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8-Amino-5-nitro-6-phenoxyquinolines: Potential Non-peptidic Neuropeptide Y Receptor Ligands

GAETANE WIELGOSZ-COLLIN[a,*], MURIEL DUFLOS[a], PATRICIA PINSON[a], GUILLAUME LE BAUT[a], PIERRE RENARD[b], CAROLINE BENNEJEAN[b], JEAN BOUTIN[c] and MICHELE BOULANGER[c]

[a]*Laboratoire de Chimie Thérapeutique et de Chimie Organique, UPRES EA 1155, Faculté de Pharmacie, 1 rue Gaston Veil–F44035 Nantes cedex 01, France;* [b]*Laboratoire SERVIER, 1 rue Carle Hébert, F92415 Courbevoie cedex, France;* [c]*IDRS, 11 rue des Moulineaux, F92150 Suresnes, France*

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The synthesis and pharmacological evaluation of analogues of PD 160170, a neuropeptide Y1 (NPY) receptor antagonist are reported. Phamacomodulation of this 8-amino-5-nitro-6-phenylsulfonylquinoline was carried out by replacing the sulfone moiety by oxygen. The corresponding ethers 11–16 were obtained by nucleophilic substitution of 8-acetamido-6-chloro-5-nitroquinoline 4 with phenols, followed by acidic hydrolysis of the intermediary amides 5–10. The test compounds 11–16 exerted no appreciable Y1 activity and they were also inactive in terms of Y5 receptor binding; their IC_{50} values were >1 μM and 10 μM, respectively. The dramatic decrease in potency resulting from replacement of the sulfone function by an ether was confirmed by IP administration of 16 to *ob/ob* mice; after a 4-day administration, no decrease in food consumption or weight was observed.

Keywords: 8-Amino-5-nitro-6-phenoxyquinolines; NPY1 and NPY5 receptor ligands; Food intake; *ob/ob* mice

INTRODUCTION

Neuropeptide Y (NPY) is a 36 amino acid polypeptide and was isolated in 1982 from porcine brain.[1] It is the most abundant peptide yet discovered in mammalian brain and its main site of biosynthesis is the arcuate nucleus (ARC).[2] In the central nervous system (CNS), NPY has been implicated in feeding and obesity; NPY is a powerful stimulant of food intake when administered directly into the hypothalamus.[3]. Among the different NPY receptors, those which most influence feeding are the Y1 and Y5 receptors, found primarily in the hypothalamus.[4] Since the discovery of the NPY peptide ligand GR 231118,[5] (Figure 1), which exhibits high Y1 receptor affinity ($IC_{50}Y1 = 0.04\,nM$ and $IC_{50}Y5 = 2050\,nM$), progress has been made in development of orally-active non peptide ligands for the Y1 and Y5 receptors. Although the arginyl derivative BIBP 3226 represents an important milestone in the receptor antagonist discovery, with high Y1 receptor affinity ($IC_{50} = 7.3\,nM$) and selectivity, its intravenous administration in pig caused a significant decrease in arterial blood pressure.[6] More recently a series of potent selective Y1 antagonists, discovered via random screening, were introduced by Parke-Davis. A representative compound, PD 160170, binds to Y1 receptors with an IC_{50} of 48 nM.[7] In the field of NPY5 receptor antagonists, Novartis and Synaptic have disclosed a potent and selective compound, CGP 71683A, whose IC_{50} NPY5 is 1.48 nM, which has been reported to inhibit food intake in rodent feeding models after IP administration of $5\,mg\,Kg^{-1}$.[8] Here we report on the synthesis and pharmacological evaluation of a series of 6-phenoxyquinolines, analogues of PD 160170, as potential NPY1 antagonists.

MATERIALS AND METHODS

Chemistry

Melting points were determined on a Tottoli-Büchi apparatus and are uncorrected. Structures were supported by IR and ^1H NMR data. IR spectra were run with KBr pellets on a Perkin Elmer-Paragon PC 1000 infrared spectrometer. ^1H NMR spectra were recorded on a Bruker AC 250 spectrometer

*Corresponding author. E-mail: gaetane.wielgosz-collin@isomer.univ-nantes.fr

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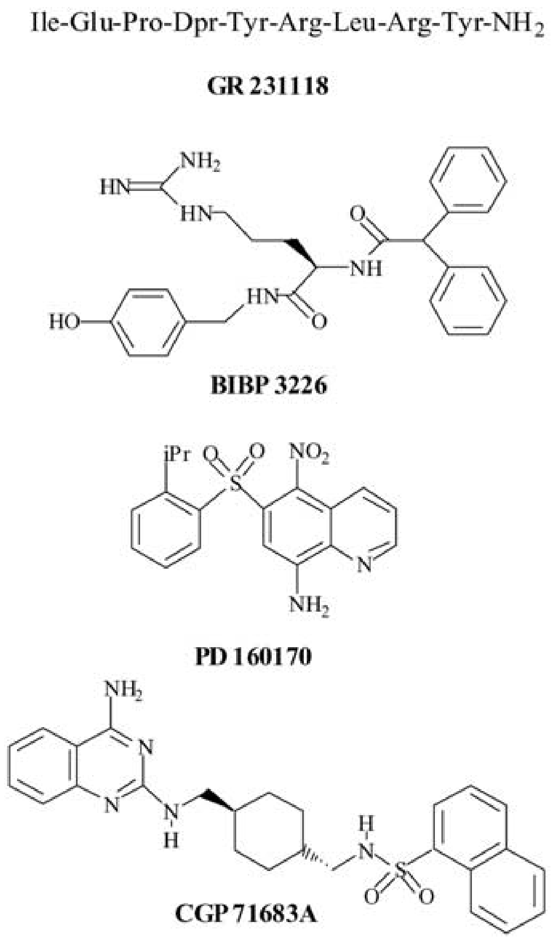


FIGURE 1 Chemical structures of NPY receptors agonists and antagonists.

(250 MHz), using $CDCl_3$ as solvent. Chemical shifts (δ ppm) are reported downfield from tetramethylsilane, the internal standard; coupling constants are in Hz. Analytical TLC was performed on precoated silica-gel aluminium plates (0.2 mm, GF254, E Merck). Spots were located by UV illumination. Evaporation (rotating evaporator) was done in vacuo. Sodium sulfate or phosphorus pentoxide were used as the drying agents. Most of the crude products were passed through columns of silica gel (silica gel 60,70–230 mesh, E Merck) with an appropriate mixture of dichloromethane and ethanol.

Starting materials were purchased from Aldrich Chimie (St Quentin-Fallavier, France) or Acros (Noisy-le-Grand, France).

Method iv. 6-Chloro-8-nitroquinoline 2

To 7 g (40 mmol) of 4-chloro-2-nitroaniline in a three-necked flask, 10 ml of concentrated hydrochloric acid were added. Then, 9.84 g (40 mmol) of p-chloranil (tetrachloro-1,4-benzoquinone) were added to the flask, along with 20 ml of n-butanol as washing agent. The mixture was stirred and heated to reflux and then, a solution of 2.7 g (60 mmol) of acrolein in 10 ml of n-butanol was added over 2 h to the refluxing solution. After refluxing for 3 h, a solution of 5.45 g (40 mmol) of zinc chloride, in 80 ml of THF, was added. The mixture was refluxed for 30 min and then cooled slowly to 0°C and maintained at 0°C for 1 h. The solid was filtered, and washed with THF. The solid collected was neutralized with a solution of sodium hydroxide and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel using dichloromethane as eluent. Crystallization from diisopropyl ether afforded 6.76 g of pure product. Yield: 81%; mp 158–159°C. ^{1}H NMR ($CDCl_3$) δ 7.51 (dd, J = 8.43 and 4.23, 1H, H_3), 7.93 (d, J = 2.71, 1H, H_7), 8.01 (d, J = 2.71, 1H, H_5), 8.14 (dd, J = 8.43 and 1.66, 1H, H_4), 8.99 (dd, J = 4.23 and 1.66, 1H, H_2).

Method vi. 8-Acetamido-6-chloroquinoline 3

A solution of 6.5 g (31 mmol) of 6-chloro-8-nitroquinoline in 20 ml of acetic anhydride was stirred for 15 h with 0.1 g of Adams oxide catalyst under hydrogen pressure. The catalyst was filtered off and the filtrate was refluxed for 2 h. The solvent was evaporated under reduced pressure and the resulting solid was recrystallized from diisopropyl ether to afford **3** (6.53 g). Yield: 95%; mp 146–147°C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CO–CH_3), 7.47 (dd, J = 8.22 and 3.97, 1H, H_3), 7.50 (d, J = 2.71, 1H, H_7), 8.01 (d, J = 2.34, 1H, H_5), 8.08 (dd, J = 8.22 and 1.52, 1H, H_4), 8.78 (dd, J = 3.97 and 1.52, 1H, H_2), 9.77 (s, 1H, NH).

Method vii. 8-Acetamido-6-chloro-5-nitroquinoline 4

To 5 ml of cold, stirred concentrated sulfuric acid was added 1.7 g (7.7 mmol) of 8-acetamido-6-chloroquinoline **3**. Then, a cooled solution of 1.01 g (10 mmol) of potassium nitrate in 15 ml of concentrated sulfuric acid was added to the stirred mixture and the whole maintained at 20–25°C for 3 h and then poured on 120 g of chopped ice. The nitro derivative formed was filtered and washed with water. The suspension was made slightly alkaline with 30 ml of a solution of 1 M sodium hydroxide and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated. Crystallization from diisopropyl ether gave 0.86 g of pure **4** as a pale yellow powder. Yield: 42%; mp 192–193°C (lit.[16] 190–193).

^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, CO–CH_3), 7.67 (dd, J = 8.53 and 4.27, 1H, H_3), 8,15 (dd, J = 8.53 and 1.52, 1H, H_4), 8.88 (dd, J = 4.27 and 1.52, 1H, H_2), 8.90 (s, 1H, H_7), 9.87 (s, 1H, NH).

Method viii. 8-Acetamido-6-(2-isopropylphenoxy)-5-nitroquinoline 5

0.1 g (2.48 mmol) of sodium hydride was added to a solution of 0.25 g (1.88 mmol) of 2-isopropylphenol in 10 ml of dimethylformamide. A solution of 0.55 g (2.07 mmol) of 8-acetamido-6-chloro-5-nitroquinoline in 10 ml of dimethylformamide was added and the mixture refluxed for 4 h and concentrated under reduced pressure. The mixture was washed with water and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel using dichloromethane as eluent and recrystallized from diisopropyl ether to give **5** (0.75 g). Yield: 42%; mp 198–199°C. ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 6.43, 6H, CH_3 isopropyl), 2.28 (s, 3H, CO–CH_3), 3.29 (sept, J = 6.43, 1H, CH isopropyl), 6.98 (m, 1H, $H_{6'}$), 7.22 (m, 2H, $H_{4'}$ and $H_{5'}$), 7.39 (m, 1H, $H_{3'}$), 7.61 (dd, J = 8.65 and 4.23, 1H, H_3), 8.29 (dd, J = 8.65 and 1.45, 1H, H_4), 8.33 (s, 1H, H_7), 8.76 (dd, J = 4.23 and 1.45, 1H, H_2), 9.86 (s, 1H, NH).

Method ix. 8-Amino-6-(2-isopropylphenoxy)-5-nitroquinoline 11

0.39 g (1 mmol) of amide **5** was added to a solution of 6 M hydrochloric acid and refluxed for 1 h. The mixture was made alkaline (pH 8) with a solution of 2 M sodium hydroxide and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel using dichloromethane as eluent and recrystallized from diethyl ether and petroleum ether to give **11** (0.32 g). Yield: 82%; mp 107–108°C. IR (KBr) 3408 (ν NH), 3052 (νCH arom.), 2976 (νCH_3), 2928 (νCH isopropyl) 1615–1315 (νCH = CH arom.), 1580 (δNH), 1512 (νasNO_2), 1315 (νsNO_2), 1226 (νC–O–C aryl) cm^{-1}. ^{1}H NMR (DMSO) δ 1.25 (d, J = 6.80, 6H, CH_3 isopropyl), 3.26 (sept, J = 6.80, 1H, CH isopropyl), 5.55 (m, 2H, NH_2), 6.22 (s, 1H, H_7), 6.99 (m, 1H, $H_{6'}$), 7.25 (m, 2H, $H_{4'}$ and $H_{5'}$), 7.38 (m, 1H, $H_{3'}$), 7.56 (dd, J = 8.85 and 4.30, 1H, H_3), 8.48 (d, J = 8.85, 1H, H_4), 8.69 (d, J = 4.30, 1H, H_2).

Pharmacology

Binding Experiments

Y1 Binding Assay

Y1 Binding of iodinated peptide YY ([^{125}I]PYY,-NEN) was carried out as described by Serradei-Le Gal *et al.*, with slight modifications.[10] Incubations were performed at 30°C for 90 min with various competitor concentrations in buffer A (Hepes-NaOH, 20 mM, pH 7.4; NaCl, 10 mM; KH_2PO_4, 0.22 nM; $CaCl_2$, 1.26 mM; $MgSO_4$, 0.81 mM; and 0.1% bovine serum albumin (BSA)) with SK-N-MC cell membranes (50 μM of protein/mL of assay) in a total volume of 500 μL. Nonspecific binding was determined in the presence of 1 μM NPY. The reaction was then stopped by filtration, through GF/B filters (Whatman, precoated in 0.3% PEI) and extensively washed with buffer A. Counting was carried out in a gamma counter (Packard). PD 160170 and CGP 71683A were used as pharmacological reference compounds.

Y5 Binding Assay

Y5 Binding of ([^{125}I]PYY,NEN) was carried out as described by Hu *et al.*[11] In summary, COS cells transfected with the human Y_5 NPY receptor were lysed and the membranes prepared by differential centrifugation. These membranes contained about 2 pmol per milligram of protein of this receptor. Incubations at 30°C for 2 h were performed in 500 μL comprising 20 pM final of [^{125}I]PYY in 50 μL, 400 μL of membrane suspension (0.15 mg/mL), and competitor dilutions in 50 μL. The reaction was stopped by filtration through GF/C filters (Whatman).

Repeated IP Administration

The effect of the studied compounds was quantified in the manner previously described by Duhault *et al.*[9] NPY-receptor antagonists were given daily for 4 days to *ob/ob* mice. The mice were kept at 22 ± 2°C under a 12 h light: 12 h dark cycle (07:00–19:00). Water and standard laboratory chowder (67.5% food flour, 26.25% saccharose, 5% gum tragacanth, 1.25% magnesium stearate) were freely available. Food intake was determined per cage and individual body weights checked daily. Tested compound in an appropriate solvent were given intraperitoneally (10 mg Kg^{-1}) at 10:30 and 16:30 each day for 4 days. Experimental procedures were approved by the Institut de Recherches Servier (IdRS) Ethical Committee and were carried out in compliance with the laws of France regulating animal experimentation.

RESULTS AND DISCUSSION

Chemistry

6-Chloro-8-nitroquinoline **2** was prepared by the classical Skraup reaction. It was obtained in 39% yield by method i, heating 4-chloro-2-nitroaniline **1** in glycerol, concentrated sulfuric acid and nitrobenzene according to Yale *et al.* (Figure 2).[12] Replacing glycerol and sulfuric acid by acrolein and phosphoric acid[12] resulted in a dramatic decrease in yield (method ii, 3%). The yield was slightly increased by using arsenic acid[12] as the oxidizing agent (method iii, yield: 29%). Finally this target quinoline could be prepared in a high yield (86%) using *para*-chloranil as

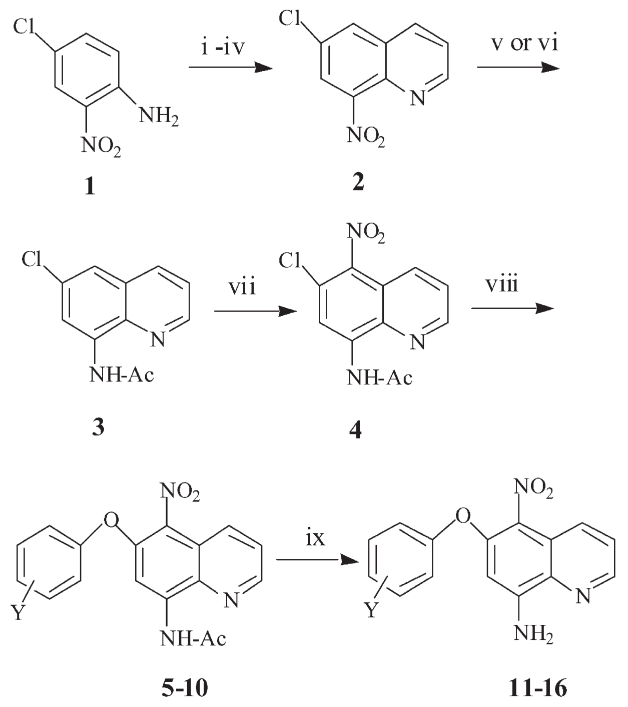


FIGURE 2 Synthetic pathways to 8-amino-6-(aryloxy)-5-nitroquinolines. Reagents: **i**. Glycerol (4 equiv.), $H_2SO_4/FeSO_4$ (3.25/0.13 equiv.), nitrobenzene (0.6 equiv.), 110°C, 7 h; **ii**. Acrolein (1.5 equiv.), $H_3PO_4/FeSO_4$ (5.3/0.06 equiv.), nitrobenzene (0.6 equiv.), 110°C, 7 h; **iii**. Acrolein (1.2 equiv.), H_3PO_4 (34 equiv.), Na_2HAsO_4 (2 equiv.), 110°C, 7 h; **iv**. Acrolein (1.5 equiv.), conc. HCl (8 equiv.), *para*-chloranil (1 equiv.), n-butanol, 117°C, 5 h; **v**. $SnCl_2$ (4 equiv.), conc. HCl, 80°C, 2 h; then acetic anhydride (1.1 equiv.), toluene reflux, 1 h; **vi**. H_2, PtO_2, acetic anhydride, r.t., 15 h; **vii**. Conc. KNO_3 (1.3 equiv), conc H_2SO_4, 0–15°C, 3 h; **viii**. NaH (1.2 equiv.), intermediary phenols (0.9 equiv.), DMF, reflux; **ix**. 6 M HCl, reflux.

oxidant[13] and isolating it as a 2:1 complex with zinc chloride.[14]

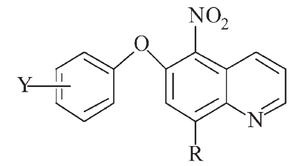
8-Acetamido-6-chloroquinoline **3** was obtained by reduction of the nitro compound **2** with stannous chloride[15] followed by acetylation of the amine with acetic anhydride in toluene (method v, yield: 30%). Reduction in acetic anhydride in presence of Adams oxide catalyst (PtO_2) afforded the acetamide **3** in excellent yield (95%). Nitration of **3**, leading to 8-acetamido-6-chloro-5-nitroquinoline **4**, was carried out according to method vii in 42% yield.[16–18] The synthesis of the intermediary 8-acetamido-6-(aryloxy)-5-nitroquinolines **5–10** was performed by condensing the corresponding phenols with **4** in the presence of sodium hydride. The final hydrolysis afforded the corresponding amines which were isolated as either base (**11**), dihydrochloride (**12–15**) or trihydrochloride (**16**) salts (Table I).

Pharmacology

Binding

The results obtained in binding of the reference compounds at the two human NPY receptor subtypes Y1 and Y5 are in good agreement with those reported in the literature (Table II). Evaluation of NPY receptor binding activity revealed that 6-phenoxyquinolines **11–16** exhibit a low affinity for NPY1 receptors (Ki > 1 μM) so it was clear that replacement of the sulfonyl group in PD 160 170 by an ether induced a deleterious effect. No gain in affinity was observed after introduction of an isopropyl group in the *ortho* position of the phenyl system (compound **11**). These quinolines were also inactive in terms of Y5 receptor binding (Ki > 10 μM).

TABLE I Physicochemical properties of 8-acetamidoquinolines **5–10** and 8-aminoquinolines **11–16**



Compounds[(a)]	Y	R	Yield (%)	Mp (°C)
5	2-CH(CH_3)$_2$	NH-Ac	42	198–199 (b)
6	2-CH_3	NH-Ac	90	159–160 (b)
7	2-OCH_3	NH-Ac	65	168–169 (b)
8	2-F	NH-Ac	67	162–163 (b)
9	2-Cl	NH-Ac	45	161–168 (b)
10	4-NHAc	NH-Ac	75	220–221 (b)
11	2-CH(CH_3)$_2$	NH_2	82	107–108 (c)
12	2-CH_3	NH_2	40	169–170 (d)
13	2-OCH_3	NH_2	69	189–190 (d)
14	2-F	NH_2	73	184–185 (d)
15	2-Cl	NH_2	77	195–196 (d)
16	4-NH_2	NH_2	58	207–208 (d)

(a): **11** was isolated as the free base, **12–15** as dihydrochloride salts and **16** as the trihydrochloride salt; recrystallization solvent: (b) (diisopropyl ether); (c) (diethyl ether/petroleum ether); (d) (diethyl ether).

Food Intake and Body Weight

This assay was performed with compound **16** in fed *ob/ob* mice allowed *ad libitum* access to food during the session. No significant decrease in food consumption was observed during administration of **16**

TABLE II NPY1 and NPY5 receptor binding affinities of 6-phenoxyquinolines **11–16**

	Ki (μM)	
Compound	Y1	Y5
11	>1	>10
12	>1	>10
13	>1	>10
14	>1	>10
15	>1	>10
16	>1	>10
PD 160170	0.05	>10
CGP 71683A	3.5	0.0015

at $10\,mg\,Kg^{-1}$ twice a day for 4 days and body weights were not significantly reduced. Under the same conditions, CGP 71683A, at $5\,mg\,Kg^{-1}$ twice a day, attenuated food intake (39%) and reduced body weight ($7.5 \pm 0.4\%$). New analogues of CGP 76183A that proved efficient as NPY5 ligands will be published in due course.

References

[1] Tatemoto, K., Carlquist, M. and Mutt, V. (1982) "Neuropeptide Y–a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide", *Nature* **296**, 659–660.

[2] Frankish, H.M., Dryden, S., Hopkins, D., Wang, Q. and Williams, G. (1995) "Neuropeptide Y, the hypothalamus, and diabetes: insights into the central control of metabolism", *Peptides* **16**, 757–771.

[3] Stanley, B.G., Kyrkouli, S.E., Lampert, S. and Leibowitz, S.F. (1986) "Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity", *Peptides* **7**, 1189–1192.

[4] Inui, Akio (1999) "Neuropeptide Y feeding receptors: are multiple subtypes involved?", *Trends in Pharmacological Sciences* **20**, 43–46.

[5] Balasubramaniam, A., Dhawan, V.C., Mullins, D.E., Chance, W.T., Sheriff, S., Guzzi, M., Prabhakaran, M. and Parker, E.M. (2001) "Highly selective and potent neuropeptide Y (NPY) Y1 receptor antagonists based on [Pro^{30}, Tyr^{32}, Leu^{34}]NPY(28–36)-NH_2 (*BW 1911U90*)", *Journal of Medicinal Chemistry* **44**, 1479–1482.

[6] Dhanoa, D.S. (1995) "Neuroropeptide Y: a promising therapeutic target", *Expert Opinion on Therapeutic Patents* **5**, 391–396.

[7] Wright, J., Bolton, G., Creswell, M., Downing, D., Georgi, L., Heffner, T., Hodges, J., Mac Kenzie, R. and Wise, L. (1996) "8-Amino-6-(arylsulphonyl)-5-nitroquinolines: novel nonpeptide neuropeptide Y1 receptor antagonists", *Bioorganic and Medicinal Chemistry Letters* **6**, 1809–1814.

[8] Buehlmayer, P. (1999) "Preparation of substituted benzenesulfonamide derivatives as antagonists of the neuropeptide NPY receptor subtype Y5". *PCT Int Appl* WO 99 32,466 (CO7D277/42).

[9] Duhault, J., Boulanger, M., Chamorro, S., Boutin, J.A., Della Zuana, O., Douillet, E., Fauchère, J.L., Félétou, M., Germain, M., Husson, B., Monge Vega, A., Renard, P. and Tisserand, F. (2000) "Food intake regulation in rodents: Y5 or Y1 NPY receptors or both?", *Canadian Journal of Physiology and Pharmacology* **78**, 173–185.

[10] Serradeil-Le-Gal, C., Valette, G., Rouby, P.E., Pellet, A., Oury-Donat, F., Brossard, G., Lespy, L., Marty, E., Neliat, G., De Cointet, P., Maffrand, J.P. and Le Fur, G. (1995) "SR 120819A, an orally-active and selective neuropeptide Y Y1 receptor antagonist", *Federation of European Biochemical Society Letters* **362**, 192–196.

[11] Hu, Y., Bloomquist, B.T., Cornfield, L.J., DeCarr, L.B., Flores-Riveros, J.R., Friedman, L., Jiang, P., Lewis Higgins, L., Sadlowski, Y., Schaefer, J., Velazquez, N. and McCaleb, M.L. (1996) "Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior", *The Journal of Biological Chemistry* **271**, 26315–26319.

[12] Yale, H.L. and Bernstein, J. (1947) "The Skraup reaction with acrolein and its derivatives", *Journal of the American Chemical Society* **70**, 254.

[13] Song, Z., Mertzman, M. and Hughes, D.L. (1993) "Improved synthesis of quinaldines by the Skraup reaction", *Journal of Heterocyclic Chemistry* **30**, 17–21.

[14] Leir, C.M. (1977) "An improvement in the Doebner-Miller synthesis of quinaldines", *Journal of Organic Chemistry* **42**(5), 911–913.

[15] Elderfield, R.C., Gensler, W.J., Williamson, T.A., Griffing, J.M., Kupchan, S.M., Maynard, J.T., Kreysa, F.J. and Wright, J.B. (1946) "Synthesis of 5-substituted derivatives of 6-methoxy-8-aminoquinoline and 5-chloro-6-methoxyquinoline", *Journal of the American Chemical Society* **68**, 1584–1587.

[16] Gilman, H., Benkeser, R.A., Gainer, G.C., Lindblad, A.E., Marshall, F.J., Massie, S.P., Myers, Jr., J.E. and Tolman, L. (1946) "8-(γ-Diethylaminopropylamino)-6-quinolyl methyl sulfide", *Journal of the American Chemical Society* **68**, 1577–1579.

[17] Dewar, M.J.S. and Maitlis, P.M. (1957) "Nitration of some six-membered nitrogen-heterocyclic compounds in sulphuric acid", *Journal of the American Chemical Society* **79**, 2521–2529.

[18] Austin, M.W. and Ridd, J.H. (1963) "The kinetics and mechanism of heteroaromatic nitration", *Journal of the Chemical Society* **85**, 4204–4210.