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Synthesis, binding affinity and intrinsic activity of new anilide derivatives of serotonin at human 5-HT_{1D} receptors

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Summary — The design and synthesis of a new series of anilide derivatives of serotonin is described. Binding affinity and intrinsic activity were evaluated at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$ and 5-HT_{1A} receptors. Modification of the terminal substituent on the aromatic moiety (R_1) was investigated and optimal affinity, activity and selectivity for 5-HT_{1D} versus 5-HT_{1A} receptors were obtained for the sulfonamide derivatives **9** and **10**. Functional activity was also assessed in the New Zealand white rabbit saphenous vein contraction model, in which most of the compounds behaved as full agonists. Further structural modifications are also described, eg, replacement of the oxygen for carbon atom at the 5-position of the tryptamine moiety or terminal *N*-dimethylation.

anilide / 5-HT_{1D} receptor agonist / serotonin / migraine / Sumatriptan

Introduction

Among the large family of serotonin (5-HT) receptors, the 5-HT_1 subfamily has probably received the most attention. The 5-HT_1 receptors appear to have the highest multiplicity with five human receptor subtypes cloned to date including 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E} and 5-HT_{1F} [1]. (Human 5-$HT_{1D\alpha}$ receptor subtype has been very recently renamed 5-HT_{1D} receptor and human 5-$HT_{1D\beta}$ receptor subtype renamed h 5-HT_{1B} [*TiPS* 17, 103, 1996].) 5-HT_{1D} receptors are promising targets for the discovery of new drug candidates since they are implicated in various important neurological disorders ranging from depression to migraine.

Migraine is a very common disease that can severely affect quality of life. Sumatriptan (**1**, fig 1) is the first (and to date the only) selective 5-HT_{1D} receptor agonist used for the acute treatment of migraine [2], which has strongly stimulated and oriented research toward the identification of more potent and selective 5-HT_{1D} ligands. The mode of action of 5-HT_{1D} receptor agonists is still unclear and debate continues on the vascular [3] versus the neurogenic [4] hypotheses of the mechanism of action of such compounds in alleviating migraine. However, of the

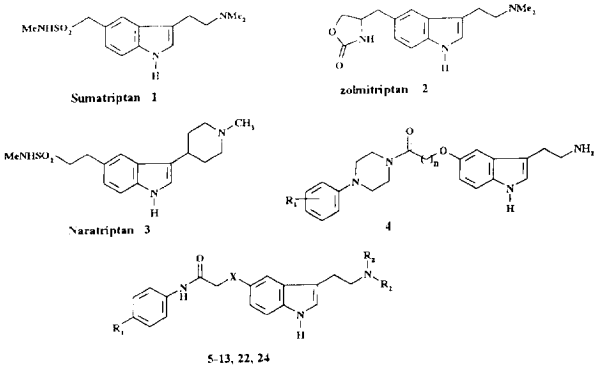

Fig 1. Structures of compounds **1**–**13**, **22** and **24**.

two human 5-HT_{1D} receptor subtypes that have been identified, the 5-$HT_{1D\beta}$ receptors appear to be more specifically involved in the vascular mechanism of migraine since human cerebral blood vessels have been shown to contain mRNA that transcripts for 5-$HT_{1D\beta}$ but not 5-$HT_{1D\alpha}$ receptors [5, 6]. On the other hand, human trigeminal ganglia possess only the 5-$HT_{1D\alpha}$ receptor mRNA which may represent a future target for more specific antimigraine drugs [7]. To date, most of the compounds described as potent 5-HT_{1D} receptor agonists do not differentiate significantly between both receptor subtypes including the three

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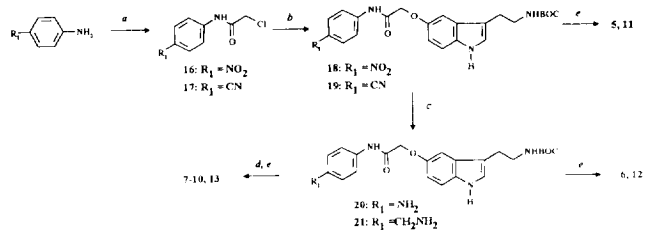
most advanced compounds (*S*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-2-oxazolidinone (zolmitriptan, **2**) [8], *N*-methyl-3-(1-methyl-4-piperidinyl)-1*H*-indol-5-ylethanesulfonamide (naratriptan, **3**) [9] a cyclic analogue of sumatriptan and *N,N*-dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethylamine (rizatriptan) [10], which are all under investigation in clinical trials. These compounds are structurally related to sumatriptan since they are all 5-C-substituted tryptamine derivatives.

As part of our program toward the identification of new, potent and selective 5-HT_{1D} receptor agonists, we have based our approach on the design of compounds which would be prepared from serotonin itself by taking advantage of the easy selective alkylation of the 5-OH residue [11]. A similar approach has been published by Glennon and coworkers who described 5-alkoxytryptamine [12] and 5-arylalkoxytryptamine [13] analogs as selective ligands at 5-$HT_{1D\beta}$ receptors.

We also recently reported a series of piperazide derivatives of serotonin (**4**) [14] as very potent and selective agonists at 5-HT_{1D} receptors. In this previous study we made the following observations: (i) the chain length between the amide and the tryptamine nucleus in derivatives of **4** has only a slight effect on binding affinity; (ii) the substitution of the terminal aromatic (R_1) was optimum on the *para* position, but the nature of the *para* substituent did not significantly influence the affinity for 5-HT_{1D} receptors; and (iii) the binding selectivity (5-HT_{1D} versus 5-HT_{1A}) was extremely sensitive to the nature of the *para* substituent. One important structural feature that has not yet been investigated is the relative importance of the piperazine ring found in derivatives of **4**. Taking advantage of the previous observations, we designed a new series of 5-*O*-substituted tryptamines and related analogs (**5–13**, **22**, **24**; fig 1) in which the linker was fixed to one methylene unit and aromatic substitution



Scheme 1. Reagents and conditions: *a*) $ClCOCH_2Cl$, MEK, $CaCO_3$, 0 to 25 °C; *b*) *N*-BOC-serotonin, MEK, K_2CO_3, KI, reflux or *N*-BOC-serotonin, DMF, Cs_2CO_3, KI, 60 °C; *c*) H_2, Pd/C, MeOH, 25 °C or H_2, Raney Ni, THF/NH_4OH, 25 °C; *d*) PhCOCl, MsCl, AC_2O, pyridine, or O_2NPhSO_2Cl, Et_3N, CH_2Cl_2, 0 to 25 °C; *e*) TFA (excess), toluene, 25 °C.

was studied at the *para* position only. The present paper describes the synthesis and pharmacological evaluation of these compounds.

Chemistry

Synthesis of compounds **5–13** (scheme 1) started with the preparation of *para*-substituted 2-chlorophenyl acetamides **16** and **17** obtained by condensing the appropriate *p*-aniline derivatives with chloroacetyl chloride. *O*-Alkylation of *N*-BOC-serotonin [14] with intermediate **16** in the presence of K_2CO_3/KI in refluxing methyl ethyl ketone gave compound **18**. Deprotection of this key intermediate (TFA/toluene) afforded compound **5**.

Catalytic hydrogenation over Pd/C of the nitro derivative **18** gave the aniline **20** in high yield, which after deprotection gave compound **6**. Tryptamines **7–10** were obtained from the aniline derivative **20** after reaction with benzoyl chloride (93%), acetic anhydride (98%) and methanesulfonyl chloride (85%) in pyridine or with 4-nitrophenylsulfonyl chloride (56%) in methylene chloride/Et_3N, followed by deprotection in the conditions described above.

Within a similar procedure, intermediate **19** was prepared by heating a solution of intermediate **17** and *N*-BOC-serotonin in DMF using Cs_2CO_3 as a base. Removal of the BOC protecting group gave compound **11**. This nitrile derivative upon catalytic hydrogenation (H_2, Raney Ni) in THF and ammonia (13:1) afforded compound **21** which was either deprotected to give anilide **12**, or treated with methanesulfonyl chloride in pyridine (70%) and deprotected to give **13**.

N,N-Dimethylation of the terminal amine of **7** using $NaCNBH_3/CH_2O$/ MeCOOH in MeOH [15] gave **22**.

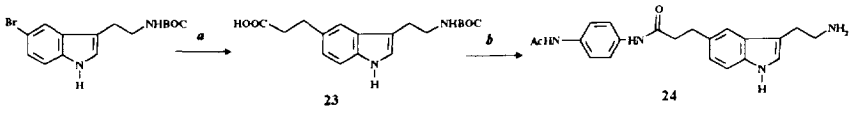
The C-5 analogue of compound **8** was prepared by first reacting methyl acrylate with *N*-BOC-5-bromotryptamine according to a Heck's procedure [16], followed by saponification and catalytic hydrogenation to give intermediate **23** (scheme 2). Condensation of the activated acid (ClCOOEt, NMM) with 4-aminoacetanilide and *N*-BOC deprotection afforded compound **24**.

All compounds were purified by column chromatography on silica gel. Subsequent treatment of the free amines with HCl in dichloromethane afforded the hydrochloride salts of the final products suitable for biological evaluation. The physical properties of compounds **5–13**, **22** and **24** are given in table I.

Pharmacology

Binding assays

The binding affinities of the described compounds and reference products (sumatriptan, zolmitriptan, naratriptan) have been measured at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$ and 5-HT_{1A} receptors [17, 18] (table II).



Scheme 2. Reagents and conditions: *a*) (i) methyl acrylate, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Et_3N, 100 °C, 86%; (ii) KOH, EtOH, reflux, 97%; (iii) H_2, Pd/C, MeOH, 96%; *b*) (i) ClCOOEt, NMM, −15 °C then 4-aminoacetanilide, 25 °C; (ii) TFA (excess), toluene, 25 °C, 1 h, 76% (two steps).

Functional activity

The functional activity of the compounds was first investigated at cloned human 5-$HT_{1D\beta}$ receptors by measuring the inhibition of forskolin-induced cAMP formation in a stably transfected CHO-K1 cell line [18]. These compounds were also tested in the in vitro New Zealand white rabbit saphenous vein contraction model [19], in order to evaluate their agonist potency (table II).

Results and discussion

As reported in table II, compounds **5–13** appear to be very potent ligands at both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (especially when compared to sumatriptan, naratriptan and zolmitriptan), with almost no selectivity between the two 5-HT_{1D} receptor subtypes (except **10** which displays a moderate but significant selectivity of sevenfold). Comparison of the binding data shows the relative importance of the nature of the *para* substituent on the affinity at 5-$HT_{1D\beta}$ receptors. Thus, sulfonylation of the terminal aniline (compare **6** to **9** or **10**) improved considerably the affinity for 5-$HT_{1D\beta}$ receptors (up to 194-fold). Surprisingly, this result is in contrast with the conclusions of our previous study concerning derivatives of **4** where we obtained very similar binding results, at 5-HT_{1D} receptors, for all the *para*-substituted compounds [14] but in accordance with the study concerning 5-(oxadiazolyl)tryptamines described by Street and coworkers [20]. Similarly, binding selectivity was improved from 8- to 94-fold upon substitution of the aromatic amine or methylamine (compare **6** with **7–10**, and **12** with **13**). It is noteworthy that going from R_1 = NHCOPh (**7**) to NHCOMe (**8**) the selectivity increases for 5-$HT_{1D\beta}$ versus 5-HT_{1A} receptors from 10 to 36, while keeping a similar affinity for 5-HT_{1D} receptor subtypes, showing the importance of the *para*-substituent for selectivity. From the binding aspect only, compound **10** is among the most potent ligand for 5-$HT_{1D\beta}$ receptors described with a pK_i of 10.1 and a selectivity versus 5-HT_{1A} of 94-fold. Adding one methylene group between the amine or sulfonamide and the terminal aromatic moiety, as illustrated in compounds **12** and **13**, gave very similar binding profiles as compared to compounds **6** and **9**, respectively.

Most of the compounds of this series behave as full agonists. First, they show a very high potency in inhibiting forskolin-induced cAMP formation at cloned human 5-$HT_{1D\beta}$ receptors, for example, compound **9** which is 215-fold more potent than sumatriptan and 82-fold more potent than naratriptan (under similar conditions). In all cases reported, the EC_{50} values are very close to the K_i values and confirmed that the two

Table I. Physical properties of compounds **5–13**, **22** and **24**.

Compound	*Mp*[a] *(°C)*	*Formula*[b]	*Anal*[c]
5	73	$C_{18}H_{18}N_4O_4$•HCl•0.75H_2O	C, H, N
6	183	$C_{18}H_{20}N_4O_2$•2HCl•1.5H_2O	C, H, N, Cl
7	230	$C_{25}H_{24}N_4O_3$•HCl•0.8H_2O	C, H, N, Cl
8	157	$C_{20}H_{22}N_4O_3$•1.4HCl•1.6H_2O	C, H, N, Cl
9	241[d]	$C_{19}H_{22}N_4O_4S$•HCl	C, H, N
10	115	$C_{24}H_{23}N_5O_6S$•HCl•1.1H_2O	C, H, N, Cl
11	161	$C_{19}H_{18}N_4O_2$•HCl•H_2O	C, H, N, Cl
12	243[d]	$C_{19}H_{22}N_4O_2$•2HCl•0.5H_2O	C, H, N, Cl
13	191	$C_{24}H_{24}N_4O_4S$•HCl•1.4H_2O	C, H, N, Cl
22	200	$C_{27}H_{28}N_4O_3$•HCl•0.2H_2O	C, H, N, Cl
24	270	$C_{21}H_{24}N_4O_2$•HCl•0.1H_2O	C, H, N, Cl

[a]All compounds were crystallized from CH_2Cl_2/Et_2O or $CHCl_3/Et_2O$. [b]Satisfactory ^{1}H-NMR spectra were obtained for all compounds. [c]The analyses are within ±0.4% of the theoretical values except for compound **5** (C: calcd 53.47; found 54.26). [d]Decomposed.

Table II. Binding profile at cloned human receptors and functional activity of compounds **5–13**, **22**, **24**, sumatriptan, zolmitriptan and naratriptan.

Compound	*R1*	*R2*	*X*	*K_i (nM)*[a]			*cAMP(nM)*[b]	*Vein contraction*	
				5-$HT_{1D\alpha}$	*5-$HT_{1D\beta}$*	*5-HT_{1A}*	*5-$HT_{1D\beta}$*	*pD_2 (CI 95%)*[c]	*Rel max*[d]
5	NO_2	H	O	1.5 ± 0.6	1.7 ± 0.2	14.0 ± 2	0.53 ± 0.25	–	–
6	NH_2	H	O	6.8 ± 0.0	13.6 ± 4.2	112 ± 17	11.7 ± 3.6	7.1 (6.9–7.2)	1.27 ± 0.13
7	NHCOPh	H	O	0.26 ± 0.13	0.23 ± 0.06	2.4 ± 0.4	0.45 ± 0.00	7.7 (7.5–7.8)	1.06 ± 0.09
8	NHCOMe	H	O	0.75 ± 0.07	1.50 ± 0.14	39.2 ± 0.0	0.74 ± 0.15	6.6 (6.5–6.8)	1.08 ± 0.11
9	$NHSO_2Me$	H	O	1.34 ± 0.82	0.44 ± 0.07	26.4 ± 1.6	0.23 ± 0.10	7.8 (7.5–8.0)	0.78 ± 0.13
10	$NHSO_2PhNO_2$	H	O	0.50 ± 0.08	0.07 ± 0.02	6.60 ± 0.07	0.55	6.9 (6.7–7.1)	0.65 ± 0.13*
11	CN	H	O	1.25 ± 0.07	1.75 ± 0.35	16.8 ± 6.6	0.46 ± 0.02	6.3 (6.1–6.5)	0.78 ± 0.12*
12	CH_2NH_2	H	O	4.3 ± 1.8	5.1 ± 2.4	93 ± 30	3.9 ± 2.1	5.9 (5.7–6.2)	1.41 ± 0.12*
13	CH_2NHSO_2Me	H	O	3.4 ± 1.4	2.04 ± 0.05	63 ± 18	0.43 ± 0.05	7.5 (7.3–7.7)	0.88 ± 0.10
22	NHCOPh	Me	O	0.32	0.55	0.93	1.3	–	–
24	NHCOMe	H	CH_2	1.55 ± 0.07	2.21 ± 0.14	18.6 ± 1.5	2.6 ± 1.0	–	–
1	Sumatriptan	–	–	8.2 ± 2.7	21.9 ± 7.2	459 ± 63	49.5 ± 11.1	5.8 (5.7–5.9)	1.26 ± 0.06*
2	Zolmitriptan	–	–	0.92	4.2 ± 0.3	78.6	15.7 ± 2.3	6.1 (5.9–6.4)	1.48 ± 0.08*
3	Naratriptan	–	–	2.9 ± 1.4	2.00 ± 0.40	53.2 ± 10.0	18.9 ± 9.2	5.7 (5.5–5.8)	1.08 ± 0.09

[a]K_i values are given as mean ± SD of two independent experiments, each performed in duplicate. [b]EC_{50} values are given as mean (**10**) or mean ± SEM of two to five independent experiments, each performed in triplicate. [c]Contraction of the New Zealand white rabbit saphenous vein with confidence interval at 95%. [d]Maximum contraction obtained relative to 5-HT (mean ± SEM); * $P<0.05$ versus 5-HT.

weakest compounds are the unsubstituted amines **6** and **12**.

The high intrinsic activity of compounds **5–13** has been confirmed by the in vitro New Zealand white rabbit saphenous vein contraction model [19] where some of the compounds, eg, **7**, **9** or **13**, are about two orders of magnitude superior (pD_2 values) to that of sumatriptan or naratriptan (table II). The other compounds **6–8** and **10–12**, are at least as potent as zolmitriptan. The comparison of the maximum contractile effect relative to 5-HT (E_{max}) is more difficult to analyze since values above one were found to be due to the presence of endothelium in the rings of rabbit saphenous vein as demonstrated for sumatriptan [21]. However, some of the compounds (**10** and **11**) demonstrate a partial agonist activity in the contraction model, with E_{max} values below one, while they behave as full agonists in the cAMP model. These results may be attributed to the difference of species (human versus rabbit) used in the two models.

From a structural point of view, compounds **5–13** present two main differences compared to the reference compounds **1–3**. First, they possess an unsubstituted terminal primary amine instead of a tertiary amine and secondly they are 5-*O* instead of 5-*C*-alkylated tryptamine derivatives which confer a much easier synthetic access.

In order to determine the effect of the *N*-disubstitution on binding and agonist profiles, we prepared and examined derivative **22**, which is the *N*-bis-methyl analog of **7**. The data obtained for compound **22** and **7** are nearly identical, for both binding level and intrinsic activity. These results are in accordance with an earlier investigation [22] concerning 5-substituted tryptamine derivatives of *o*-tolylpiperazine.

We also tried to investigate the relative importance of the oxygen versus carbon atoms at the 5-position of the tryptamine moiety. Comparison of the binding values obtained for compound **8** and the C-5 derivative **24** shows that the latter retains affinity for both $5\text{-}HT_{1D}$ receptor subtypes, but is a less selective agent versus $5\text{-}HT_{1A}$ receptors and a weaker agonist in the cAMP assay compared to the ether compound (EC_{50} 2.6 and 0.7 nM, respectively).

Conclusion

The new anilide derivatives of serotonin **5–13** reported in this paper appear as very potent agonists at cloned human $5\text{-}HT_{1D}$ receptors, thus demonstrating that the piperazine ring found in previously reported derivatives **4** is not necessary for $5\text{-}HT_{1D}$ receptor agonist properties. Moreover, bis-methylation of the 3-ethylamino side chain or modification from 5-*O*- to 5-*C*-alkylated tryptamine derivatives did not alter the pharmacological profile. However, binding affinity and selectivity (versus $5\text{-}HT_{1A}$) was shown to be highly dependent of the nature of the *para*-substituent (R_1) leading to a new class of compounds with potential use in the treatment of migraine.

Experimental protocols

Chemistry

Melting points were recorded on a electrothermal 9200 apparatus and were uncorrected. ^{1}H-NMR spectra were obtained on a Brüker 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 SDS) with the eluent 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; analyses indicated by the symbols of the elements or functions were within ±0.4% of theoretical values. Chlorine was determined by the oxygen flask method: combustion in a Schöniger flask and dosage of chloride with $AgNO_3$. 3-[2-[*N*-*tert*-Butoxycarbonyl)amino]ethyl]-1*H*-indol-5-ol (*N*-BOC-serotonin) was prepared according to a literature procedure [14].

*General procedure for the preparation of chloroamides **16** and **17**. 2-Chloro-N-(4-nitrophenyl)acetamide **16***

A mixture of 4-nitroaniline (1 g, 7.24 mmol) and $CaCO_3$ (2.17 g, 21.72 mmol) in methyl ethyl ketone (MEK) (15 mL) was treated at 0 °C by chloroacetyl chloride (0.58 mL, 7.24 mmol). The mixture was stirred at room temperature 2 h 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 (1.48 g, 95%) was used for the next step without further purification.

*2-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol-5-yloxy]-N-(4-nitrophenyl)acetamide **18***

A mixture of *N*-BOC-serotonin (500 mg, 1.81 mmol), α-chloroamide **16** (777 mg, 3.62 mmol), K_2CO_3 (625 mg, 4.52 mmol) and KI (30 mg, 0.18 mmol) in MEK (10 mL) was refluxed for 7 h. After that time, compound **16** was added again (390 mg, 1.81 mmol) and the mixture was refluxed overnight. The mixture was then diluted with EtOAc, washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated. The crude product was chromatographed (CH_2Cl_2/acetone 20:1) to give **18** (430 mg, 55%): ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, *t*Bu), 2.74 (t, 2H, J = 7.8 Hz, CH_2), 3.16 (m, 2H, CH_2N), 4.75 (s, 2H, CH_2O), 6.86 (m, 2H, CH + NHBOC), 7.11 (m, 2H, Ar), 7.26 (d, 1H, J = 8.8 Hz, 7-CH), 7.95 (d, 2H, J = 9.2 Hz, 2',6'-CH), 8.25 (d, 2H, J = 9.2 Hz, 3',5'-CH), 10.67 (s, 1H, NH), 10.71 (s, 1H, NH).

*2-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol-5-yloxy]-N-(4-cyanophenyl)acetamide **19***

A mixture of *N*-BOC-serotonin (3.0 g, 10.86 mmol), α-chloroamide **17** (3.8 g, 19.55 mmol), Cs_2CO_3 (5.3 g, 28.29 mmol) and KI (0.18 g, 1.08 mmol) was heated at 60 °C for 96 h. The mixture was 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 was chromatographed (hexane/EtOAc 1:1) to give **19** (3.01 g, 64%): ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, *t*Bu), 2.74 (t, 2H, J = 8.0 Hz, CH_2), 3.16 (m, 2H, CH_2N), 4.72 (s, 2H, CH_2O), 6.86 (m, 2H, CH + NHBOC), 7.11 (m, 2H, Ar), 7.26 (d, 1H, J = 8.6 Hz, 7-CH), 7.79 (d, 2H, J = 8.8 Hz, 2',6'-CH), 7.89 (d, 2H, J = 9.2 Hz, 3',5'-CH), 10.49 (s, 1H, NH), 10.71 (s, 1H, NH).

*2-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol-5-yloxy]-N-(4-aminophenyl)acetamide **20***

Compound **18** (2 g, 4.40 mmol) in suspension in MeOH (130 mL) was hydrogenated over Pd/C (10%) (200 mg, 0.20 mmol) under 1 atm of H_2 at room temperature for 4 h. The mixture was filtered through celite and the filtrate was concentrated. The crude product was chromatographed (CH_2Cl_2/acetone, 7:1) to give **20** (1.86 g, 99%): ^{1}H-NMR (DMSO-d_6) δ: 1.40 (s, 9H, *t*Bu), 2.76 (t, 2H, J = 7.8 Hz, CH_2), 3.19 (m, 2H, CH_2N), 4.58 (s, 2H, CH_2O), 4.92 (s, 2H, $ArNH_2$), 6.52 (d, 2H, CH + NHBOC), 6.90 (m, 2H, Ar), 7.12 (broad s, 2H, Ar), 7.27 (m, 3H, Ar), 9.59 (s, 1H, NH), 10.71 (s, 1H, NH).

*2-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol-5-yloxy]-N-(4-aminomethylphenyl) acetamide **21***

Compound **19** (3.0 g, 6.93 mmol) in solution in a mixture of THF (100 mL) and NH_4OH (8.7 mL) was hydrogenated over Raney nickel (catalytic) under 1 atm of H_2 at room temperature for 7 h. The mixture was filtered through celite and the filtrate was concentrated. The crude product was chromatographed (CH_2Cl_2/MeOH/NH_4OH ; 80:19.5:0.5) to give **21** (2.12 g, 70%): ^{1}H-NMR (DMSO-d_6) δ: 1.39 (s, 9H, *t*Bu), 2.76 (t, 2H, J = 7.6 Hz, CH_2), 3.20 (m, 2H, CH_2N), 3.33 (broad s, 2H, NH_2), 3.67 (s, 2H, CH_2N), 4.65 (s, 2H, CH_2O), 6.88 (m, 2H, CH + NHBOC), 7.12 (broad s, 2H, Ar), 7.27 (d, 3H, Ar), 7.60 (d, 2H, J = 8.0 Hz, Ar), 9.94 (s, 1H, NH), 10.71 (s, 1H, NH).

*General procedure for sulfonylation or acylation of compounds **20–21** followed by N-BOC deprotection to give **7–10** and **13**. N-[4-(Benzoylamino)phenyl]-2-[3-(2-amino)ethyl]-1H-indol-5-yloxyacetamide **7***

Compound **20** (0.56 g, 1.32 mmol) in pyridine (15 mL) was treated with benzoyl chloride (0.15 mL, 1.32 mmol) at 0 °C. The reaction mixture was stirred from 0 °C to room temperature for 3 h, diluted with EtOAc, washed with saturated $CuSO_4$, H_2O and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated to give the desired product (0.65 g, 93%). The crude product in toluene (14 mL) was then deprotected by treatment at 25 °C with excess TFA (2 mL). After 3 h, the mixture was diluted with CH_2Cl_2, washed with NaOH (2 N) and H_2O, dried (Na_2SO_4) and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 80:18.5:1.5) to give **7** (396 mg, 82%) isolated as the hydrochloride salt: mp 230 °C, ^{1}H-NMR (DMSO-d_6) δ 3.00 (m, 4H, CH_2), 4.70 (s, 2H, CH_2O), 6.90 (dd, 1H, J = 2.1 and 8.7 Hz, 6-CH), 7.21 (d, 2H, J = 2.0 Hz, Ar), 7.30 (d, 1H, J = 8.7 Hz, Ar), 7.49–7.76 (m, 7H, Ar), 7.94–7.98 (m, 4H, Ar + NH_3^+), 10.19 (s, 1H, NH), 10.27 (s, 1H, NH), 10.89 (s, 1H, NH). Anal $C_{25}H_{25}N_4O_3Cl{\cdot}0.8H_2O$ (C, H, N, Cl).

Compounds **8–10** and **13** were prepared using a similar procedure.

*N-[4-(Acetylamino)phenyl]-2-[3-(2-amino)ethyl]-1H-indol-5-yloxyacetamide hydrochloride **8***. ^{1}H-NMR (DMSO-d_6) δ 2.01 (s, 3H, CH_3CO), 2.85–3.05 (m, 4H, CH_2), 4.66 (s, 2H, CH_2O), 6.86 (dd, 1H, J = 2.3 and 8.7 Hz, 6-CH), 7.20 (d, 2H, J = 2.2 Hz, Ar), 7.28 (d, 1H, J = 8.7 Hz, Ar), 7.49–7.62 (m, 4H, Ar), 8.03 (broad s, 3H, NH_3^+), 9.98 (s, 1H, NH), 10.12 (s, 1H, NH). Anal $C_{20}H_{22}N_4O_3{\cdot}1.4HCl{\cdot}1.6H_2O$ (C, H, N, Cl).

*N-[4-(Methanesulfonylamino)phenyl]-2-[3-(2-amino)ethyl]-1H-indol-5-yloxyacetamide hydrochloride **9***. ^{1}H-NMR (DMSO-d_6) δ 2.90–3.10 (m, 7H, CH_3 + CH_2), 4.71 (s, 2H, CH_2O), 6.89 (d, 1H, J = 8.6 Hz, 6-CH), 7.16–7.32 (m, 6H, Ar), 7.66 (d, 2H, J = 8.0 Hz, Ar), 8.30 (broad s, 3H, NH_3^+), 10.22 (s, 1H, NH), 10.88 (s, 1H, NH). Anal $C_{19}H_{22}N_4O_4S{\cdot}HCl$ (C, H, N).

*N-[4-(4-Nitrobenzenesulfonylamino)phenyl]-2-[3-(2-amino)-ethyl]-1H-indol-5-yloxyacetamide hydrochloride **10***. ^{1}H-NMR (DMSO-d_6) δ 2.90–3.10 (m, 4H, CH_2), 4.66 (s, 2H, CH_2O),

6.86 (dd, 1H, J = 2.0 and 8.8 Hz, 6-CH), 7.04 (d, 2H, J = 8.6 Hz, Ar), 7.20 (m, 2H, Ar), 7.27 (d, 1H, J = 8.8 Hz, 7-CH), 7.57 (d, 2H, J = 8.8 Hz, Ar), 7.94–7.98 (m, 4H, Ar + NH_3^+), 8.36 (d, 2H, J = 8.4 Hz, Ar), 10.19 (s, 1H, NH), 10.52 (s, 1H, NH), 10.87 (s, 1H, NH). Anal $C_{24}H_{23}N_5O_6S$•HCl•1.1H_2O (C, H, N, Cl).

N-[4-(Methanesulfonylaminomethyl)phenyl]-2-[3-(2-amino)-ethyl]-1H-indol-5-yloxyacetamide hydrochloride ***13***. ^{1}H-NMR (DMSO-d_6) δ 2.83 (s, 3H, CH_3SO_2), 2.90–3.10 (m, 4H, CH_2), 4.10 (d, 2H, J = 6.2 HZ, CH_2NH), 4.70 (s, 2H, CH_2O), 6.89 (dd, 1H, J = 2.0 and 8.8 Hz, 6-CH), 7.20 (m, 2H, Ar), 7.29 (d, 3H, J = 8.4 Hz, Ar), 7.53 (t, 1H, J = 6.2 Hz, NH), 7.66 (d, 2H, J = 8.4 Hz, Ar), 7.96 (broad s, 3H, NH_3^+), 10.18 (s, 1H, NH), 10.87 (s, 1H, NH). Anal $C_{24}H_{24}N_4O_4S$•HCl•1.4H_2O (C, H, N, Cl).

N-[4-(Acetylamino)phenyl]-3-[3-(2-amino)ethyl]-1H-indol-5-ylpropionamide ***24***

N-BOC-5-bromotryptamine (7.0 g, 20.6 mmol) was heated overnight, in a sealed tube, with Et_3N (15 mL), methyl acrylate (2.78 mL, 30.9 mmol), $Pd(OAc)_2$ (46 mg, 0.21 mmol) and tri-*o*-tolyl phosphine (126 mg, 0.42 mmol). After that time, the same amounts of $Pd(OAc)_2$ and tri-*o*-tolyl phosphine were added and the mixture was further heated 7 h. The mixture was then diluted with AcOEt and filtered through celite. The filtrate was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated. The crude product was chromatographed (CH_2Cl_2/EtOAc 15:1) to give the desired product (6.24 g, 88%).

This intermediate (2.0 g, 5.80 mmol), in EtOH (20 mL), was treated with KOH (0.65 g, 11.6 mmol) at refluxed for 3 h. The solution was diluted with EtOAc, washed with HCl (1 N), H_2O and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated to give the acid derivative (1.85 g, 97%). The crude product (547 mg, 1.65 mmol), in suspension in MeOH (11 mL), was hydrogenated over Pd/C (10%) (catalytic) under 1 atm of H_2 at room temperature for 4 h. The mixture was filtered through celite and the filtrate was concentrated. The crude product was chromatographed (CH_2Cl_2/MeOH, 20:1) to give the saturated acid (489 mg, 89%). This acid (361 mg, 1.08 mmol), in solution in CH_2Cl_2 (36 mL) in the presence of *N*-methyl morpholine (0.18 mL, 1.62 mmol) at −15 °C, was then treated with ethyl chloroformate (0.134 mL, 1.40 mmol) for 0.5 h. 4-Aminoacetanilide (245 mg, 1.62 mmol) was then added and the mixture was stirred from −15 to 25 °C for 2 h. The solution was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, H_2O and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated. The crude product was chromatographed (CH_2Cl_2/acetone, 3:1) to give the desired anilide in a quantitative yield (500 mg), which was deprotected with TFA in the conditions described above to give compound **24** (411 mg, 76%) isolated as the hydrochloride salt: mp 270 °C, *m/z* 365 (MH^+), ^{1}H-NMR (DMSO-d_6) δ 2.01 (s, 3H, CH_3CO), 2.65 (t, 2H, J = 8.0 Hz, CH_2), 2.95–3.01 (m, 6H, CH_2), 7.00 (d, 1H, J = 8.3 Hz, 6-CH), 7.20 (d, 1H, J = 2.0 Hz, 4-CH), 7.28 (d, 1H, J = 8.3 Hz, 7-CH), 7.44–7.55 (m, 5H, Ar), 8.02 (broad s, 3H, NH_3^+), 9.93 (s, 1H, NH), 9.96 (s, 1H, NH), 10.87 (s, 1H, NH). Anal $C_{21}H_{25}N_4O_2Cl$•0.1H_2O (C, H, N, Cl).

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). Right and left lateral saphenous veins were cleaned of surrounding adipose and connective tissue in situ. The veins were then excised and placed in cold oxygenated Krebs bicarbonate buffer solution, then cut into rings of approximately 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}), 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_2/5% 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) to assess the functional integrity of the rings. Then, 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 non-linear least-square algorithm of Marquardt [23]. 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 Dunnett's test (StatView™, Abacus Concepts, Inc, Berkeley, CA).

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