, before NPY stimulation.

Calculation of IC_{50} values

IC_{50} values were calculated from at least two antagonist concentrations [B], inhibiting the NPY-stimulated increase in intracellular $[Ca^{2+}]$ between 20 and 80%. The mean percentual inhibition values P with SEM < 10%, determined from at least 3 independent experiments, performed on different days, were logit transformed, according to the equation

$$\text{logit}(P) = \log \frac{P}{100 - P} \qquad (2)$$

and IC_{50} values (logit $P = 0$) were determined from the plot logit (P) versus log [B] with the slope n according to

$$\log \frac{P}{100 - P} = n \cdot \log [B] - n \cdot \log IC_{50} \qquad (3)$$

by linear regression with Fig. P (Biosoft, Cambridge, UK).

Calculation of pK_B- values

Complete concentration response curves of NPY were only constructed in the presence of selected compounds, which showed promising antagonist activity in the screening procedure. The increase in intracellular $[Ca^{2+}]$ after stimulation with 100 nM NPY was taken as 100%. EC_{50} values were determined from fitted curves (100% and 0% values fixed, slope variable) with Fig. P (Biosoft, Cambridge, UK). The pK_B value was calculated according to the Schild equation[40] with the slope taken as 1.

$$\log (\text{concentration ratio} - 1) = \log [B] + pK_B \qquad (4)$$

where concentration ratio = EC_{50} in the presence of the antagonist/EC_{50} in the absence of the antagonist and [B] is the concentration of the antagonist.

Histamine H_1 receptor antagonism and histamine H_2 receptor agonism

Selected compounds were investigated for histamine H_1 receptor antagonist activity at the isolated guinea pig ileum and for histamine H_2 receptor agonist activity at the isolated spontaneously beating guinea pig right atrium according to the experimental protocols described elsewhere [41]. The pD_2 (pEC_{50}) values for agonists and the pK_B values for antagonists (mean of at least three independent experiments) were determined from isometrically (atrium) or isotonically (ileum) recorded cumulative concentration-response curves using histamine as the reference agonist.

Molecular modeling

Superpositions of compound **73** with NPY and of compound **119** with BIBP 3226 were performed with the modeling software SYBYL 6.3 (Tripos Ass.) on a Silicon Graphics Indigo^2. The putative 3D structure of NPY was produced by homology modeling, starting from the crystal structure of the avian pancreatic polypeptide, APP[25]. The conformation obtained after minimization (Kollman force field) corresponds to that independently derived by Allen et al.[23] and by Beck-Sickinger et al.[22]. Compound **73** was first investigated by conformational analysis (rigid rotor principle), including all rotatable bonds except those adjacent to the rings. Only conformations were accepted where the distances between the basic centers as well as between each basic group and one of the phenyl rings were within the constraints of the corresponding distances in NPY (Arg^{33}, Arg^{35}, Tyr^1).

About 5000 resulting conformations and their antipodes were superimposed with NPY according to this model. Well-fitting candidates were freely energy-minimized (TRIPOS force field without electrostatics) and again fitted to NPY.

The putative binding conformation of BIBP 3226 originates from the paper of Sautel et al.[25]. More than 1000 local energy minima (TRIPOS force field without electrostatics) of compound **119** were generated by the SYBYL randomsearch routine (Monte-Carlo method). The three phenyl rings and the guanidino group of each conformation were superimposed to the corresponding groups of BIBP 3226.

References

☆ Dedicated to Prof. Dr. W. Wiegrebe on the occasion of his 65th birthday

[1] K. Tatemoto, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 5485–5489.

[2] C. Wahlestedt, D.J. Reis, *Annu. Rev. Pharmacol. Toxicol.* **1993**, *32*, 309–352.

[3] W.F. Colmers, C. Wahlestedt (Eds.), *The Biology of Neuropeptide Y and Related Peptides*, Humana Press, Totowa, **1993**.

[4] M.C. Michel, W. Rascher, *J. Hypertens.* **1995**, *13*, 385–395.

[5] R.J. Playford, H.M. Cox, *Trends Pharmacol. Sci.* **1996**, *17*, 436–438.

[6] S.P.H. Alexander, J.A. Peters, *Receptor and Ion Channel Nomenclature*, 8th ed., *Trends Pharmacol. Sci. Suppl.* **1997**.

[7] K. Rudolf, W. Eberlein, W. Engel, H.A. Wieland, K.D. Willim, M. Entzeroth, W. Wienen, A.G. Beck-Sickinger, H.N. Doods, *Eur. J. Pharmacol.* **1994**, *271*, R11–R13.

[8] C. Serradeil-Le Gal, G. Valette, P.-E. Rouby, A. Pellet, F. Oury-Donat, G. Brossard, L. Lespy, E. Marty, G. Neliat, P. De Cointet, J.-P. Maffrand, G. Le Fur, *FEBS Lett.* **1995**, *362*, 192–196.

[9] J. Wright, G. Bolton, M. Creswell, D. Downing, L. Georgic, T. Heffner, J. Hodges, R. MacKenzie, L. Wise, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1809–1814.

[10] L. Grundemar, S.R. Bloom (Eds.), *Neuropeptide Y and Drug Development*, Academic Press, San Diego, **1997**.

[11] A. Buschauer, *J. Med. Chem.* **1989**, *32*, 1963–1970.

[12] M.C. Michel, H.J. Motulsky, *Ann. N. Y. Acad. Sci.* **1990**, *611*, 392–394.

[13] M.C. Michel, S. Dove, A. Buschauer, *Arch. Pharm. (Weinheim)* **1993**, *326*, 664.

[14] A. Uffrecht, W. Wernet, L. Unger, S. Dove, A. Buschauer, *Eur. J. Pharm. Sci.* **1994**, *2*, 137.

[15] S. Knieps, M.C. Michel, S. Dove, A. Buschauer, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2065–2070.

[16] S. Knieps, S. Dove, M.C. Michel, K. Rottmeier, W. Wernet, G. Bernhardt, A. Buschauer, *Pharm. Pharmacol. Lett.* 1996, *6*, 27–30.

[17] H.J. Motulsky, M.C. Michel, *Am. J. Physiol.* **1988**, *255*, E880–E885.

[18] V. Zingel, S. Elz, W. Schunack, *Eur. J. Med. Chem.* **1990**, *25*, 673–680.

[19] W. Black, W.A. Duncan, G.J. Durant, C.R. Ganellin, E.M. Parsons, *Nature (London)* **1972**, *236*, 385–390.

[20] I. Aiglstorfer, A. Uffrecht, K. Rottmeier, A. Meister, B. Malawska, M. Müller, S. Dove, G. Bernhardt, A. Buschauer *Abstracts of Papers*, XIVth International Symposium on Medicinal Chemistry, Maastricht, The Netherlands, **1996**, Abstract P-7.21.

[21] P. Walker, M. Munoz, R. Martinez, M. Peitsch, *J. Biol. Chem.* **1994**, *269*, 2863–2869.

[22] A.G. Beck-Sickinger, H.A. Wieland, H. Wittneben, K.-D. Willim, K. Rudolf, G. Jung, *Eur. J. Biochem.* **1994**, *225*, 947–958.

[23] J. Allen, J. Novotny, J. Martin, G. Heinrich, *Proc. Natl. Acad. Sci. USA* **1987**, *84*: 2532–2536.

[24] I. Glover, D.J. Barlow, J.E. Pitts, S.P. Wood, I.J. Tickle, T.L. Blundell, K. Tatemoto, J.R. Kimmerl, A. Wollmer, W. Strassburger, Y.S. Zhang, *Eur. J. Biochem.* **1985**, *142*, 379–385.

[25] M. Sautel, K. Rudolf, H. Wittneben, H. Herzog, R. Martinez, M. Munoz, W. Eberlein, W. Engel, P. Walker, A.G. Beck-Sickinger, *Mol. Pharmacol.* **1996**, *50*, 285–292.

[26] P. Du, J.A. Salon, J.A. Tamm, C. Hou, W. Cui, M.W. Walker, N. Adham, D.S. Dhanoa, I. Islam, P.J.-J. Vaysse, B. Dowling, Y. Shifman, N. Boyle, H. Rueger, T. Schmidlin, Y. Yamaguchi, T.A. Branchek, R.L. Weinshank, C. Gluchowski, *Prot. Eng.* **1997**, *10*, 109–117.

[27] A. Buschauer, *Arch Pharm (Weinheim)* **1989**, *322*, 165–171.

[28] T.B. Johnson, W.M. Scott, *J. Am. Chem. Soc.* **1913**, *35*, 1130–1136.

[29] S. Chodroff, R. Kapp, C.O. Beckmann, *J. Am. Chem. Soc.* **1947**, *69*, 256–258.

[30] K. Balenovic, I. Jambresic, B. Urbas, *J. Org. Chem.* **1954**, *19*, 1589–1590.

[31] W.F. Fones, R.S. Stander, J. White, *J. Org. Chem.* **1951**, *16*, 708–710.

[32] B. Taub, H.A. Leipold, J.B. Hino, *J. Org. Chem.* **1959**, *24*, 2062–2063.

[33] K. Itoh, M. Kori, Y. Inada, K. Nischikawa, Y Kawamatsu, H. Sugihara, *Chem. Pharm. Bull.* **1986**, *34*, 5: 2078–2089.

[34] J. Engels, M. Lamchen, A.J. Wicken, *J. Chem. Soc.* **1959**, 2694–2699.

[35] R. Ing, R.H.F. Manske, *J. Chem. Soc.* **1926**, 2348–2351.

[36] C.E. Braun, *J. Biol. Chem.* **1930**, *89*, 97–108.

[37] R.J. Hay in *Animal Cell Culture: A Practical Approach* (Ed.: R.I. Freshney), 2nd edn., IRL Press, Oxford, **1992**, pp 95–148.

[38] A.P. Thomas, F. Delaville in *Cellular Calcium: A Practical Approach* (Eds.: J. G. McCormack, P. H. Cobbold), IRL Press, Oxford, **1991**, pp. 1–54.

[39] G. Grynkiewicz, M. Poenie, R.Y. Tsien, *J. Biol. Chem.* **1985**, *260*, 3440–3450.

[40] O. Arunlakshana, H.O. Schild, *Br. J. Pharmacol.*, **1959**, *14*, 48–58.

[41] A. Buschauer, A. Friese-Kimmel, G. Baumann, W. Schunack, *Eur. J. Med. Chem.* **1992**, *27*, 321–330.

Received: June 16, 1997 [FP229]