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New 2-pyridylethylamines with dopaminergic activity: synthesis and radioligand-binding evaluation

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Summary — In order to determine whether the pyridine nucleus could replace the catechol moiety of the neurotransmitter dopamine or the phenol ring of the dopaminergic pharmacophore *m*-hydroxyphenylethylamine, the 2-(3-pyridyl)ethylamine **7**, 2-(4-pyridyl)ethylamine **8**, 2-(2-hydroxy-4-pyridyl)ethylamine **10** and their *N,N*-di-*n*-propyl- and *N*-*n*-propyl-*N*-2-phenylethyl derivatives were synthesized. The affinities of the new compounds for D_1 and D_2 dopamine receptors were evaluated by displacement of [^{3}H]SCH 23390 (D_1 selective) and [^{3}H]spiperone (D_2 selective) on rat neostriatum sections. The 2-(4-pyridyl)ethylamine **8** and its *N,N*-di-*n*-propyl derivative **18** showed the same affinity for the D_1 and D_2 receptors. Other compounds bound to the D_1 receptor with higher affinity than to the D_2 receptor. The possibility that the above compounds act as agonists and antagonists at the dopamine D_1 and D_2 receptors is discussed on the basis of guanosine-5'-triphosphate and Na^+ displacement curves.

2-pyridylethylamine / synthesis / dopamine D_1 and D_2 receptor binding

Introduction

The catecholamine dopamine (DA) is an important neurotransmitter in the mammalian brain and is involved in several central nervous system (CNS) activities, such as motor (extrapyramidal) and behavioural (limbic) control, and neuroendocrine regulation. It has been proposed that alterations of the dopaminergic system play a major role in several diseases, such as schizophrenia, tardive dyskinesia, Parkinson's disease, and hyperprolactinemia. Initially, it was widely accepted that DA produces its effects through only two types of receptors, D_1 and D_2 [1]. The application of molecular biology techniques has allowed the identification of two D_1-like (D_1 and D_5) and three D_2-like (D_2, D_3, and D_4) receptors [2].

During the past years significant efforts have been dedicated to the design and synthesis of new compounds selective for the two main DA receptor subtypes. Ergoline derivatives, such as bromocriptine and pergolide, have been extensively studied as DA agonists. Structural modification of the ergoline skeleton has led to bicyclic and tricyclic ergoline partial structures such as tetrahydroindazole **1** [3], octahydro-1*H*-pyrrolo[3,4-*g*]quinoline **2**, octahydro-2*H*-pyrazolo[3,4-*g*]quinoline **3** (Quinpirole) [4], octahydro-6-propylpyrido[2,3-*g*]quinazolin-2-amine **4** (Quinerolane) [5] and tetrahydrobenzothiazole **5** (Pramipexole) [6] (fig 1). All compounds were found to be potent and selective D_2-like agonists.

It is interesting to note that compounds **1–5** do not contain aromatic hydroxy functionalities. DA agonists containing catechol or phenol rings have a limited clinical utility, because of their low oral bioavailability and their short duration of action. Catechols are degraded by the enzyme catechol-*O*-methyltransferase (COMT) and, like the phenols, are susceptible to conjugation (as glucuronides and/or sulphates) [7, 8].

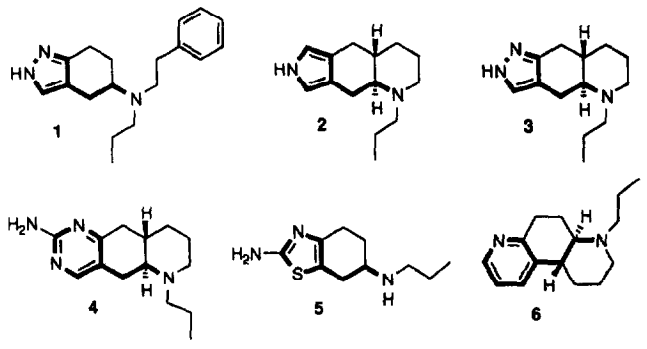
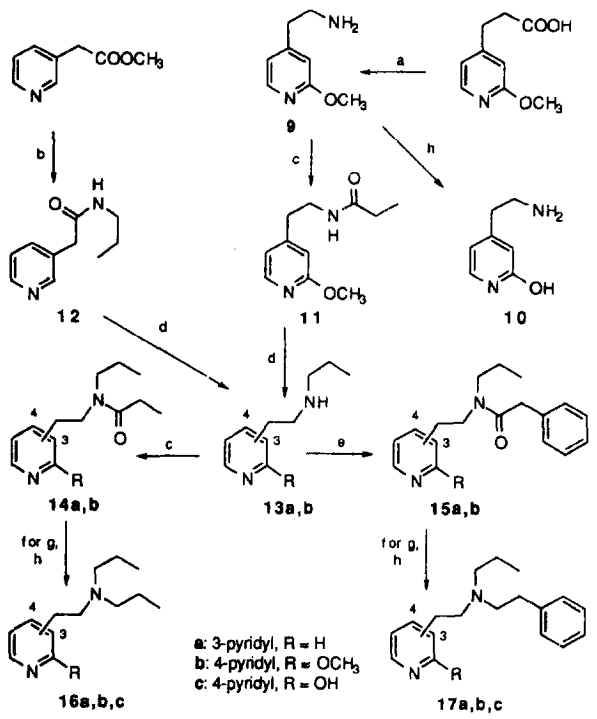


Fig 1. Structures of compounds **1–6**.



Scheme 1. a: NaN_3, H_2SO_4. b: *n*-$C_3H_7NH_2$, CH_3ONa. c: C_2H_5COCl, $N(C_2H_5)_3$. d: $NaBH_4$, CH_3COOH. e: $PhCH_2COCl$, $N(C_2H_5)_3$. f: **14a**, **15a** + $BH_3{\cdot}S(CH_3)_2$, $BF_3{\cdot}O(C_2H_5)_2$. g: **14b**, **15b** + $LiAlH_4$. h: HBr 48%, CH_3COOH, **16c** and **17c** from **16b** and **17b**.

A simple comparison of compounds **1–5** suggests that the molecular substructure required for a selective activation of the D_2-like DA receptors is a rigid heteroarylethylamine, which mimics the '3-hydroxyphenylethylamine' pharmacophore, embedded in various classes of DA agonists [9]. The heteroaromatic systems of pyrrole, pyrazole, 2-aminopyrimidine and 2-aminothiazole can function as catechol/phenol bioisosteres. Moreover, the dopaminergic pharmacophore in **3** is a 2-(4-pyrazolyl)ethylamine. The pyrazole nitrogens might be involved in hydrogen bonding, donor (NH) and acceptor (N), with the two serine residues in the fifth transmembrane domain of the D_2 receptor [10].

In a previous work, we described the synthesis of *trans*-4-propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline **6** [11]. Biological assays showed that this compound is a fully effective D_2-like dopaminergic agonist, able to produce an almost complete inhibition of prolactin release in reserpinized male rats. These results suggest that the pharmacophore in **6** is the 2-(3-pyridyl)ethylamine moiety. The pyridine (as the pyrazole) nucleus could interact by π-π stacking with the phenylalanine residues in the fifth and sixth transmembrane domains of the D_2 receptor [10], and by the nitrogen atom which can function as a hydrogen bond acceptor with its electron lone pair in the sp^2 orbital. The presence of a basic sp^2 nitrogen could be a critical pharmacophoric element since Quinpirole **3** shows higher dopaminergic activity in prolactin inhibition than **2** [4].

These findings prompted us to explore whether or not the pyridine nucleus could replace the catechol moiety of DA or the phenol ring of the 3-hydroxyphenylethylamine pharmacophore. We have therefore synthesized 2-(3-pyridyl)ethylamine **7**, 2-(4-pyridyl)-ethylamine **8** and 2-(2-hydroxy-4-pyridyl)ethylamine **10** and their *N*,*N*-di-*n*-propyl- and *N*-propyl-*N*-2-(phenylethyl) derivatives. The dopaminergic activity of all compounds was evaluated with binding assays on the D_1 and D_2 DA receptors.

Chemistry

2-(3-Pyridyl)ethylamine **7** and 2-(4-pyridyl)ethylamine **8** were prepared by reduction of the 3-pyridylethanamide or 4-pyridylethanamide [12] with sodium borohydride and acetic acid. 2-(2-Hydroxy-4-pyridyl)-ethylamine **10** was synthesized from 3-(2-methoxy-4-pyridyl)propanoic acid by a modification of the Curtius degradation of the azide. The 2-(3-pyridyl)- and 2-(2-hydroxy-4-pyridyl)ethylamine derivatives were obtained as outlined in scheme 1. *N*-*n*-Propyl-*N*-(2-phenylethyl)-2-(4-pyridyl)ethylamine **19** was synthesized by reaction of 4-vinylpyridine with *N*-*n*-propyl-*N*-(2-phenylethyl)amine as reported for *N*,*N*-di-*n*-propyl-2-(4-pyridyl)ethylamine **18** [13].

Compound **10** was synthesized as an isostere of 3-hydroxyphenylethylamine pharmacophore. It is well established that 2-hydroxypyridine exists also as a 2(1*H*)-pyridinone tautomer [14]. A similar situation can be envisaged in **10**. Analytical data show that it produces a dihydrobromide, thus indicating the presence of a 2-hydroxypyridine nucleus, confirmed by the chemical shifts of pyridine hydrogens (δ (Me_2SO-d_6) 7.56 (H_6), 6.40 (H_3 and H_5)). On the other hand, the free base shows chemical shifts (δ (Me_2SO-d_6) 7.27 (H_6), 6.13 (H_3), and 6.08 (H_5)) which are identical to those of 4-methyl-2-pyridinone (δ (Me_2SO-d_6) 7.24 (H_6), 6.15 (H_3), and 6.02 (H_5)) [15].

Results and discussion

The *in vitro* binding affinity of the synthesized compounds toward D_1 and D_2 subtypes of DA receptors was measured by displacement of [^{3}H]SCH 23390 (D_1 selective) and [^{3}H]Spiperone (D_2 selective)

from a frozen preparation of sections of rat neostriatum. Since rat neostriatum expresses primarily D_1 and D_2 DA receptors [16], the compounds were analyzed for their D_1 and D_2 DA receptor affinity.

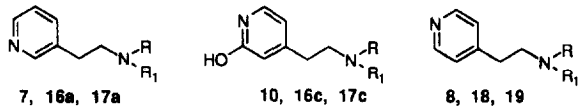
Reference compounds included SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride), Quinpirole (*trans*-(–)-4a-*R*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline), PPHT ((±)-2-(*N*-phenylethyl-*N*-propyl)amino-5-hydroxytetralin hydrochloride), and DA. The data obtained are summarized in table I.

The results of binding studies indicate that the new compounds are more powerful competitors of [^{3}H]SCH 23390 than of [^{3}H]Spiperone binding, with the exception of **8** and **18** which are equipotent. In displacing [^{3}H]SCH 23390, both 2-(3-pyridyl)- and 2-(2-hydroxy-4-pyridyl)ethylamine **7** and **10** are more active than the 2-(4-pyridyl)ethylamine **8**. *N,N*-Dipropylation increases the D_1 affinity of 4-pyridyl- and 2-(2-hydroxy-4-pyridyl)ethylamines **18** and **16c**. Compounds **7**, **10**, **16a**, **16c**, **17a** and **18** show affinities for the D_1 sites similar to that of the selective antagonist SCH 23390.

In the [^{3}H]Spiperone assay the 2-hydroxy-4-pyridyl derivative **10** is the less effective among the primary amines, but all the compounds show higher affinities than the D_2-like selective agonist Quinpirole. Substitution of the amino group with two propyl groups increases the D_2 affinity in the 4-pyridyl series (compound **18**) but does not change that of the other series. The replacement of a propyl with a 2-phenylethyl group decreases the D_1 and D_2 affinities. The 4-pyridyl derivatives **8** and **18** cannot discriminate between the D_1 and D_2 sites, while **16c** is the most D_1 selective.

The D_1 binding data of primary amines **7** and **10** reveal that a 3-pyridyl or a 2-hydroxy-4-pyridyl system is required for optimal interaction at D_1 sites. These systems contain a nitrogen atom or a hydroxyl group at the position *meta* to ethylamine chain which seem to be discriminant for the best interaction at D_1 sites. The cloned catecholaminergic G protein-coupled receptors contain two serine residues in transmembrane domain five, which represent the binding partners of the catechol group [10]. A similar situation can also be supposed for the D_1 receptor. Most likely the sp^2 nitrogen lone pair of the 3-pyridyl nucleus interacts, as an acceptor hydrogen bond, with the serine residue which binds the *meta*-hydroxy group of DA. This serine residue has a much greater impact on the D_1 affinity than the other which forms a hydrogen bond with the *para*-hydroxy group of DA. On the other hand, the hydroxy group of the 2-hydroxy-4-pyridyl system can interact with the same serine resi-

Table I. Inhibition of [^{3}H]SCH 23390 and [^{3}H]Spiperone binding to sections of rat neostriatum.



Compound	*R*	R_1	K_i (nM)		
			[^{3}H]SCH 23390	*[^{3}H]Spiperone*	D_2/D_1
7	H	H	0.4 ± 0.01	3.3 ± 0.18	8.25
10	H	H	0.99 ± 0.1	8.43 ± 0.91	8.51
8	H	H	2.83 ± 0.1	2.77 ± 0.07	0.97
16a	*n*-C_3H_7	*n*-C_3H_7	0.5 ± 0.01	2.55 ± 0.07	5.1
16c	*n*-C_3H_7	*n*-C_3H_7	0.29 ± 0.02	9.21 ± 0.41	31.75
18	*n*-C_3H_7	*n*-C_3H_7	0.56 ± 0.01	0.33 ± 0.07	0.59
17a	*n*-C_3H_7	$(CH_2)_2Ph$	0.85 ± 0.04	9.75 ± 0.9	11.47
17c	*n*-C_3H_7	$(CH_2)_2Ph$	1.69 ± 0.2	28.5 ± 1.2	16.86
19	*n*-C_3H_7	$(CH_2)_2Ph$	1.19 ± 0.1	22.3 ± 1.31	18.73
Quinpirole			> 1000	108 ± 10.2	
PPHT			> 1000	0.17 ± 0.03	
SCH 23390			0.72 ± 0.04	> 1000	
Dopamine			> 1000	848 ± 39	

Values are the means ± SEM of 5–7 independent triplicate experiments performed as indicated in the test.

due as a hydrogen bond acceptor or donor. If the 2-hydroxy-4-pyridyl system interacts as 2-pyridinone tautomer, the carbonyl group is a hydrogen bond acceptor. The pyridine nitrogen of **7** and the 2-hydroxyl or 2-carbonyl group of **10** induce a higher D_1 affinity than the nitrogen of the 4-pyridyl system, as can be seen by the affinity value of **8**. Primary amines **7**, **8** and **10**, which have the catechol system of DA replaced by a 3-pyridyl, 4-pyridyl and 2-hydroxy-4-pyridyl group respectively, display higher D_1 and D_2 receptor affinities than DA. This suggests that the basic nitrogen lone pair binds DA receptor more closely than the hydroxyl group.

Compounds displaying the highest D_1 (**7**, **16a**, **16c**, and **18**) or D_2 (**7**, **8**, **16a**, and **18**) affinities were selected to evaluate the DA receptor's possible agonist or antagonist properties. D_1 or D_2 DA receptor agonist activity was assayed by incubating sections with [^{3}H]SCH 23390 or [^{3}H]Spiperone and the compound under examination (see below) in the presence of a 100 μM concentration of guanosine-5'-triphosphate (GTP) or a 100 mM NaCl concentration, respectively. It is known that: a) the presence of GTP in the incubation medium has no effect on antagonist potency in [^{3}H]SCH 23390-binding experiments, but causes a shift to the right of agonist displacement curve; and b) the presence of Na^+ in the incubation medium has no effect on agonist potency in [^{3}H]Spiperone-binding experiments, but causes a shift to the left of the antagonist displacement curve [17, 18].

Analysis of the influence of GTP on [^{3}H]SCH 23390 binding to sections of rat neostriatum revealed a shift to the right on the competition displacement curve of the compound **16a** (K_i values: 0.5 ± 0.01 nM without GTP, and 6.2 ± 0.3 nM with GTP, $P < 0.01$). The presence of GTP in the incubation medium did not change the K_i values of other competitors tested (**7**, **16c** and **18**, data not shown). This suggests that only compound **16a** has a DA D_1 receptor agonist activity.

Analysis of the influence of Na^+ on [^{3}H]Spiperone binding to sections of rat neostriatum caused a shift to the left of the competition displacement curve of the compounds **7** (K_i values 3.3 ± 0.18 nM without Na^+ and 0.18 ± 0.01 nM with Na^+, $P < 0.01$) and **8** (K_i values: 2.77 ± 0.07 nM without Na^+ and 0.25 ± 0.01 with Na^+, $P < 0.01$). The presence of Na^+ in the incubation medium did not change the K_i values of other competitors tested (**16a** and **18**, data not shown). This suggests that the compounds **7** and **8** have probably a DA D_2 receptor antagonist activity.

Analysis of displacement curves showed that the primary amine **7** displayed a DA D_1 and D_2 antagonist profile, whereas the tertiary amine **16a** displayed a DA D_1 and D_2 agonist profile. On the other hand, the tertiary amines **16c** and **18** displayed a DA D_1 antagonist profile, and a DA D_1 antagonist and D_2 agonist profile respectively. Our agonist/antagonist profiles were evaluated by radioligand binding experiments only. It cannot be excluded that the use of functional assays may disclose partial agonist and/or antagonist receptor activities of these compounds. Further work is in progress to clarify this apparent discrepancy of radioligand-binding data.

The preliminary data presented in this paper highlight the possibility that 3-pyridyl and 2-hydroxy-4-pyridyl moieties could replace the catechol ring of DA or the phenol ring of 3-hydroxyphenylethylamine, thus affording compounds with a high affinity for D_1 DA receptors but with poor selectivity.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Microanalyses were performed on a 1106 Carlo Erba CHN Analyzer, and the results were within ±0.4% of the calculated values. Proton magnetic resonance (NMR) spectra were recorded on a Varian VXR 200 MHz spectrometer and are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si). The IR spectra were run on a Perkin-Elmer Model 297 spectrometer as nujol mulls or liquid films. The identity of all new compounds was confirmed by both elemental analysis and NMR data; homogeneity was confirmed by TLC on silica gel Merck 60 F_{254}. Chromatographic purifications were accomplished on Merck silica gel 60 (70-230 mesh ASTM) columns with the reported solvent.

2-(3-Pyridyl)ethylamine hydrochloride 7
A solution of acetic acid (2.28 ml, 40 mmol) in dry dioxane (10 ml) was added dropwise to an ice-cooled suspension of 2-(3-pyridyl)ethanamide (1 g, 7.3 mmol) and $NaBH_4$ (1.55 g, 40 mmol) in dry dioxane (25 ml). After the addition was complete, the ice bath was removed and the mixture was stirred at reflux for 4 h. The solvent was evaporated and CH_3OH (25 ml) was added. The mixture was heated at reflux for 12 h. The solvent was evaporated and the resulting residue was partitioned between $CHCl_3$ and H_2O. The organic layers were washed with 5% sodium bicarbonate and brine, dried (anhydrous Na_2SO_4), filtered, and evaporated. The residue was dissolved in anhydrous EtOH and HCl gas was bubbled into the solution. The solid was collected and recrystallized from anhydrous EtOH: mp 207–209°C; yield 45%. ^{1}H NMR (CD_3OD) δ 8.94 (d, 1H, H-2 Pyr), 8.83 (m, 1H, H-6 Pyr), 8.68 (m, 1H, H-5 Pyr), 8.41 (bs, 1H, NH^+), 8.12 (m, 1H, H-4 Pyr), 4.90 (s, 3H, NH_3^+), 3.34 (m, 4H, CH_2). Anal $C_7H_{10}N_2 \cdot 2HCl \cdot 1/2H_2O$ (C, H, N).

2-(4-Pyridyl)ethylamine hydrochloride 8
Compound **8** was synthesized as described for **7** starting from 2-(4-pyridyl)ethanamide (2 g, 14.6 mmol), $NaBH_4$ (3.1 g, 80 mmol) in dry dioxane (40 ml), acetic acid (4.56 ml, 80 mmol) in dry dioxane (10 ml), CH_3OH (25 ml). The salt was recrystallized from anhydrous EtOH: mp 212–214°C; yield 65%. ^{1}H NMR (Me_2SO-d_6) δ 8.89 (dd, 2H, H-2,6 Pyr),

8.30 (bs, 1H, NH^+), 8.0 (dd, 2H, H-3,5 Pyr), 3.90 (bs, 3H, NH_3^+), 3.25 (m, 4H, CH_2). Anal $C_7H_{10}N_2 \cdot 2HCl \cdot 1/2H_2O$ (C, H, N).

2-(2-Methoxy-4-pyridyl)ethylamine hydrochloride ***9***

3-(2-Methoxy-4-pyridyl)propanoic acid (1.5 g, 8.3 mmol) was added to concentrated H_2SO_4 (5 ml) and stirred at 70°C. When the solution became clear, sodium azide was slowly added over a period of 2 h. The mixture was stirred for 2 h at 70°C, for 12 h at room temperature, and then poured onto ice. The solution was basified with 40% NaOH and extracted with $CHCl_3$. The combined organic layers were dried (anhydrous Na_2SO_4), and evaporated under reduced pressure. The oily residue was dissolved in anhydrous EtOH and HCl gas was bubbled into the solution. The solvent was evaporated and the residue was recrystallized from anhydrous EtOH: mp 149–151°C; yield 93%. ^1H NMR (Me_2SO-d_6) δ 9.12 (bs, 1H, NH^+), 8.38 (bs, 3H, NH_3^+), 8.20 (d, 1H, H-6 Pyr), 7.10 (dd, 1H, H-5 Pyr), 7.02 (d, 1H, H-3 Pyr), 3.97 (s, 3H, OCH_3), 3.08 (m, 4H, CH_2). Anal $C_8H_{12}N_2O \cdot 2HCl \cdot H_2O$ (C, H, N).

2-(2-Hydroxy-4-pyridyl)ethylamine hydrobromide ***10***

A solution of the amine **9** (0.96 g, 4 mmol) was dissolved in 48% HBr (7.5 ml) and acetic acid (7.5 ml). The mixture was heated to reflux for 4 h, after which the solvent was removed under reduced pressure. The residue was recrystallized from anhydrous EtOH: mp 221–223°C; yield 71%. ^1H NMR (Me_2SO-d_6) δ 7.82 (bs, 2H, NH^+, OH), 7.56 (d, 1H, H-6 Pyr), 6.40 (m, 2H, H-3,5 Pyr), 4.88 (bs, 3H, NH_3^+), 3.10 (m, 2H, NCH_2), 2.79 (t, 2H, $PyrCH_2$). Anal $C_7H_{10}N_2O \cdot 2HBr \cdot H_2O$ (C, H, N).

N-2-(2-Methoxy-4-pyridyl)ethylpropanamide ***11***

Propionyl chloride (1.8 ml, 21 mmol) in dry THF (15 ml) was added dropwise to an ice-cooled solution of the amine **9** (3 g, 19.7 mmol) and triethylamine (2.95 ml, 21 mmol) in dry THF (60 ml). After the addition was complete, the ice bath was removed and the mixture was stirred at room temperature for 12 h. The precipitate was filtered and the solution was evaporated. The residue was partitioned between $CHCl_3$ and H_2O. The organic layers were washed with saturated sodium carbonate solution and brine, dried (anhydrous Na_2SO_4), filtered, and evaporated. The residue was recrystallized from cyclohexane: mp 64–66°C; yield 68%. ^1H NMR ($CDCl_3$) δ 8.10 (d, 1H, H-6 Pyr), 6.75 (dd, 1H, H-5 Pyr), 6.59 (d, 1H, H-3 Pyr), 5.51 (bs, 1H, NH), 3.94 (s, 3H, OCH_3), 3.51 (q, 2H, NCH_2), 2.78 (t, 2H, CH_2) 2.18 (q, 2H, CH_2), 1.15 (t, 3H, CH_3). Anal $C_{11}H_{16}N_2O_2$ (C, H, N).

N-n-Propyl-2-(3-pyridyl)ethanamide ***12***

A mixture of methyl-2-(3-pyridyl)acetate (4 g, 24 mmol), propylamine (2.4 ml, 29 mmol), and sodium methoxide (1.65 g, 29 mmol) in anhydrous benzene (20 ml) was heated to reflux for 6 h. The reaction mixture was poured into ice and stirred. Benzene was separated and the water layer was extracted with $CHCl_3$. The combined organic layers were dried (anhydrous Na_2SO_4), filtered, and evaporated. The residue was recrystallized from Et_2O: mp 65–66°C; yield 42%. ^1H NMR ($CDCl_3$) δ 8.52 (m, 2H, H-2,6 Pyr), 7.68 (m, 1H, H-5 Pyr), 7.29 (m, 1H, H-4 Pyr), 5.50 (bs, 1H, NH), 3.53 (s, 2H, CH_2CO), 3.20 (q, 2H, NCH_2), 1.48 (m, 2H, CH_2), 0.87 (t, 3H, CH_3). Anal $C_{10}H_{14}N_2O$ (C, H, N).

N-n-Propyl-2-(3-pyridyl)ethylamine ***13a***

A solution of acetic acid (2.1 ml, 36.4 mmol) in dry dioxane (20 ml) was added dropwise to an ice-cooled suspension of the amide **12** (1.3 g, 7.2 mmol) and $NaBH_4$ (1.38 g, 36.4 mmol) in dry dioxane (50 ml). After the addition was complete, the ice bath was removed and the mixture was stirred at reflux for 3 h. The solvent was evaporated and 2 N HCl (30 ml) was added. The mixture was stirred at room temperature for 12 h. The solution was made basic with a saturated solution of sodium carbonate and extracted with $CHCl_3$. Combined organic layers were dried (anhydrous Na_2SO_4), filtered, and evaporated. The oily residue was purified by column chromatography with ethyl acetate/hexane/CH_3OH/NH_4OH (6:3:0.9:0.1) as eluent; yield 85%. ^1H NMR ($CDCl_3$) δ 8.47 (m, 2H, H-2,6 Pyr), 7.51 (m, 1H, H-5 Pyr), 7.22 (m, 1H, H-4 Pyr), 2.82 (m, 4H, NCH_2), 2.61 (t, 2H, $PyrCH_2$), 2.18 (bs, 1H, NH), 1.52 (m, 2H, CH_2), 0.90 (t, 3H, CH_3). Anal $C_{10}H_{16}N_2$ (C, H, N).

N-n-Propyl-2-(2-methoxy-3-pyridyl)ethylamine hydrochloride ***13b***

Compound **13b** was synthesized as described for **7** starting from amide **11** (2.92 g, 14 mmol), $NaBH_4$ (2.65 g, 70 mmol) in dry THF (60 ml), acetic acid (4.07 ml, 70 mmol) in dry THF (20 ml), and CH_3OH (35 ml). The residue was dissolved in anhydrous EtOH and HCl gas was bubbled into the solution. The precipitate was collected and recrystallized from anhydrous EtOH: mp 132–134°C; yield 95%. ^1H NMR (Me_2SO-d_6) δ 10.51 (bs, 1H, NH^+), 9.52 (bs, 2H, NH_2^+), 8.22 (d, 1H, H-6 Pyr), 7.16 (m, 2H, H-3,5 Pyr), 3.98 (s, 3H, OCH_3), 3.13 (m, 4H, NCH_2), 2.82 (m, 2H, $PyrCH_2$), 1.70 (m, 2H, CH_2), 0.91 (t, 3H, CH_3). Anal $C_{11}H_{18}N_2O \cdot 2HCl$ (C, H, N).

N-n-Propyl-N-[2-(3-pyridyl)ethyl]propanamide ***14a***

Compound **14a** was synthesized as described for **11** starting from **13a** (0.9 g, 5.5 mmol), triethylamine (1.1 ml, 8 mmol) in dry THF (15 ml), and propionyl chloride (0.71 ml, 8 mmol) in dry THF (10 ml). The residue was chromatographed on silica gel eluting with $CHCl_3$/MeOH (9:1, TLC: R_f = 0.49) to afford an oil; yield 78%. IR (nujol) 1640 cm^{-1} (C=O). ^1H NMR ($CDCl_3$) δ 8.42 (m, 2H, H-2,6 Pyr), 7.55 (m, 1H, H-5 Pyr), 7.20 (m, 1H, H-4 Pyr), 3.48 (m, 2H, NCH_2), 3.30 and 3.10 (2m, 2H, NCH_2), 2.82 (m, 2H, $PyrCH_2$), 2.31 and 2.15 (2q, 2H, $COCH_2$), 1.53 (m, 2H, CH_2), 1.02 (t, 3H, CH_3), 0.89 (t, 3H, CH_3). Anal $C_{13}H_{20}N_2O$ (C, H, N).

N-n-Propyl-N-[2-(2-methoxy-4-pyridyl)ethyl]propanamide ***14b***

Compound **14b** was synthesized as described for **11** starting from **13b** (1.5 g, 7.8 mmol), triethylamine (1.08 ml, 7.8 mmol) in dry Et_2O (25 ml), and propionyl chloride (0.67 ml, 7.8 mmol) in dry Et_2O (20 ml). The residue was chromatographed on silica gel eluting with ethyl acetate (TLC: R_f = 0.41) to afford an oil; yield 57%. IR (nujol) 1640 cm^{-1} (C=O). ^1H NMR ($CDCl_3$) δ 8.10 (m, 1H, H-6 Pyr), 6.73 (m, 1H, H-5 Pyr), 6.58 (m, 1H, H-3 Pyr), 3.93 (s, 3H, OCH_3), 3.50 (m, 2H, NCH_2), 3.30 and 3.11 (2m, 2H, NCH_2), 2.80 (m, 2H, $PyrCH_2$), 2.32 and 2.23 (2q, 2H, $COCH_2$), 1.56 (m, 2H, CH_2), 1.17 and 1.10 (2t, 3H, CH_3), 0.90 (t, 3H, CH_3). Anal $C_{14}H_{22}N_2O_2$ (C, H, N).

N-n-Propyl-N-[2-(3-pyridyl)ethyl]-2-phenylethanamide ***15a***

Compound **15a** was synthesized as described for **11** starting from **13a** (0.9 g, 5.5 mmol), triethylamine (1.1 ml, 8 mmol) in dry THF (10 ml), and phenylacetyl chloride (1.1 ml, 8 mmol) in dry THF (5 ml). The residue was chromatographed on silica gel eluting with $CHCl_3$/MeOH (9:1, TLC: R_f = 0.53) to afford an oil; yield 80%. IR (nujol) 1640 cm^{-1} (C=O). ^1H NMR ($CDCl_3$) δ 8.41 (m, 2H, H-2,6 Pyr), 7.50 (m, 1H, H-5 Pyr), 7.26 (m, 6H, ArH, H-4 Pyr), 3.68 and 3.57 (2s, 2H, $COCH_2$), 3.50 and 3.45 (2m, 2H, NCH_2), 3.32 and 3.10 (2m, 2H, NCH_2), 2.85 and 2.68 (2m, 2H, $PyrCH_2$), 1.52 (2m, 2H, CH_2), 0.90 and 0.82 (2t, 3H, CH_3). Anal $C_{18}H_{22}N_2O$ (C, H, N).

N-n-Propyl-N-[2-(2-methoxy-4-pyridyl)ethyl]-2-phenylethanamide 15b
Compound **15b** was synthesized as described for **11** starting from **13b** (2 g, 10.2 mmol), triethylamine (2.8 ml, 20 mmol) in dry Et_2O (20 ml), and phenylacetyl chloride (2 ml, 15 mmol) in dry Et_2O (10 ml). The residue was chromatographed on silica gel eluting with ethyl acetate/cyclohexane (8:2, TLC: R_f = 0.49) to afford an oil; yield 62%. IR (nujol) 1640 cm^{-1} (C=O). ^1H NMR ($CDCl_3$) δ 8.09 and 8.0 (2d, 1H, H-6 Pyr), 7.13 (m, 5H, ArH), 6.69 and 6.60 (2m, 1H, H-5 Pyr), 6.57 and 6.47 (2d, 1H, H-3 Pyr), 3.68 and 3.59 (2s, 2H, $COCH_2$), 3.54 and 3.46 (2m, 2H, NCH_2), 3.31 and 3.10 (2m, 2H, CH_2), 2.80 and 2.61 (2m, 2H, $PyrCH_2$), 1.52 (m, 2H, CH_2), 0.90 and 0.85 (2t, 3H, CH_3). Anal $C_{19}H_{24}N_2O_2$ (C, H, N).

N,N-Di-n-propyl-2-(3-pyridyl)ethylamine 16a
A distillation apparatus with a 10 ml flask, a septum capped inlet and a Vigreux column, connected to a source of nitrogen, was charged with the amide **14a** (0.95 g, 4.3 mmol) and dry THF (2 ml). Boron trifluoride etherate (0.53 ml, 4.3 mmol) was added and the mixture was heated under reflux. When the solution became clear, borane-dimethylsulfide (0.33 ml, 3.1 mmol) was slowly added over a period of 10 min. The liberated dimethylsulfide and Et_2O were distilled off. After 1 h the solvent was removed *in vacuo* and the residue was heated at 100°C for 1 h. Then 6 N HCl (0.7 ml) was added and the mixture was heated under reflux for 1 h. The solution was cooled to 0°C and 6 N NaOH (1.1 ml) was added. The aqueous layer was saturated with sodium carbonate and extracted with $CHCl_3$. The organic extracts were dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness to give an oil; yield 72%. ^1H NMR ($CDCl_3$) δ 8.44 (m, 2H, H-2,6 Pyr), 7.50 (m, 1H, H-5 Pyr), 7.19 (m, 1H, H-4 Pyr), 2.68 (m, 4H, NCH_2), 2.40 (m, 4H, CH_2), 1.41 (m, 4H, CH_2), 0.82 (t, 6H, CH_3).
A solution of oxalic acid dihydrate in EtOH was added to the residue dissolved in EtOH. The precipitate was filtered and recrystallized from anhydrous EtOH: mp 159–161°C. Anal $C_{13}H_{22}N_2 \cdot 2C_2H_2O_4$ (C, H, N).

N,N-Di-n-Propyl-2-(2-methoxy-4-pyridyl)ethylamine 16b
A solution of the amide **14b** (1 g, 3.9 mmol) in dry THF (10 ml) was added to a suspension of $LiAlH_4$ (0.16 g, 4.2 mmol) in dry THF (30 ml). The mixture was heated to reflux for 32 h by adding $LiAlH_4$ (4.2 mmol) every 8 h. The mixture was cooled at room temperature and excess $LiAlH_4$ was quenched by successive dropwise additions of 0.6 ml H_2O, 0.6 ml 15% NaOH, and 1.8 ml H_2O. The mixture was filtered, the precipitate was washed with THF. The solution was dried (anhydrous Na_2SO_4), filtered, and evaporated. The oily residue was chromatographed on silica gel with $CHCl_3$/MeOH 9:1, (TLC: R_f = 0.5) as eluent; yield 81%. ^1H NMR ($CDCl_3$) δ 8.03 (d, 1H, H-6 Pyr), 6.72 (m, 1H, H-5 Pyr), 6.58 (d, 1H, H-3 Pyr), 3.91 (s, 3H, OCH_3), 2.68 (s, 4H, CH_2), 2.42 (m, 4H, NCH_2), 1.46 (m, 4H, CH_2), 0.85 (t, 6H, CH_3). Anal $C_{14}H_{24}N_2O$ (C, H, N).

N,N-Di-n-propyl-2-(2-hydroxy-4-pyridyl)ethylamine 16c
Compound **16c** was synthesized as described for **10** starting from **16b** (0.65 g, 2.7 mmol), 48% HBr (5 ml) and acetic acid (5 ml). A saturated solution of sodium carbonate was added to the residue obtained after evaporation of the solvent. The solution was extracted with $CHCl_3$. The organic layer was dried (anhydrous Na_2SO_4), filtered and evaporated to dryness to give an oil: yield 65%. ^1H NMR ($CDCl_3$) δ 13.38 (bs, 1H, NH), 7.25 (d, 1H, H-6 Pyr), 6.38 (s, 1H, H-3 Pyr), 6.17 (d, 1H, H-5 Pyr), 2.60 (m, 4H, CH_2), 2.38 (m, 4H, CH_2), 1.39 (m, 4H, CH_2), 0.80 (t, 6H, CH_3).

A saturated solution of maleic acid in EtOH was added to the residue dissolved in EtOH. The precipitate was decanted, triturated, washed with EtOH, and dried over P_2O_5 to give an amorphous uncrystallizable solid. Anal $C_{13}H_{22}N_2O \cdot C_4H_4O_4 \cdot H_2O$ (C, H, N).

N-n-Propyl-N-(2-phenylethyl)-2-(3-pyridyl)ethylamine 17a
Compound **17a** was synthesized as described for **16a** starting from **15a** (2.63 g, 9.3 mmol) in dry THF (3 ml), boron trifluoride etherate (1.15 ml, 9.3 mmol), borane-dimethylsulfide (0.33 ml, 3.1 mmol), 6 N HCl (1.5 ml), and 6 N NaOH (2.4 ml); oil; yield 75%. ^1H NMR ($CDCl_3$) δ 8.44 (m, 2H, H-2,6 Pyr), 7.47 (m, 1H, H-5 Pyr), 7.22 (m, 6H, ArH, H-4 Pyr), 2.74 (m, 8H, NCH_2), 2.53 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 0.90 (t, 3H, CH_3).
The oxalate was prepared as for **16a** and recrystallized from anhydrous EtOH: mp 174–176°C. Anal $C_{18}H_{24}N_2 \cdot 2C_2H_2O_4$ (C, H, N).

N-Propyl-N-(2-phenylethyl)-2-(2-methoxy-4-pyridyl)ethylamine 17b
Compound **17b** was synthesized as described for **16b** starting from **15b** (0.7 g, 2.2 mmol) in dry THF (10 ml), $LiAlH_4$ (0.09 g, 2.2 mmol) in dry THF (15 ml). The mixture was heated to reflux for 32 h, by adding $LiAlH_4$ (2.2 mmol) every 8 h. The mixture was cooled at room temperature and the excess $LiAlH_4$ was quenched by successive dropwise additions of H_2O (1.2 ml), 15% NaOH (1.2 ml), and H_2O (3.6 ml). The oily residue was chromatographed on silica gel with ethyl acetate as eluent (TLC: R_f = 0.48); yield 91%. ^1H NMR ($CDCl_3$) δ 8.04 (d, 1H, H-6 Pyr), 7.21 (m, 5H, ArH), 6.71 (m, 1H, H-5 Pyr), 6.58 (d, 1H, H-3 Pyr), 3.92 (s, 3H, OCH_3), 2.73 (m, 8H, $4CH_2$), 2.52 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 0.91 (t, 3H, CH_3). Anal $C_{19}H_{26}N_2O$ (C, H, N).

N-n-Propyl-N-(2-phenylethyl)-2-(2-hydroxy-4-pyridyl)ethylamine 17c
Compound **17c** was synthesized as described for **16c** starting from **17b** (1 g, 3.3 mmol), 48% HBr (10 ml) and acetic acid (10 ml). The oily residue was purified by column chromatography with $CHCl_3$/hexane/CH_3OH (90:5:5, TLC: R_f = 0.23) as eluent; yield 75%. ^1H NMR ($CDCl_3$) δ 12.47 (s, 1H, NH), 7.21 (m, 6H, ArH, H-6 Pyr), 6.39 (s, 1H, H-3 Pyr), 6.08 (d, 1H, H-5 Pyr), 2.72 (m, 6H, CH_2), 2.50 (m, 4H, CH_2), 1.48 (m, 2H, CH_2), 0.88 (t, 3H, CH_3).
The maleate was prepared in the same manner as for **16c** to give an amorphous uncrystallizable solid. Anal $C_{13}H_{22}N_2O \cdot C_4H_4O_4 \cdot H_2O$ (C, H, N).

N,N-Di-n-Propyl-2-(4-pyridyl)ethylamine 18
The synthetic method described by Reich *et al* was followed [13]: bp 80–81°C/0.1 mm Hg; yield 36%. ^1H NMR ($CDCl_3$) δ 8.48 (m, 2H, H-2,6 Pyr), 7.12 (m, 2H, H-3,5 Pyr), 2.68 (m, 4H, CH_2), 2.41 (m, 4H, CH_2), 1.42 (m, 4H, CH_2), 0.85 (t, 6H, CH_3).
The maleate was prepared in the same manner as for **16c** and recrystallized from ethyl acetate: mp 123–125°C. Anal $C_{13}H_{22}N_2 \cdot 2C_4H_4O_4 \cdot H_2O$ (C, H, N).

N-n-Propyl-N-(2-feniletil)-2-(4-pyridyl)ethylamine 19
Compound **19** was synthesized as described for **18** starting from 4-vinylpyridine (2.1 g, 20 mmol), *N*-propyl-*N*-(2-phenylethyl)amine (3.32 g, 20 mmol) and acetic acid (1 ml): bp 118–125°C/0.05 mm Hg; yield 36%. ^1H NMR ($CDCl_3$) δ 8.45 (m, 2H, H-2,6 Pyr), 7.20 (m, 5H, ArH), 7.08 (m, 2H, H-3,5 Pyr), 2.73 (m, 8H, CH_2), 2.51 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 0.88 (t, 3H, CH_3).

The maleate was prepared in the same manner as for **16c** and recrystallized from ethyl acetate: mp 92–94°C. Anal $C_{13}H_{22}N_2 \cdot 2C_4H_4O_4 \cdot H_2O$ (C, H, N).

Radioligand-binding studies

Male Sprague–Dawley rats (250–300 g body weight) were obtained from Charles River (Calco, Italy). [^{3}H]Spiperone (specific activity 42 Ci/mmol) and [^{3}H]SCH 23390 (specific activity 70 Ci/mmol) were purchased from Amersham Radiochemical Centre (Buckinghamshire, UK). Quinpirole, PPHT, SCH 23390 and (+)-butaclamol hydrochloride were obtained from Research Biochemicals International (Natick, MA, USA).

Rats were killed by decapitation and brains were removed and frozen at –40°C. Sections of the striatum (7 μm thick) were cut serially at –26°C using a microtome cryostat, mounted on pre-weighed gelatin-coated microscope slides which were air-dried and stored at –20°C until the binding assay. Sections were thawed and washed in the recently reported assay buffers [19]. Sections were then incubated for 45 min at 25°C in assay buffer containing [^{3}H]SCH 23390 (0.25 nM) or [^{3}H]Spiperone (0.25 nM), to label D_1 and D_2 receptors, respectively, and in the presence of various concentrations of the compounds tested. Incubation of some compounds was performed with [^{3}H]SCH 23390 plus 100 μM GTP or with [^{3}H]Spiperone plus 100 mM NaCl to identify a possible DA receptor agonist or antagonist activity [17, 18]. Non-specific binding was defined by adding a 1 μM concentration of SCH 23390 to the incubation medium for DA D_1 receptor labelling and a 1 μM concentration of (+)-butaclamol for DA D_2 receptor labelling. After incubation, sections were washed in ice-cold (4°C) incubation buffer (2 x 5 min) and wiped onto scintillation vials. The radioactivity was counted in a liquid scintillation spectrometer at an efficiency of 40%. Specific binding was defined as the difference between total and nonspecific binding (with and without [^{3}H]drug). K_i values were calculated according to the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + L/K_D)$ with L the concentration and K_D the apparent dissociation constant of [^{3}H]ligand obtained from Scatchard analysis of saturation experiments [20]. The K_i value was determined at least in duplicate with nine concentrations of each drug in triplicate.

Competitor displacement curves in the presence or in the absence of GTP and Na^+ were evaluated by quantitative interactive non-linear regression computer-fitted analysis. Radioligand-binding experiments were performed on slide-mounted sections of rat neostriatum instead of membrane preparations in order to have enough tissue samples available from a single animal for several assays. In a series of preliminary experiments no important differences in the affinity or density of [^{3}H]SCH 23390 or [^{3}H]Spiperone binding sites were noticeable using slide-mounted sections or membrane preparations of rat neostriatum (data not shown).

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