Laboratory of Molecular Pharmacology[1], Synthetic Chemical Research Laboratory II.[2], Gedeon Richter Ltd., Budapest, Hungary

Substituted phenoxyalkylpiperazines as dopamine D_3 receptor ligands

I. Laszlovszky[1], T. Ács[2], B. Kiss[1] and Gy. Domány[2]

A novel series of potential antipsychotic agents were prepared by combination of condensed heterocycles containing bridgehead nitrogen with (2-methoxyphenyl)piperazine using phenoxyalkyl spacer of variable length. The affinity of the compounds was determined at rat cloned D_3 and rat D_2, 5-HT_{1A} receptors by an vitro receptor binding assay. The D_3 selectivity of the compounds was calculated from the K_i values.

1. Introduction

Dysfunction of the dopaminergic and serotonergic neurotransmitter systems is involved in the pathology of several neuropsychiatric disorders such as schizophrenia, Parkinson's disease and anxiety. The effect of dopamine is mediated via at least five distinct dopamine receptors belonging to the D_1- (D_1, D_5) or the D_2- (D_2, D_3, D_4) families. D_2 receptor antagonits have successfully been used for several years in the management of schizophrenia, a disease associated with hyperfunction of the mesolimbic dopaminergic system [1]. However, extrapyramidal side-effects and hyperprolactinaemia, mediated through the nigrostriatal and tuberoinfundibular dopaminergic systems, respectively, seriously limited the usefulness of classical D_2 antagonist drugs [2]. D_3 receptors show characteristic distribution in the cerebral dopaminergic systems. Namely, high densities were found in certain limbic structures such as nucleus accumbens and islands of Calleja. Therefore, selective targeting of the D_3 receptors may be a promising approach for more selective modulation of dopaminergic functions and consequently for a successful therapeutic intervention in several abnormalities such as schizophrenia, emotional or cognitive dysfunctions [3–5]. Possible usefulness of drugs with significant D_3 affinity is also supported by the findings that all the effective antipsychotics, especially the atypical ones (those with less EPS liability), in addition to their D_2 antagonism show remarkable binding potential to the D_3 receptors as well.

The (2-methoxyphenyl)piperazine moiety is frequently used in drug design, mainly in the therapeutic fields of cardiovascular and CNS disorders. Several compounds of the general formula **3** with CNS activity have been prepared earlier. In these compounds n used to be 3 and Q stood for halogen, methoxy or a substituted amino group [6–10] while in some papers different condensed heterocycles were described as Qs [11–13].

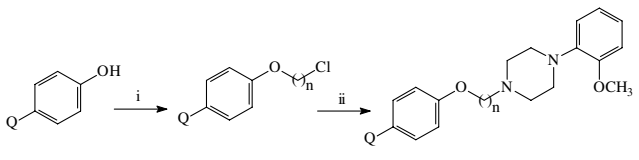
In this paper we report the synthesis and the results of some characteristic binding assays of a new series of compounds having the general formula **3** where Q is a condensed heterocycle containing a bridgehead nitrogen atom. The value of n varies from 2 to 4. This series was designed by means of combinatorial approach, selecting group Q and the length of the spacer as variables, while keeping the (2-methoxyphenyl)piperazine group unchanged. The neurochemical and behavioural properties of the selected compound [14–17] will be presented in a separate article.

2. Investigations, results and discussion

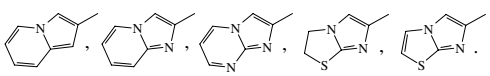
The straightforward synthesis of the compounds is shown in the Scheme. Compounds **1** and/or some of compounds **2** are known from the literature [18–22].

For the characterization of receptor affinity of compounds **4**–**18** to D_3, D_2 and 5-HT_{1A} receptors the K_i values were determined. The calculated pK_i values are shown in the Table. It is obvious that derivatives with chain lengths of 3 and 4 methylene groups were potent ligands at rD_3 receptors (pK_is between 7.1 and 9.5 for compounds **5** and **9**, respectively). The D_3 receptor affinity of the compounds showed a clear-cut structure activity relationship (SAR) with the number of carbon atoms (n) in the lipophilic alkyl chain giving a $4 > 3 > 2$ rank order. Replacement of the 2-indolizinyl group (**4**–**6**) by condensed imidazoles led to derivatives (**7**–**18**) with increased D_3 receptor affinities reaching the low nanomolar range. The most active compounds were **9**, **12**, **8** and **18** with pK_i values of 9.5, 9.3, 9.1 and 9.1, respectively. The binding affinities to D_2 receptors from rat striatum were also in the nanomolar range (pK_is between 6.9 and 8.6). The length of the alkyl chain showed a $3 \geq 4 > 2$ rank order in the D_2 potency of imidazole derivatives. Again, the 2-indolizinyl derivatives turned out to be less active at D_2 receptors like in the case of D_3 and 5-HT_{1A} receptor subtypes. Segregation of the length optimum of the alkyl chain at the D_3 (n=4) versus D_2 ($n = 3$) sites preference resulted in highly D_3 receptor selective (from 10.2 to 42.3 fold) compounds (**9**, **12**, **15** and **18**) in case of $n = 4$. Reduction of the length of the alkyl chain markedly decreased the D_3

Scheme

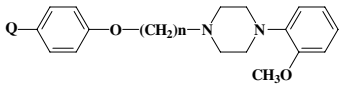


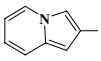
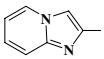
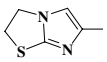
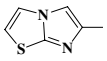
1 **2** **3**

Q: 

(i) α-bromo-ω-chloro-alkanes/K_2CO_3/5-methyl-2-hexanone, reflux, 10 h
(ii) 2-methoxyphenyl-piperazine hydrochloride/K_2CO_3/5-methyl-2-hexanone, reflux, 10 h

Table: Chemical properties, affinities and D_3 selectivity of compounds 4–18 at D_3, D_2 and 5-HT_{1A} receptors



Compd.	Q	n	Formula	MW	mp (°C)	Receptor affinity (pK_i)			Selectivity* D_3 versus D_2
						D_3	D_2	5-HT_{1A}	
4		2	$C_{27}H_{29}N_3O_2$	427.6	159–161	6.6	6.9	5.9	0.4
5		3	$C_{28}H_{31}N_3O_2$	441.6	157–158	7.1	7.4	6.0	0.5
6		4	$C_{29}H_{33}N_3O_2$	455.6	153–155	7.5	7.6	6.8	1.0
7		2	$C_{26}H_{28}N_4O_2$	428.5	151–153	7.4	7.6	7.6	0.6
8		3	$C_{27}H_{30}N_4O_2$	442.6	149–150	9.1	8.6	6.8	3.3
9		4	$C_{28}H_{32}N_4O_2$	456.6	144–145	9.5	7.9	7.7	42.3
10		2	$C_{25}H_{27}N_5O_2$	429.5	150–152	7.1	7.5	7.9	0.4
11		3	$C_{26}H_{29}N_5O_2$	443.5	156–158	8.8	8.0	7.2	6.8
12		4	$C_{27}H_{31}N_5O_2$	457.6	151–153	9.3	7.8	8.3	32.8
13		2	$C_{24}H_{28}N_4O_2S$	436.6	145–147	<6.7	7.7	7.6	<0.1
14		3	$C_{25}H_{30}N_4O_2S$	450.6	142–144	7.8	8.0	<6.4	0.6
15		4	$C_{26}H_{32}N_4O_2S$	464.6	139–140	9.0	7.8	8.0	14.7
16		2	$C_{24}H_{26}N_4O_2S$	434.6	158–161	7.2	7.8	7.5	0.2
17		3	$C_{25}H_{28}N_4O_2S$	448.6	130–132	8.9	8.1	<6.6	7.3
18		4	$C_{26}H_{30}N_4O_2S$	462.6	128–130	9.1	8.1	8.0	11.2
Haloperidol						7.7	9.0	5.8	0.04
Nafadotride**						8.7	7.8	n.d.	7.2

* Selectivity ratio is defined as the antilogarithm of the difference between D_3 and D_2 pK_i values. The pK_i values represent the mean of at least two experiment, each within 0.3 of the mean.
** gift from P. Sokoloff (INSERM)
n. d. = not determined

receptor selectivity of the compounds. The best compounds were 1.6- to 5.9-times more selective than the known D_3 antagonist nafadotride. All the three 2-indolizinyl derivatives showed D_2 but not D_3 receptor preference regardless to the length of the alkyl chain. These data direct the attention to the advantageous property of imidazole nitrogen in the D_3 receptor affinity of these types of compounds.

It has also been found that linking of the condensed heterocycle to the piperazine moiety through a lipophilic chain, as in NAN-190 or buspirone, yields compounds with 5-HT_{1A} affinity. This affinity was also influenced by the length of the lipophilic chain. In this respect the rank order was $n = 4 > 2 > 3$ (except the 2-indolizinyls). The K_i values of the most active compounds (**12**, **15**, **18**, **10**) were in the nanomolar range (pK_i between 7.9 and 8.3). As it was stated earlier the 2-indolizinyl derivatives showed very low affinity to the 5-HT_{1A} receptor subtype.

In summary, we have disclosed a novel series of substituted phenoxyalkylpiperazines, which show high affinity to the dopamine D_3 receptor. Some of the compounds displayed up to 42-fold selectivity over D_2 receptors. Compounds with a 2-methoxy-phenyl moiety showed marked 5-HT_{1A} affinity. These new compounds may be useful tools for further understanding of the physiological role of D_3 receptors and their therapeutical potential in the treatment of psychiatric disorders.

3. Experimental

3.1. Chemistry

3.1.1. Equipment

Structures of the new compounds were determined by their IR (using KBr pellets on a Nicolet 20 DXC FT-IR spectrophotometer), and ^{1}H NMR (recorded at 300 MHz on a VARIAN VXR-300 spectrometer using tetramethylsilane as internal standard) spectra, while their purity was assessed by TLC, LC/MS (the LC/MS analyses were performed using an HP 1100 binary gradient system (Hewlett Packard, Waldbronn, Germany) controlled by ChemStation software. A HP diode array detector was used to acquire UV spectra at $\lambda = 254$ nm. Analytical chromatographic experiments were made on a Discovery C_{16}-Amide, 5 cm X 4.6 mm X 5 μm column (Supelco, Bellefonte, PA) with a flow rate of 1 ml/min. A 10 min linear gradient of 95% acetonitrile in 0.1% aqueous TFA was applied. Equilibration time between analysis was 2 min. All experiments were performed using a HP MSD single quadropole mass spectrometer equipped with an electrospray ionization source). Functional group analysis was done by acidi- or alkalimetry. The purity of all studied compounds was higher than 95%. Melting points were taken on a Büchi 535 capillary apparatus.

3.1.2. Typical procedure for the preparation of 1-(4-substituted-phenoxy)-alkyl-4-(2-methoxy-phenyl)piperazines (**4–18**)*; 1-{2-[4-(indolizin-2-yl)phenoxy]ethyl}-4-(2-methoxyphenyl)-piperazine* (**4**)

A suspension of 2-[4-(indolizin-2-yl)phenoxy]ethylchloride (2.72 g, 10 mmol; prepared from the known 2-(4-hydroxyphenyl)indolizine [18] by a standard procedure using 1-bromo-2-chloroethane as alkylating agent), (2-methoxyphenyl)piperazine hydrochloride (2.74 g, 12 mmol), sodium carbonate (2.54 g, 24 mmol) and sodium iodide (0.3 g, 2 mmol) in 5-methyl-2-hexanone (40 ml) was stirred and refluxed for 10 h. The reaction mixture was evaporated under reduced pressure and the residue was suspended in water (20 ml) and extracted with chloroform (80 ml). The organic layer was washed with water (2 × 15 ml), dried on sodium sulfate then filtered. The filtrate was stirred with charcoal (1 g) and aluminum oxide (0.5 g) for 20 min at room temperature, than it was filtered and the filtrate was concentrated to the half of its original volume. Ethanol was added to the residue and the solution was again concentrated under reduced pressure to 15 ml. The precipitated solid was filtered and dried to yield 2.9 g raw product. It was dissolved in chloroform (30 ml), ethanol (30 ml) was added to the solution which was then concentrated under reduced pressure to 15 ml. The precipitated solid was filtered and dried to yield 2.61 g (61%) of the title compound (mp: 159–161 °C).

4: ^{1}H NMR(DMSO-d_6, 30 °C): 2.66 m (4H) (N−CH_2); 2.78 t (2H) 2.98 m (4H) (N−CH_2); 3.77 s (3H) (OMe); 4.14 t (2H) (O−CH_2); 6.50 m (1H) (Ar); 6.63–6.69 m (2H) (Ar); 6.83–6.95 m (4H) (Ar); 6.98 m (2H) (Ar); 7.36 m (1H) (Ar); 7.61 m (2H) (Ar); 7.86 s (1H) (Ar); 8.19 m (1H) (Ar).

IR (KBr pellet): 2803, 1610, 1498, 1457, 1247, 1237, 1029, 832, 780, 741.

LC/MS: $k' = 3.943$ min, Area% = 99.307, MW + H^+ = 428.

Compounds **5–18** were prepared analogously starting from known materials like 2-(4-hydroxyphenyl)imidazo[1,2-1*a*]pyridine [19], 2-(4-hydroxyphe-

nyl)imidazo[1,2-*a*]pyrimidine [20], 6-(4-hydroxyphenyl)-2,3-dihydro-imidazo[2,1-*b*]thiazole [21], 6-[4-(3-chloropropoxy)]-imidazo[1,2-a]pyridine and 6-[4-(3-chloropropoxy)]-imidazo[1,2-*a*]pyrimidine [22] or from their analogues using standard procedures, like 6-(4-hydroxyphenyl)imidazo[2,1-*b*]-thiazole that were prepared as follows first in our laboratory:

4′-Hydroxyacetophenone (27.2 g, 0.2 mol) was dissolved in a mixture of chloroform (400 ml) and ethyl acetate (400 ml). Copper(II)bromide (84 g, 0.376 mol) and 48% aqueous HBr was added to the solution then it was stirred under reflux for 2 h. Having cooled the reaction mixture to 60 °C charcoal (3 g) was added, after filtration the filtrate was concentrated to 50–60 ml. Ethanol (100 ml) and 2-aminothiazol (19 g, 0.19 mol) was added to the residue, the reaction mixture was refluxed for 30 min then it was cooled to 50–60 °C. Tetraethylammonium bromide (1 g) and sodium carbonate (12 g, 0.113 mol) was added cautiously and the reflux and stirring was continued further for 6 h. Water (40 ml) and ethyl acetate (70 ml) was added to the cooled reaction mixture, the precipitated solid was filtered off, washed with ethyl acetate and water to yield 20 g product (49%, m.p.: 262–264 °C) that was used without further purification.

Analytical data of some selected compounds:

9: ^{1}H NMR ($CDCl_3$, 30 °C): 1.74 m (2H) (CH_2); 1.83 m (2H) (CH_2), 2.49 t (2H) (N−CH_2); 2.67 m (4H) (N−CH_2); 3.11 m (4H) (N−CH_2); 3.86 s (3H) (OMe); 4.04 t (2H) (O−CH_2); 6.74 m (1H) (Ar); 6.83–7.04 m (6H) (Ar); 7.13 m (1H) (Ar); 7.60 m (1H) (Ar); 7.76 s (1H) (Ar); 7.87 m (2H) (Ar); 8.08 m (1H) (Ar). IR (KBr pellet): 2951, 2820, 1612, 1502, 1486, 1284, 1249, 1173, 1032, 839, 745, 736. LC/MS: k′ = 4.058 min, Area% = 98.171, MW + H^+ = 457.

12: ^{1}H NMR ($CDCl_3$, 30 °C): 1.73 m (2H) (CH_2); 1.86 m (2H) (CH_2); 2.49 t (2H) (N−CH_2); 2.67 m (4H) (N−CH_2); 3.11 m (4H) (N−CH_2); 3.86 s (3H) (OMe); 4.04 t (2H) (O−CH_2); 6.79 dd (1H) (Ar); 6.83–7.02 m (6H) (Ar); 7.70 s (1H) (Ar); 7.94 m (2H) (Ar); 8.38 dd (1H) (Ar); 8.52 dd (1H) (Ar). IR (KBr pellet): 2941, 1615, 1498, 1488, 1475, 1251, 1238, 1227, 835, 763, 753. LC/MS: k′ = 3.746 min, Area% = 98.345, MW + H^+ = 458.

17: ^{1}H NMR ($CDCl_3$, 30 °C): 2.02 m (2H) (CH_2); 2.51 t (2H) (CH_2); 2.69 m (4H) (N−CH_2); 3.11 m (4H) (N−CH_2); 3.86 s (3H) (OMe); 4.08 t (2H) (O−CH_2); 6.78 d (1H) (Ar); 6.85–7.03 m (6H) (Ar); 7.39 d (1H) (Ar); 7.62 s (1H) (Ar); 7.75 m (2H) (Ar). IR (KBr pellet): 2808, 1543, 1500, 1464, 1302, 1242, 1145, 834, 747. LC/MS: k′ = 3.843 min, Area% = 98.224, MW + H^+ = 449.

18: ^{1}H NMR (DMSO-d_6, 30 °C): 1.61 m (2H) (CH_2); 1.76 m (2H) (CH_2); 2.39 t (2H) (N−CH_2); 2.51 m (4H) (N−CH_2); 2.95 m (4H) (N−CH_2); 3.76 s (3H) (OMe); 4.02 t (2H) (O−CH_2); 6.84–6.94 m (4H) (Ar); 6.95 m (2H) (Ar); 7.22 d (1H) (Ar); 7.73 m (2H) (Ar); 7.90 d (1H) (Ar); 8.08 s (1H) (Ar). IR (KBr pellet): 3103, 2817, 1612, 1546, 1499, 1463, 1239, 1021, 998, 832, 742, 731. LC/MS: k′ = 4.009 min, Area% = 98.074, MW + H^+ = 463.

3.2. Pharmacological screening

3.2.1. D_3 receptor binding

The binding studies were carried out on rat recombinant D_3 receptors expressed in Sf9 cells using [^{3}H]-spiperone (0.4 nM) as a ligand and haloperidol (10 μM) for the determination of non-specific binding. The assay was performed according to the Research Biochemical International assay protocol for rD_3 receptor (Cat. No. D-181).

3.2.2. D_2 receptor binding

Binding of [^{3}H]-spiperone (0.5 nM) to rat striatal tissue was measured according to the method of Seeman [23]. The non-specific binding was determined in the presence of (±)-sulpiride (10 μM).

3.2.3. 5-HT_{1A} receptor binding

The binding assay was performed according to Peroutka [24] on rat hippocampal tissue using [^{3}H]-8-OH-DPAT (0.5 nM) as ligand. The non-specific binding was determined in the presence of serotonin (10 μM).

The K_i values were calculated on the basis of the Cheng-Prusoff equation [25].

Acknowledgement: The authors are indebted to Béla Hegedüs for the IR spectra, to Gábor Tárkányi for the NMR spectra, to Györgyi Szendrei for the LC/MS measurements and to Ilona Szilágyi and Erika Varga for the technical assistance in the binding studies.

References

1 Hartman, D.; Monsma, F.; Civelli, O.; in: Csernansky, J. G. (Ed.): Antipsychotics, p. 43, Springer, Berlin, 1996
2 Peacock, L.; Gerlach, J.; in: Csernansky, J. G. (Ed.): Antipsychotics, p. 359, Springer, Berlin, 1996
3 Sokoloff, P.; Giros, B.; Martres, M-P.; Bouthenet, M-L.; Schwartz, J.-C.: Nature **347**, 146 (1990)
4 Schwartz, J.-C.; Levesque, D.; Martres, M.-P.; Sokoloff, P.: Clin. Neuropharmacol. **16**, 295 (1993)
5 Levant, B.: Pharmacol. Rev. **49**, 231 (1997)
6 Ratouis, R.; Boissier, J. R.; Dumont, C.: J. Med. Chem. **8**, 271 (1965)
7 Agarwal, S.K.; Kumar, Y.; Saxena, A. K.; Jain, P. C.; Anand, N.: Indian J. Chem. Sect. B **21**, 435 (1982)
8 Agarwal, S. K.; Saxena, A. K.; Jain, P. C.; Anand, N.: Indian J. Chem. Sect. B **21**, 914 (1982)
9 Agarwal, S. K.; Saxena, A. K.; Jain, P. C.; Anand, N.: Indian J. Chem. Sect. B **24**, 733 (1985)
10 Agarwal, S. K.; Saxena, A. K.; Jain, P. C.; Anand, N.; Sur, R. N.: Indian J. Chem. Sect. B **29**, 80 (1990)
11 Terán, C.; Santana, L.; Uriarte, E.; Fall, Y.; Unelius, L.; Tolf, B.: Bioorg. Med. Chem. Letters **8**, 3567 (1998)
12 Sparatore, A.; Goegan, M.; Cagnotto, A.; Sparatore, F.: Farmaco **54**, 402 (1999)
13 Wright, J.; Heffner, T.; Pugsley, T.; MacKenzie, R.; Wise, L.: Bioorg. Med. Chem. Letters **5**, 2547 (1995)
14 Laszlovszky, I.; Csejtei, M.; Kovács, K. J.; Kiss B.: Fundament. Clin. Pharmacol. **13** (Suppl. 1), 382s (1999)
15 Kiss, B.; Auth, F.; Laszlovszky, I.: Fundament. Clin. Pharmacol. **13** (Suppl. 1), 382s (1999)
16 Sághy, K.; Laszlovszky, I.; Gyertyán, I.: Fundament. Clin. Pharmacol. **13** (Suppl. 1), 378s (1999)
17 Laszy, J.; Gyertyán, I.; Laszlovszky, I.: Fundament. Clin. Pharmacol. **13** (Suppl. 1), 381s (1999)
18 Buu-Hoï, N. P.; Xuong, N. D.; Lavit, D.: J. Chem. Soc. 1034 (1954)
19 Ito, Y.; Kato, H.; Ogawa, N.; Etsutchu, E.; Kurata, S.: Jpn. Kokai Tokkyo Koho JP 60,233,074; C.A. **104**, 186412e (1986)
20 Pentimalli, L.; Milani, G.: Gazz. Chim. Ital. **100**, 1106 (1970)
21 Koyama, K.; Oishi, T.; Ishii, A.; Deguchi, T.: Oyo Yakuri **26**, 869 (1983); C.A. **101**, 32746t (1984)
22 Sanfilippo, P. J.; Urbanski, M.; Press, J. B.; Dubinsky, B.; Moore Jr., J. B.: J. Med. Chem. **31**, 2221 (1988)
23 Seeman, P.; in: Parvez, S.; Nagatsu, T.; Nagatsu, I.; Parvez H. (Eds.): Methods in Biogenic Amine Research, p. 591, Elsevier, Amsterdam 1984
24 Peroutka, S. J.: J. Neurochem. **47**, 529 (1986)
25 Cheng, Y.; Prusoff, W. H.: Biochem. Pharmacol. **22**, 3099 (1973)

Received March 29, 2000
Accepted September 13, 2000

Dr. István Laszlovszky
Gedeon Richter Ltd.
P. O. Box 27
1475 Budapest 10
Hungary
i.laszlovszky@richter.hu