Synthesis and Serotonergic Activity of Arylpiperazide Derivatives of Serotonin: Potent Agonists for 5-HT_{1D} Receptors

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A series of new arylpiperazide derivatives of serotonin has been prepared and evaluated as 5-HT_{1D} receptor agonists. Binding experiments at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, and 5-HT_{1A} receptors show that all the compounds are very potent and selective ligands for 5-HT_{1D} receptor subtypes. Functional activity studies (contraction of the New Zealand white rabbit saphenous vein) demonstrate that most of the derivatives behave as full agonists. Among them, the aryl sulfonamide derivative **5q** (pD_2 = 8.33 compare to 5.75 for sumatriptan) was also identified as a very potent agonist in inhibiting the forskolin-mediated cyclase coupled to 5-$HT_{1D\beta}$ receptors (EC_{50} = 0.52 nM).

A migraine is a common neurological disorder that can severely affect quality of life and daily functioning. Serotonin (**1**, Figure 1) has long been implicated in the pathophysiology of migraine,[1] and the recent discovery that a selective 5-HT_{1D} receptor agonist such as sumatriptan (**2**) is a highly effective, rapid-acting, and well-tolerated drug for the treatment of acute attacks of migraine[2] has stimulated extensive research efforts in this area. Very recently, new 5-HT_{1D} receptor agonists such as **3** (MK-462) and **4** (311C90) have also been reported[3] to be clinically effective in the treatment of migraine headache, thus confirming the therapeutic potential of this class of compounds. The 5-HT_{1D} receptors belong to the 5-HT_1 subfamily of serotonin receptors[4] and have been recently divided into two further receptor subtypes, both negatively coupled to adenylate cyclase and identified in human tissues as 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors on the basis of molecular biology and cloning studies.[5] To date, no clear functional distinction between 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes has been reported, and their respective importance in migraine pathology remains to be determined.[6]

From a structural point of view, it is noteworthy that, with the noticeable exception of SR27592,[7] almost all potent and selective 5-HT_{1D} agonists reported to date are tryptamine derivatives substituted in position 5 of the indole nucleus. Extensive work has subsequently been done in designing conformationally restricted analogs of the amino ethyl side chain of tryptamine derivatives, leading to promising new selective 5-HT_{1D} agonists like naratriptan,[8] BRL56905,[9] or CP-122,288.[10]

From a synthetic point of view, 5-substituted tryptaminergic 5-HT_{1D} agonists can be divided into 5-C-, 5-N-, and 5-O-alkylated derivatives; the synthesis and pharmacological properties of 5-C-alkylated tryptamine derivatives have been extensively studied as exemplified not only by the three antimigraine agents **2**, **3**, or **4** but also by numerous 5-heterocyclic-substituted tryptamine derivatives including L694,247.[11] Recently, some 5-N-substituted tryptamine derivatives incorporating a nitropyridyl moiety,[12] as well as some 5-O alkylated tryptamine derivatives including S-CM-$GTNH_2$[13] and NOT,[14] have also been reported as potent 5-HT_{1D} agonists. The latter molecules are attractive since they can be synthesized from serotonin itself. This, of course, differs considerably from the synthetic chemistry required to prepare 5-C-alkylated tryptamine derivatives.

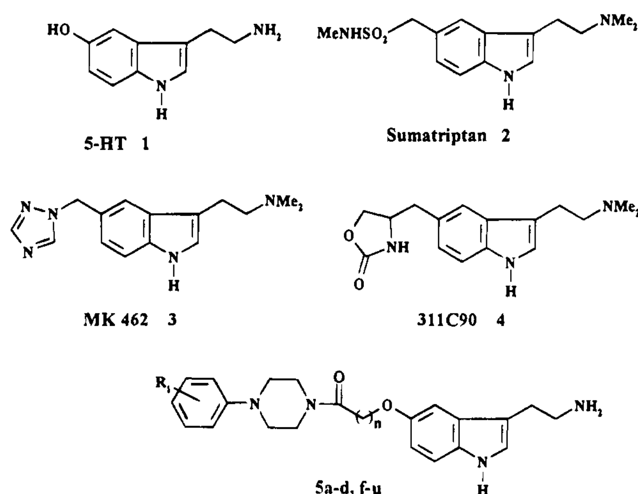

Figure 1.

During the course of our studies concerning the identification of new, potent, and selective 5-HT_{1D} agonists, we have considered the introduction of an arylpiperazide moiety as a 5-O substituent on serotonin in order to design new, easily prepared, potent, and selective 5-HT_{1D} agonists. We describe in this paper[15] the synthesis of a series of 5-arylpiperazide derivatives of serotonin of formula **5**. In order to explore the structural requirements to achieve high affinity and selective 5-HT_{1D} binding, we have studied changes in the arylpiperazide substituent (R_1) and the length of the linking chain (n). The pharmacological properties of these compounds have been compared to sumatriptan, MK462, and 311C-C90 at the cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, and 5-HT_{1A} receptors, and their agonist potency has been determined in a functional assay based on 5-HT_{1D} receptor-mediated contractions in the isolated rabbit saphenous vein.

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Table 1. Binding Profile at Cloned Human Receptors and Functional Activity of Compounds 1–5

compd	R^1	n	K_i (nM)[a] 5-HT1D[α]	5-HT1D[β]	5-HT1A	vein contraction pD_2 (CI 95%)[b]	rel max[c]
5a	H	1	1.2	0.4	10.4	7.36 (7.22–7.50)	0.68 ± 0.22
5b	o-Me	1	2.08	0.8	8.0	5.86 (5.40–6.35)	1.01 ± 0.19
5c	o-NO_2	1	0.58	0.30	5.2	6.33 (6.00–6.66)	0.87 ± 0.17
5d	o-CN	1	0.92	0.39	15.0	7.42 (7.17–7.64)	1.22 ± 0.17
5f	p-NO_2	3	1.2	5.0	15.1	6.91 (6.38–7.31)	1.06 ± 0.18
5g	p-NO_2	1	0.42	0.83	24	6.12 (5.54–6.64)	0.52 ± 0.15*
5h	p-NO_2	4	2.5	1.8	15.9	6.74 (6.26–7.16)	0.71 ± 0.17*
5i	p-NO_2	5	0.34	0.47	12.8	7.00 (6.37–7.55)	0.73 ± 0.12
5j	p-NH_2	1	2.92	1.64	607	6.88 (6.60–7.08)	0.83 ± 0.10
5k	p-NH_2	4	1.4	1.4	50.7	7.05 (6.79–7.28)	0.73 ± 0.11*
5l	p-NH_2	5	0.38	0.31	42.6	8.09 (7.78–8.37)	0.79 ± 0.13
5m	p-NHCOPh	1	1.7	1.3	61	7.31 (7.17–7.46)	0.90 ± 0.11
5n	p-NHCOMe	1	1.4	2.8	77.8	6.67 (6.51–6.85)	1.01 ± 0.14
5o	p-$NHSO_2Me$	1	0.56	2.7	68.9	7.62 (7.39–7.80)	1.21 ± 0.11
5p	p-$NHSO_2NMe_2$	1	0.26	1.9	40.3	7.20 (6.88–7.51)	0.96 ± 0.16
5q	p-$NHSO_2Me$	5	1.30	1.4	10.2	8.33 (7.84–8.65)	1.05 ± 0.18
5r	p-CH_2NH_2	1	0.93	2.5	130	6.80 (6.47–7.14)	0.92 ± 0.18
5s	o-CH_2NH_2	1	6.9	1.8	13.6	6.52 (6.26–6.80)	0.90 ± 0.13
5t	p-CH_2NHSO_2Me	1	1.0	2.2	95	6.92 (6.76–7.09)	0.88 ± 0.18
5u	o-CH_2NHSO_2Me	1	1.8	0.53	5.1	6.00 (5.45–6.52)	0.93 ± 0.20
1	5-HT		4.8	7.1	2.5	6.95 (6.85–7.06)	1.00 ± 0.05
2	sumatriptan		8.5	23.1	440	5.75 (5.68–5.87)	1.26 ± 0.06*
3	MK462		13.5	40	293	6.15 (6.04–6.26)	1.42 ± 0.11*
4	311C90		0.92	4	78.6	6.13 (5.91–6.37)	1.48 ± 0.08*

[a] K_i values are given as mean of one or two independent experiments each performed in duplicate. [b] Contraction of the New Zealand white rabbit saphenous vein with confidence interval at 95%. [c] Maximum contraction obtained relative to 5-HT (mean ± SEM);* $p < 0.05$ versus 5-HT.

Table 2. Physical Properties of Compounds Listed in Table 1

compd	mp,[a] °C	formula[b]	anal.[c]
5a	229	$C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot H_2O \cdot 0.7MeOH$	C,H,N
5b	146–148	$C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 1.8H_2O$	C,H,N,Cl*
5c	130	$C_{22}H_{25}N_5O_4 \cdot HCl \cdot 1.3H_2O$	C,H,N,Cl
5d	107	$C_{23}H_{25}N_5O_2 \cdot HCl \cdot 1.4H_2O$	C,H,N,Cl
5f	120	$C_{24}H_{29}N_5O_4 \cdot HCl \cdot H_2O$	C,H,N,Cl*
5g	225	$C_{22}H_{25}N_5O_4 \cdot HCl \cdot H_2O$	C,H,N,Cl
5h	120	$C_{25}H_{31}N_5O_4 \cdot HCl \cdot 1.7H_2O$	C,H,N,Cl*
5i	100	$C_{26}H_{33}N_5O_4 \cdot 1.1HCl \cdot H_2O$	C,H,N,Cl
5j	196	$C_{22}H_{27}N_5O_2 \cdot 3HCl \cdot 2H_2O$	C,H,N
5k	170	$C_{25}H_{33}N_5O_2 \cdot 3HCl \cdot 1.5H_2O$	C,H,N,Cl*
5l	150	$C_{26}H_{35}NO_2 \cdot 3HCl \cdot 1.4H_2O$	C,H,N,Cl*
5m	184–185	$C_{29}H_{31}N_5O_3 \cdot 2HCl \cdot 2H_2O$	C,H,N
5n	171	$C_{24}H_{29}N_5O_3 \cdot 2HCl \cdot 1.8H_2O$	C,H,N,Cl
5o	229	$C_{23}H_{29}N_5O_4S \cdot 2HCl \cdot 0.8H_2O$	C,H,N,Cl
5p	170	$C_{24}H_{32}N_6O_4S \cdot 2HCl \cdot 1.5H_2O$	C,H,N,Cl
5q	141	$C_{27}H_{37}N_5O_4S \cdot 2HCl \cdot 0.5H_2O \cdot 0.3EtOH$	C,H,N,Cl
5r	185	$C_{23}H_{29}N_5O_2 \cdot 3HCl \cdot 1.5H_2O$	C,H,N,Cl
5s	198	$C_{23}H_{29}N_5O_2 \cdot 3HCl \cdot 1.6H_2O$	C,H,N,Cl*
5t	154	$C_{24}H_{31}N_5O_4S \cdot 2HCl \cdot 0.3H_2O$	C,H,N,Cl*
5u	250	$C_{24}H_{31}N_5O_4S \cdot 1.5HCl \cdot H_2O$	C,H,N,Cl

[a] All compounds were crystallized from CH_2Cl_2/Et_2O or $CHCl_3/Et_2O$. [b] Satisfactory ^1H-NMR were obtained for all compounds. [c] The analyses are within ±0.4% of the theoretical values (compounds with an asterisk (*) do not agree with calculated values for Cl possibly due to the accuracy of the method used).

Chemistry

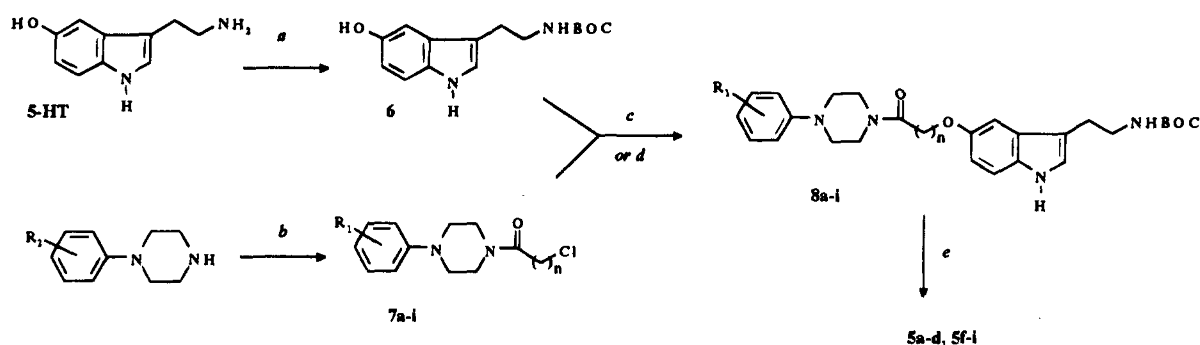
All the compounds of formula **5** (Table 1) have been prepared through a linear sequence from serotonin itself.

The synthesis of compounds **5a–d,f–i** (Scheme 1) starts by treating the commercially available creatinine salt of 5HT with $(BOC)_2O$/NaOH to give *N*-BOC-serotonin **6** in 89% yield. This intermediate was then condensed with the chloro amides **7a–i** (prepared by treatment of the corresponding arylpiperazine with the appropriate acid chloride ($ClCO(CH_2)_nCl$) in the presence of $CaCO_3$ in methyl ethyl ketone) to give the expected tryptamines **8a–i** by one of the two following methods: compounds **8a–g** were synthesized by refluxing the solution of *N*-BOC-serotonin **6** and the appropriate chloro amides **7a–g** in MEK (4–6h), in the presence of K_2CO_3 as a base and a catalytic amount of KI. Intermediates **8h,i** were prepared by heating a solution of **6** and **7h,i** in DMF (24–66 h) using Cs_2CO_3 as a base. Finally, removal of the BOC protecting group upon reaction with excess TFA in toluene at room temperature afforded the arylpiperazide derivatives of serotonin **5a–d,f–i**. The nitro derivatives **8g–i** were found to be very interesting synthetic intermediates since, upon reduction by catalytic hydrogenation (hydrogen over Pd/C), they are easily transformed into the corresponding aniline derivatives **8j–l**. These compounds were then deprotected in the conditions described above to give the final products **5j–l** or, alternatively, they are used to further modify the arylpiperazine moiety as illustrated in Scheme 2. Thus, treatment of intermediate **8j** with methanesulfonyl chloride in pyridine afforded **8o** (92% yield). As demonstrated by ^1H-NMR analysis, the methylsulfonylation takes place exclusively at the aniline nitrogen with no sulfonylation observed at the indole nitrogen. The only byproduct (<10%) obtained under these conditions was identified as the bis-sulfonylation product at the aniline nitrogen. Within similar conditions, the aniline derivative **8j** can be transformed into the intermediates **8n**, **8m**, and **8p** after reaction with acetic anhydride (73% yield), benzoylchloride (92% yield), and dimethylsulfamoyl chloride (58%), respectively.

By analogy, the nitriles **8d,e** were also used as precursors of benzylamine derivatives **8r,s** upon catalytic hydrogenation (H_2, Raney Ni) in THF and ammonia (13/1). These compounds treated with methanesulfonyl chloride in pyridine afforded **8t,u** which upon deprotection gave **5t,u** (Scheme 3).

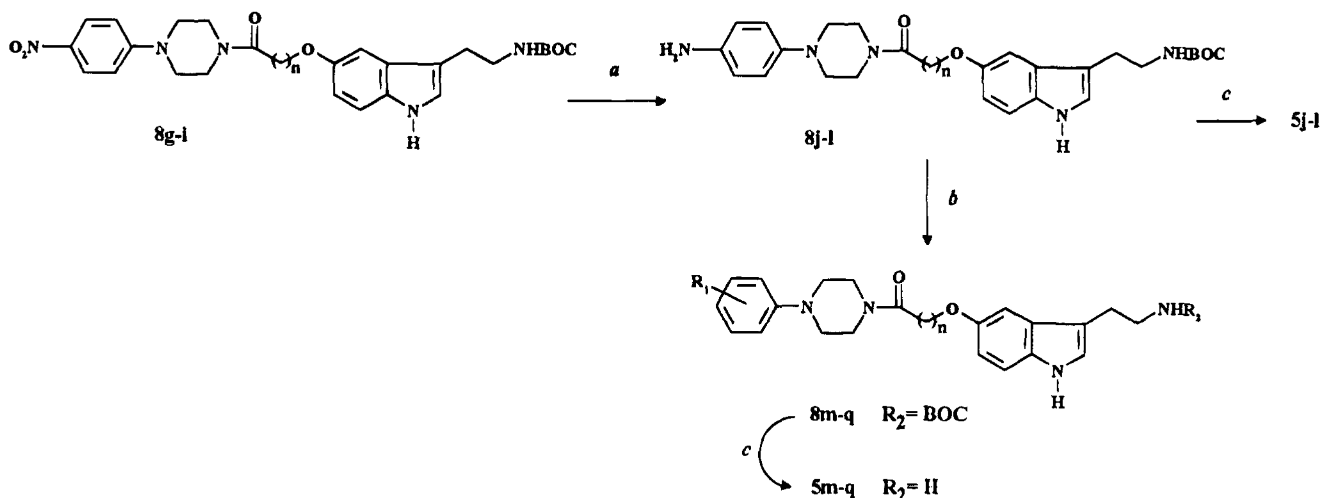
All compounds were purified by column chromatography on silica gel. Subsequent treatment of the free amines with HCl in dichloromethane afforded the

Scheme 1[a]



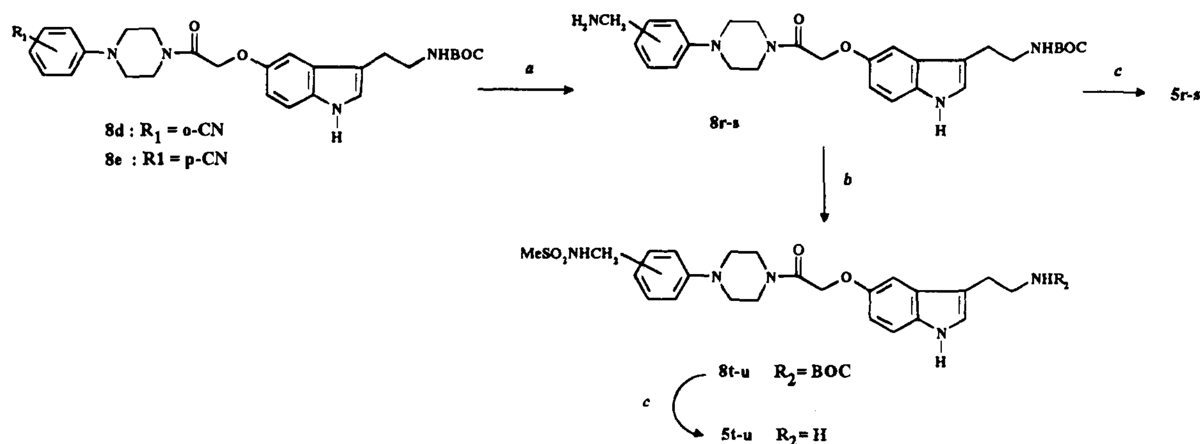
[a] Reagents and conditions: (a) $(BOC)_2O$ (1.5 equiv), NaOH, H_2O, 25 °C, 89%; (b) $ClCO(CH_2)_nCl$ (1.2 equiv), MEK, $CaCO_3$ (1.5 equiv), 0 °C, 29–99%; (c) MEK, K_2CO_3 (2.5 equiv), KI (0.1 equiv), reflux, 4–6 h, 62–93%; (d) Cs_2CO_3, DMF, 25–80 °C, 24–66 h, 42–64%; (e) TFA (excess), toluene, 25 °C, 1–2 h, 50–91%.

Scheme 2[a]



[a] Reagents and conditions: (a) H_2, Pd/C, MeOH, 59–99%; (b) PhCOCl, Ac_2O, MsCl or Me_2NHSO_2Cl, pyridine, 0 °C to room temperature, 1–12 h, 58–95%; (c) TFA (excess), toluene, 25 °C, 1 h, 69–99%.

Scheme 3[a]



[a] Reagents and conditions: (a) H_2, Raney Ni, THF/NH_4OH (13/1), 59–99%; (b) MsCl, pyridine, 0 °C to room temperature, overnight, 97–99%; (c) TFA (excess), toluene, 25 °C, 1 h, 56–93%.

hydrochloride salts of the arylpiperazides derivatives of serotonin **5** suitable for biological evaluation.

Results and Discussion

Structure–Affinity Relationships. The binding affinities of compounds **5** and reference compounds have been measured at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, and 5-HT_{1A} receptors.[16,17] The results obtained are summarized in Table 1. The arylpiperazides derivatives of serotonin **5** show a very good affinity at both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, especially when compared to reference compounds including serotonin itself. The introduction of substituents (H-bond acceptors or donors) on the aromatic ring attached to the piperazine moiety has only very little influence on the binding of these compounds at 5-HT_{1D} receptors (compare **5a** to **5b–d,g,j,m–p,r–u**), a result which is opposite to the observations of an earlier SAR study concerning C-5-

substituted tryptamine derivatives where the same type of modifications leads to a dramatic increase in affinity.[11d] Similarly, modification of the chain length linking the amide to the tryptamine nucleus have only a small effect on binding (**5g** vs **5f** vs **5h** vs **5i**). On the contrary, it can be observed from the data of Table 1 that the binding selectivity (5-HT_{1D} vs 5-HT_{1A}) is extremely sensitive to the nature of the para substituent (compare **5a** to **5g,j,m−p,r,t**). This observation is not verified for the ortho position (compare **5a** to **5b,c,s,u**). Modification of the chain length has some slight but significant effect on the binding at 5-HT_{1A} receptor (compare **5g** to **5f,h,i**; **5j** to **5k,l**, **5o** to **5q**); as a general trend, an increase of the chain length slightly increases the affinity at these receptors. In considering the binding aspect only, compound **5l** is among the best 5-HT_{1D} ligands described to date with a pK_i of 9.5 (for both 5-HT_{1D} receptor subtypes) and a selectivity over 5-HT_{1A} of 142-fold; interestingly enough, compound **5j** which is also a (*p*-aminoaryl)piperazide derivative is the most selective 5-$HT_{1D\beta}$ ligand of the series (selectivity = 370).

Functional Activity. The 5-HT_{1D} ligands **5**, sumatriptan, MK462, 311C90, and 5-HT have been tested in the *in vitro* New Zealand white rabbit saphenous vein contraction model[18] in order to evaluate their agonist potency. When comparing the maximum effect observed for the compounds of this series with 5-HT, it can be noticed that some compounds behave as partial agonists (**5a,g**) while other compounds (**5b−f** and **5m−u**) were found to be full agonist.[19] Most of these compounds show a very high potency, as, for example, compound **5q** (pD_2 = 8.33) which is about 2600-fold more potent than sumatriptan and 150-fold more potent than MK462 or 311C90 (under similar conditions). Analysis of the functional activity from a structural point of view shows that changing R_1 from *p*-NO_2 to *p*-NH_2 increases the potency of about 1 order of magnitude (compare **5g** to **5j** and **5i** to **5l**), and moreover, changing R_1 from *p*-NH_2 to *p*-$NHSO_2Me$ improves again the potency in the two series (n = 1 or 5; compare **5j** to **5o** and **5l** to **5q**). The intrinsic activity of the two best compounds of this series, **5o** and **5q**, was also assessed for their ability to inhibit the forskolin-stimulated activity of adenylate cyclase coupled to human 5-$HT_{1D\beta}$ receptors in CHO-K_1 cells. Compounds **5o** and **5q** were found to be highly efficient 5-$HT_{1D\beta}$ receptor agonists with EC_{50} values of 6.5 and 0.52 nM, respectively. This represents again a clear improvement when compared to the EC_{50} values for sumatriptan (40 nM), MK462 (105 nM), and 311C90 (15 nM) under similar conditions.

Conclusions

A new class of highly potent 5-HT_{1D} receptor agonists is described. The synthesis takes advantage of the use of serotonin as starting material to obtain the piperazide derivatives through a short sequence. Binding affinity of compounds of formula **5** is only slightly altered by substitution of the arylpiperazine moiety (R_1) or by modification of the linker chain length (n), possibly suggesting that a deep pocket is available in the binding domain of the 5-HT_{1D} receptors. On the contrary, binding selectivity (versus 5-HT_{1A}) and intrinsic activity (as measured by the ability of compounds to contract rabbit saphenous vein) are highly dependent on these structural features, altogether most of the new arylpiperazide derivatives of serotonin **5** compare very favorably with reference compounds as 5-HT_{1D} agonists.

Experimental Section

Melting points were recorded on a electrothermal 9200 apparatus and were uncorrected. ^{1}H NMR spectra were obtained on a Bruker AC200 (200 MHz) instrument. IR spectra were obtained on a Nicolet FT510P. Mass spectra were recorded on a Nermag R10-10B spectrometer. Purification by chromatography refers to flash chromatography on silica gel (0.04−0.063 mm supplied by S.D.S.) with the eluant indicated applied at a pressure of 0.5 atm. Elemental analyses for carbon, hydrogen, and nitrogen were determined with a Fisons EA 1108/CHN instrument.

Preparation of 3-[2-[*N*-(*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-ol (6). To a solution of serotonin creatinine sulfate monohydrate (102 g, 252 mmol) in H_2O (2.1 L) and NaOH 2N (420 mL) was added di-*tert*-butyl dicarbonate (82.6 g, 378 mmol) at room temperature. The mixture was stirred for 1 h, then extracted with EtOAc (3 × 1 L), and washed with H_2O. The combined organic phases were dried (Na_2SO_4) and concentrated. The crude oil was purified by chromatography (CH_2Cl_2/MeOH, 20/1) to give **6** (65.9 g, 95%): ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H, tBu), 2.86 (t, 2H, J = 8.2 Hz, CH_2), 3.45 (dt, 2H, J = 5.4 and 8.2 Hz, CH_2N), 6.56 (dd, 1H, J = 2.2 and 8.6 Hz, 6-CH), 6.80 (d, 1H, J = 2.2 Hz, 2-CH), 6.87 (t, 1H, J = 5.4 Hz, NHBOC), 7.00 (d, 1H, J = 2.2 Hz, 4-CH), 7.09 (d, 1H, J = 8.6 Hz, 7-CH), 8.57 (s, 1H, OH), 10.45 (d, 1H, J = 2.2 Hz, NH). Anal. ($C_{15}H_{20}N_2O_3$) C, H, N.

General Procedure for the Preparation of Chloro Amides 7a−i. 2-Chloro-1-[4-(4-nitrophenyl)piperazin-1-yl]acetamide (7g). A mixture of 4-(4-nitrophenyl)piperazine (30 g, 0.144 mol) and $CaCO_3$ (43.5 g, 0.432 mol) in methyl ethyl ketone (MEK) (1 L) was treated at 0 °C by chloroacetyl chloride (20.8 mL, 0.26 mol). The mixture was stirred at room temperature overnight and then diluted with EtOAc and filtered through celite. The filtrate was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The crude product (39.2 g, 95%) was used for the next step without further purification.

General Procedures for the Preparation of Serotonin Derivatives 8a−i. General Method A: 1-[2-[[3-[2-[*N*-(*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-yl]oxy]acetyl]-4-(4-nitrophenyl)piperazide (8g). This procedure was used for the preparation of **8a−g**. A mixture of *N*-BOC-serotonin **6** (21.2 g, 76.9 mmol), α-chloroamide **7g** (39.2g, ~138 mmol), K_2CO_3 (26.5 g, 192 mmol), and KI (1.2 g, 7.69 mmol) in MEK (880 mL) was refluxed for 16 h. Then the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO4), and concentrated. The crude product was chromatographed (CH_2Cl_2/acetone, 10/1) to give **8g** (35.2 g, 87%): ^{1}H NMR (DMSO-d_6) δ 1.34 (s, 9H, tBu), 2.71 (t, 2H, J = 7.8 Hz, CH_2), 3.14 (dt, 2H, J = 5 and 7.8 Hz, CH_2N), 3.51−3.60 (m, 8H, CH_2), 4.79 (s, 2H, CH_2O), 6.75 (dd, 1H, J = 2 and 8.7 Hz, 6-CH), 6.88 (t, 1H, J = 5 Hz, NHBOC), 6.99−7.04 (m, 4H, Ar), 7.08 (d, 1H, J = 2 Hz, 4-CH), 7.20 (d, 1H, J = 8.7 Hz, 7-CH), 8.06 (d, 2H, J = 9.3 Hz, 3′,5′-CH), 10.65 (s, 1H, NH).

General Method B: 6-[[3-[2-[*N*-(*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-yl]oxy]-1-[4-(4-nitrophenyl)piperazin-1-yl]hexan-1-one (8i). This procedure was used for the preparation of **8h−i**. A mixture of compound **6** (1.87 g, 6.77 mmol) and Cs_2CO_3 (3.3 g, 10.15 mmol) in DMF (11 mL) was stirred for 15 min at room temperature and then treated with **7i** (4.14 g, ~12 mmol). The mixture was warmed at 70 °C for 24 h, diluted with EtOAc, washed with H_2O (2×) and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The crude product was chromatographed (CH_2Cl_2/EtOAc, 2/1) to give **8i** (2.52 g, 64%): ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 9H, tBu), 1.44−1.72 (m, 6H, CH_2), 2.36 (t, 2H, J = 7.0 Hz, CH_2-CO), 2.70 (t, 2H, J = 9 Hz, CH_2), 3.14 (dt, 2H, J = 4.5 and 9 Hz), 3.46 (m, 6H, CH_2N), 3.57 (m, 4H, CH_2N), 3.92 (t, 2H, J = 6.2 Hz, CH_2O), 6.67 (dd, 1H, J = 2.2 and 8.6 Hz, 6-CH), 6.84 (t, 1H, J = 4.5 Hz, NHBOC), 6.94−6.98 (m, 3H, CH), 7.04 (d,

1H, $J = 2.2$ Hz, 4-CH), 7.17 (d, 1H, $J = 8.6$ Hz, 7-CH), 8.03 (d, 2H, $J = 9.4$ Hz, 3′,5′-CH), 10.58 (s, 1H, NH).

General Procedure for the Deprotection of Compounds 8 To Give 5: 1-[2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]acetyl]-4-(4-nitrophenyl)piperazide] (5g). Compound **8g** (1.55 g, 2.96 mmol) in toluene (40 mL) was treated with excess TFA (5.3 mL) at 25 °C. After 1 h, the mixture was diluted with CH_2Cl_2, washed with NaOH (2N) and H_2O, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography ($CHCl_3$/MeOH/NH_4OH, 80/19/1) to give **5g** (839 mg, 67%) isolated as the hydrochloride salt: mp 225 °C, ^1H NMR (DMSO-d_6) δ 2.97 (m, 4H, CH_2), 3.58 (m, 8H, CH_2), 4.81 (s, 2H, CH_2O), 6.77 (dd, 1H, $J = 2.2$ and 8.6 Hz, 6-CH), 7.00 (d, 2H, $J = 9.4$ Hz, 2′,6′-CH), 7.17 (m, 2H, CH), 7.24 (d, 2H, $J = 8.8$ Hz, 7-CH), 8.05 (d, 2H, $J = 9.4$ Hz, 3′,5′-CH), 8.07 (broad s, 3H, NH_3^+), 10.84 (s, 1H, NH). Anal ($C_{22}H_{26}N_5O_4Cl{\cdot}H_2O$) C, H, N, Cl.

General Procedure for the Reduction of Nitro Compounds 8g–i To Give 8j–l: 1-[2-[[3-[2-[*N*-(*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-yl]oxy]acetyl]-4-(4-aminophenyl)piperazide] (8j). Compound **8g** (5 g, 9.55 mmol) in suspension in MeOH (300 mL) was hydrogenated over Pd/C (10%) (500 mg, 0.47 mmol) under 1 atm of H_2 at room temperature for 9 h. The mixture was filtered through Celite, and the filtrate was concentrated. The crude product was chromatographed (CH_2Cl_2/Acetone, 3/1, + 0.2% Et_3N) to give **8j** (2.78 g, 59%): ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H, tBu), 2.89 (t, 2H, $J = 7.0$ Hz, CH_2), 3.02 (m, 4H, CH_2), 3.42 (m, 2H, CH_2), 3.77 (m, 4H, CH_2), 4.63 (broad s, 1H, NH), 4.75 (s, 2H, CH_2O), 6.61–6.66 (m, 2H, Ar), 6.76–6.81 (m, 2H, Ar), 6.89 (dd, 1H, $J = 2.2$ and 8.7 Hz, 6-CH), 7.01 (d, 1H, $J = 2$ Hz, Ar), 7.09 (d, 1H, $J = 2.2$ Hz, Ar), 7.25 (d, 1H, $J = 8.7$ Hz, Ar), 8.08 (s, 1H, NH).

General Procedure for the Reduction of Nitriles 8d,e To Give 8r,s: 1-[2-[[3-[2-[*N*-(*tert*-butoxycarbonyl)amino]ethyl]-1*H*-indol-5-yl]oxy]acetyl]-4-[4-(aminomethyl)phenyl]piperazide (8r). Compound **8e** (800 mg, 1.59 mmol) in solution in a mixture of THF (30 mL) and NH_4OH (2 mL) was hydrogenated over Raney nickel (catalytic) under 1 atm of H_2 at room temperature for 1.5 day. The mixture was filtered through Celite, and the filtrate was concentrated. The crude product was chromatographed (CH_2Cl_2/MeOH/NH_4OH, 80/19/1) to give **8r** (804 mg, 99%): ^1H NMR (DMSO-d_6) δ 1.35 (s, 9H, tBu), 2.71 (t, 2H, $J = 8$ Hz, CH_2), 3.05–3.20 (m, 6H, CH_2), 3.58 (m, 4H, CH_2), 4.76 (s, 2H, CH_2O), 6.73 (dd, 1H, $J = 2.2$ and 8.7 Hz, 6-CH), 6.87 (m, 3H, Ar + NHBOC), 7.01 (d, 1H, $J = 2$ Hz, Ar), 7.07 (d, 1H, $J = 2$ Hz, Ar), 7.13–7.22 (m, 3H, Ar), 10.65 (s, 1H, NH).

General Procedure for Sulfonylation or Acylation of Compounds 8j,l,r,s To Give 8m–p,t,u: *N*-[4-[4-[2-[[3-[2-[N-(*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-yl]oxy]acetyl]piperazin-1-yl]phenyl]methanesulfonamide (8o). Compound **8j** (2 g, 4.05 mmol) in pyridine (54 mL) was treated with methanesulfonyl chloride (0.31 mL, 4.05 mmol) at 0 °C. The reaction mixture was stirred from 0 °C to room temperature for 1 h, diluted with EtOAc, washed with saturated $CuSO_4$, H_2O, and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated to give the pure compound **8o** (2.09 g, 90%): ^1H NMR (DMSO-d_6) δ 1.35 (s, 9H, tBu), 2.71 (t, 2H, $J = 7.8$ Hz, CH_2), 2.84 (s, 3H, CH_3SO_2), 3.14 (m, 6H, CH_2), 3.62 (m, 4H, CH_2), 6.74 (dd, 1H, $J = 2.3$ and 8.7 Hz, 6-CH), 6.85–6.94 (m, 3H, Ar + NHBOC), 7.02-7.10 (m, 4H, Ar), 7.20 (d, 1H, $J = 8.7$ Hz, Ar), 9.28 (s, 1H, $NHSO_2$), 10.65 (s, 1H, NH).

Biological Methods. Male New Zealand white rabbits (ESD, France) weighing 2.2–3.1 kg were killed by overdose of intravenous pentobarbital sodium (Sanofi Laboratories, France). The right and left lateral saphenous veins were cleaned of surrounding fat and connective tissue *in situ*. The veins were excised, placed in cold oxygenated Krebs-bicarbonate buffer solution, and then cut into rings approximatively 5 mm in length. The buffer solution used for preparing the vascular rings and the organ bath studies had the following composition (mM): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 D-(+)-glucose. In addition, the solution contained (M): idazoxan (10^{-6}), indomethacin (10^{-5}), ketanserin (10^{-7}), pargyline (10^{-6}), prazosin (10^{-5}) and N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10^{-5}). Each ring was suspended between two stainless steel wire hooks and mounted in an organ bath filled with 20 mL of Krebs-bicarbonate solution maintained at 37 °C and continuously gassed with 95% O_2 and 5% CO_2. Changes in isometric force were measured by means of a transducer (Statham) connected to an amplifier (Gould Instruments, France) and a computerized data acquisition system (AcqKnowledge, BIOPAC Systems, Inc., Goleta, CA). Following tension adjustments for stress relaxation and a 15 min stabilization period, tissues were successively challenged with a submaximal concentration of KCl (50 mM) and 5-HT (10^{-6} M) in order to provide a reference contraction by which subsequent agonist concentration–effect curves could be normalized. After recovery from these initial challenges, cumulative concentration–effect curves to the different agonists (1 nM–0.1 mM) were constructed. One concentration–effect curve was carried out per ring.

Calculations and Logistic Curve Fitting. Concentration–response curve fitting was performed using the nonlinear least-square algorithm of Marquardt.[20] $pD_2 = -\log EC_{50}$ where EC_{50} refers to the geometric mean agonist concentration (with 95% confidence intervals in parentheses) inducing 50% of its maximal effect. Maximum contractions were compared to 5-HT using one way analysis of variance followed by the Fisher test as posthoc test (StatView, Abacus Concepts, Inc., Berkeley, CA).

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