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Original article

Synthesis and binding affinities for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors of a series of 1- and 2-(4-arylpiperazinylalkyl)-4-(benzoyl)-1,2,3-triazole derivatives

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Abstract – A number of 1- and 2-(4-arylpiperazinylalkyl)-4-(benzoyl)-1,2,3-triazole derivatives (1–4) were prepared in order to obtain compounds with a high affinity and selectivity for 5-HT_{1A} receptors. 5-$HT_{1A,}$ 5-HT_{2A} and 5-HT_{2C} affinities were determined by radioligand binding experiments and the most active compounds were also tested for binding affinities on dopaminergic D-1, D-2 and adrenergic α_1, α_2 receptors. The modification of aromatic substituents, the length of the alkyl chain and its position on the 4-benzoyl-1,2,3-triazole ring were explored. Most of the considered compounds generally showed moderate to high affinity for the 5-HT_{1A} receptor binding site. Three derivatives **2c**, **3c** and **3e** bind to 5-HT_{1A} receptors in the nanomolar range (IC_{50} values = 2, 7.2 and 2.6 nM respectively). The most active compound, **2c**, presented a high degree of selectivity versus all considered receptors. It was found that the benzoyltriazole derivatives **1h** and **4c** are new selective ligands for 5-HT_{2A} (IC_{50} = 89 nM) and 5-HT_{2C} receptors (IC_{50} = 17 nM), respectively. © 1999 Éditions scientifiques et médicales Elsevier SAS

arylpiperazines / synthesis / 5-HT receptor / serotonin

1. Introduction

The neurotransmitter serotonin is involved in various physiological (e.g. sleep and thermoregulation) and pathological processes (e.g. migraine and depression). A classification of 5-HT receptor subtypes, their role in various CNS activities and their respective ligands were recently reviewed [1, 2]. One of the serotonin receptor subtypes, 5-HT_{1A}, plays an important role as the somatodendritic autoreceptor (presynaptic) in the dorsal raphe nucleus and as a postsynaptic receptor for 5-HT in terminal areas [3]. Buspirone and ipsapirone, which are agonists and display high affinity to the 5-HT_{1A} receptor (IC_{50} = 60 and 35 nM, respectively), are presently being used as anti-anxiety agents [4, 5]. It seems useful to develop ligands that are selective for the 5-HT_{1A} subtype to facilitate the study and characterization of this receptor, but also in view of their potential use as anxiolytics. The most commonly used ligand, 8-OH-DPAT (8-hydroxy-2-(N-N-di-*n*-propylamino)tetralin), is a potent 5-HT_{1A} agonist, and in fact, the tritium-labelled compound is the ligand of choice for 5-HT_{1A} receptor binding studies (Kd = 0.5 nM, rat hippocampal homogenates).

One class of compounds with affinity for the 5-HT_{1A} receptor is represented by the arylpiperazine derivatives [6] (general structure **I**) where R is a heterocyclic nucleus.

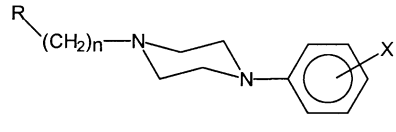

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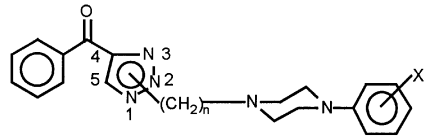
Table I. Physicochemical properties of 4-benzoyl-1,2,3-triazole derivatives.



				1-Substituted 4-benzoyl-1,2,3-triazoles			2-Substituted 4-benzoyl-1,2,3-triazoles		
X	n	Formula[a]	M.W.	Compound[b]	M.p. (°C)	Yield[c] %	Compound[b]	M.p. (°C)	Yield[c] %
H	2	$C_{21}H_{23}N_5O\cdot HCl$	397.91	**1a**	201 – 202	25	**2a**	206 – 207	35
o-Cl	2	$C_{21}H_{22}ClN_5O\cdot HCl$	432.34	**1b**	208 – 210	23	**2b**	212 – 214	32
m-Cl	2	$C_{21}H_{22}ClN_5O\cdot HCl$	432.34	**1c**	204 – 206	24	**2c**	180 – 182	35
p-Cl	2	$C_{21}H_{22}ClN_5O\cdot HCl$	432.34	**1d**	236 – 238	25	**2d**	191 – 193	37
o-OCH_3	2	$C_{22}H_{25}N_5O_2\cdot HCl$	428.01	**1e**	210 – 212	28	**2e**	206 – 208	40
p-OCH_3	2	$C_{22}H_{25}N_5O_2\cdot HCl$	428.01	**1f**	218 – 220	20	**2f**	200 – 201	38
o-F	2	$C_{21}H_{22}N_5OF\cdot HCl$	415.89	**1g**	142 – 143	28	**2g**	164 – 165	42
p-F	2	$C_{21}H_{22}N_5OF\cdot HCl$	415.89	**1h**	216 – 217	24	**2h**	196 – 197	38
H	3	$C_{22}H_{25}N_5O\cdot HCl$	411.93	**3a**	183 – 184	27	**4a**	251 – 252	42
o-Cl	3	$C_{22}H_{24}ClN_5O\cdot HCl$	446.35	**3b**	334 – 335	27	**4b**	177 – 179	45
m-Cl	3	$C_{22}H_{24}ClN_5O\cdot HCl$	446.35	**3c**	231 – 232	22	**4c**	178 – 180	38
p-Cl	3	$C_{22}H_{24}ClN_5O\cdot HCl$	446.35	**3d**	217 – 219	27	**4d**	248 – 249	40
o-OCH_3	3	$C_{23}H_{27}N_5O_2\cdot HCl$	441.95	**3e**	207 – 209	28	**4e**	153 – 155	41
p-OCH_3	3	$C_{23}H_{27}N_5O_2\cdot HCl$	441.95	**3f**	220 – 221	22	**4f**	208 – 210	34
o-F	3	$C_{22}H_{24}N_5OF\cdot HCl$	429.92	**3g**	188 – 189	28	**4g**	179 – 180	42
p-F	3	$C_{22}H_{24}N_5OF\cdot HCl$	429.92	**3h**	187 – 188	25	**4h**	208 – 209	39

[a]Satisfactory microanalyses obtained: C, H, N, Cl, F values are within ± 0.4% of the theoretical ones; [b]All compounds were crystallized by methyl alcohol and diethyl ether. [c]Yield refers to the single structural isomer after separation by chromatography as free bases.

Affinity and specificity depend critically on the nature of the specific heterocyclic nucleus (R), on the (X) aromatic substitution and on the length (n) of the polymethylene chain. In particular, it was observed that different terminal groups (R) play an important role in the interaction with a corresponding hydrophobic region of the 5-HT_{1A} receptor [7].

We recently reported a series of arylpiperazines of general structure **I** [8] having a benzotriazole group as a terminal (R) moiety with mixed 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptor affinity.

Structure-affinity relationships in this series [8] showed that nanomolar affinity towards the 5-HT_{1A} receptor requires a methoxy group at the *ortho*-position of the phenyl ring. However, the most potent benzotriazole ligands of the 5-HT_{1A} receptor have poor selectivity, e.g., they possess affinities for the 5-HT_{2A} receptor only 10–50 times lower than those measured for 5-HT_{1A}.

Considering the important role played by the (R) terminal group, in an attempt to increase both affinity and selectivity for the 5-HT_{1A} receptor the benzotriazole nucleus of these arylpiperazine derivatives [8], was replaced by a 4-benzoyl-1,2,3-triazole group. These compounds (**1–4**) represent open chain analogues of their benzotriazole counterparts with bioisosteric properties.

Most of the arylpiperazine moieties used are those previously reported [8] displaying the highest affinity for the 5-HT_{1A} receptor, typified by 1-(2- or 4-methoxyphenyl)-, 1-phenyl-, 1-(2- or 3- or 4-chlorophenyl)piperazine. In this work we considered also a new electron-withdrawing substituent such as a fluoro group introduced into the 2- or 4- position of the phenylpiperazine moiety. The piperazine is connected to the terminal benzoyltriazole system via bridges of two (series **1** and **2**) or three (series **3** and **4**) methylene groups.

Herein we report the synthesis of a series of 1- and 2-(4-arylpiperazinylalkyl)-4-(benzoyl)-1,2,3-triazole derivatives **1–4**, *(table I)* and the affinities for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors obtained by radioligand binding studies. The most active compounds were also tested for their affinity at dopaminergic D-1, D-2 and adrenergic α_1 and α_2 receptors to substantiate their pharmacological profile in view of potential therapeutic uses.

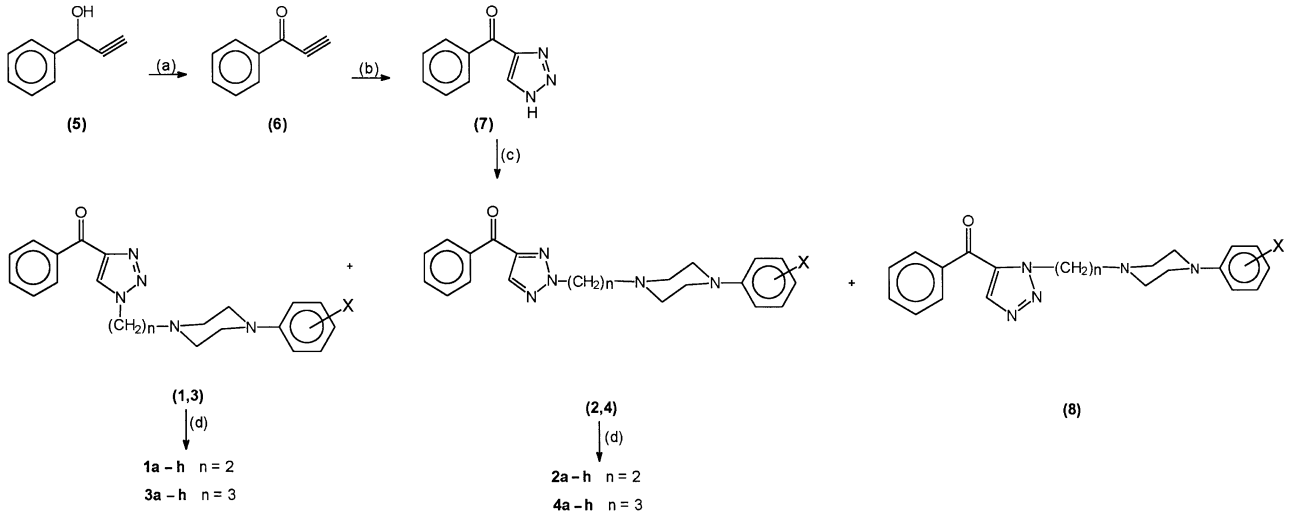


Figure 1. Reagents: (a) CrO_3/H_2SO_4; (b) NaN_3; (c) N-arylpiperazine/K_2CO_3; (d) HCl (anhydrous).

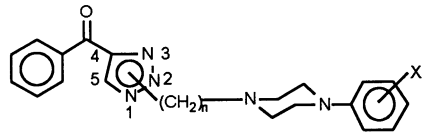
2. Chemistry

4-benzoyl-1,2,3-triazole derivatives **1a**–**h**, **2a**–**h**, **3a**–**h** and **4a**–**h** *(table I)* were synthesized as described in *figure 1*. The parent compound 4-benzoyl-1,2,3-triazole (**7**) was obtained in 95% yield by a modified method described in the literature [9, 10]. Oxidation of the phenylethynylcarbinol **5** with chromium trioxide and concentrated sulphuric acid produced phenyl ethynyl ketone **6,** which was successively treated with NaN_3 in anhydrous dimethylacetamide providing the desired compound. Alkylation of the aromatically substituted 1-(2-chloroethyl)- or 1-(3-chloropropyl)-4-phenylpiperazines, prepared as described in the literature [8], with 4-benzoyl-1,2,3-triazole in butan-2-one in the presence of potassium carbonate afforded a mixture of the expected 1-, 2- and 3- triazole isomers with an overall yield in the range of 45–80%. The three isomers were separated by chromatography on a silica gel column using *n*-hexane/diethyl ether 95:5 as eluent. The faster moving 2-substituted isomers (**2** and **4**) were collected with a higher yield with respect to the slower moving 1-substituted isomers (**1** and **3**). The yield of intermediate moving 3-substituted isomers (**8**) was so low that they were not further considered. The free bases were converted into their corresponding hydrochlorides by usual methods. All the final products were further purified by crystallization from a mixture of diethyl ether and ethyl alcohol. Synthesized compounds listed in *table I* were charcterized by ^{1}H-NMR spectroscopy. ^{1}H-NMR differentiated clearly between 1-, 2- and 3- substituted 4-benzoyl-1,2,3-triazole derivatives. In fact, it should be pointed out that there is a difference in the chemical shift values among the protons in the 5- position of the benzoyltriazole ring in the series of 1-, 2- and the proton in 4-position of the benzoyltriazole ring of the 3-substituted compounds. The triazole proton of the 1-isomer appears always as a singlet at lower field with respect to the position of the same proton of analogues 2-substituted, whereas, that of the 3-isomer resonated as a singlet at higher field with respect to the corresponding 1- and 2-isomers. This evidence is in accordance with to literature data [11, 12]. All compounds gave satisfactory analyses (C, H, N, Cl, F).

3. Biological activity

The compounds reported in *table I* (**1**–**4)** were tested for in vitro affinity on serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors by radioligand binding assays. The more active compounds on serotonin receptors have been selected and evaluated for their affinity on dopaminergic (D-1 and D-2) and adrenergic (α_1 and α_2) receptors. All the compounds were used as hydrochloride salts and were water-soluble. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptors, [^{3}H]-8-OH-DPAT, rat brain cortex membranes; (b) serotonin 5-HT_{2A} receptors, [^{3}H] ketanserin, rat brain cortex membranes; (c) serotonin 5-HT_{2C} receptors, [^{3}H] mesulergine, rat brain cortex membranes; (d) dopamine D-1 receptors [^{3}H]SCH-23390, rat strial membranes; (e)

Table II. Binding affinities and selectivities



Compound	X	n	IC_{50} nM (± SEM)			Selectivity vs. 5-HT_{1A} receptor IC_{50} ratio	
			5-HT_{1A} [^{3}H] 8-OH-DPAT	5-HT_{2A} [^{3}H] ketanserin	5-HT_{2C} [^{3}H] mesulergine	5-HT_{2A}	5-HT_{2C}
1-substituted							
1a	H	2	3 200 ± 300	24 000 ± 1 900	2 900 ± 350	7.5	0.9
1b	*o*-Cl	2	24 ± 2	> 10^5	37 ± 6	> 4 10^3	1.5
1c	*m*-Cl	2	240 ± 20	30 000 ± 2 850	960 ± 86	125	4.0
1d	*p*-Cl	2	180 ± 16	9 600 ± 810	480 ± 34	53	2.7
1e	*o*-OCH_3	2	480 ± 35	46 000 ± 4 400	180 ± 20	96	0.4
1f	*p*-OCH_3	2	> 10^5	> 10^5	> 10^5	1	1
1g	*o*-F	2	10 000 ± 1 200	1 100 ± 170	610 ± 55	0.1	0.06
1h	*p*-F	2	> 10^5	89 ± 6	> 10^5	> 9 10^{-4}	1
2-substituted							
2a	H	2	73 ± 5.8	> 10^5	1 100 ± 170	> 10^3	15
2b	*o*-Cl	2	36 ± 3	> 10^5	12 000 ± 1 100	> 3 10^3	333
2c	*m*-Cl	2	2 ± 0.2	> 10^5	1 900 ± 290	> 10^4	950
2d	*p*-Cl	2	16 000 ± 1 400	> 10^5	250 ± 23	> 6.2	0.02
2e	*o*-OCH_3	2	62 ± 7	> 10^5	3 000 ± 280	> 10^3	48.4
2f	*p*-OCH_3	2	> 10^5	> 10^5	> 10^5	1	1
2g	*o*-F	2	4 800 ± 390	240 ± 20	8 800 ± 780	0.08	1.8
2h	*p*-F	2	13 000 ± 1 200	1 000 ± 120	580 ± 57	0.05	0.04
1-substituted							
3a	H	3	20 ± 1.8	1 300 ± 140	17 ± 2.0	65	0.8
3b	*o*-Cl	3	660 ± 60	2 000 ± 360	120 ± 19	3.0	0.2
3c	*m*-Cl	3	7.2 ± 0.8	770 ± 67	230 ± 30	107	32
3d	*p*-Cl	3	200 ± 15	3 000 ± 250	310 ± 30	15	1.5
3e	*o*-OCH_3	3	2.6 ± 0.2	8 100 ± 700	980 ± 72	3115	377
3f	*p*-OCH_3	3	> 10^5	> 10^5	> 10^5	1	1
3g	o-F	3	5 200 ± 480	7 800 ± 670	> 10^5	1.5	> 19
3h	*p*-F	3	11 000 ± 1 000	6 800 ± 620	29 000 ± 2 750	0.6	3
2-substituted							
4a	H	3	91 ± 6.8	22 000 ± 2 100	9 500 ± 880	242	104
4b	*o*-Cl	3	180 ± 20	34 000 ± 3 100	930 ± 72	189	5.2
4c	*m*-Cl	3	710 ± 65	8 200 ± 740	17 ± 2	11.5	0.02
4d	*p*-Cl	3	10 000 ± 1 150	12 000 ± 1 100	1 100 ± 120	1.2	0.1
4e	*o*-OCH_3	3	93 ± 6.8	> 10^5	2 200 ± 150	> 10^3	24
4f	*p*-OCH_3	3	> 10^5	> 10^5	> 10^5	1	1
4g	*o*-F	3	95 ± 7.2	11 ± 1.2	460 ± 40	0.1	5
4h	*p*-F	3	1 500 ± 160	800 ± 74	4 400 ± 380	0.5	3
8-OH-DPAT			2.1 ± 0.1	–	–	–	–
ketanserin			–	1.7 ± 0.3	–	–	–
cinanserin			–	1.6 ± 1.0	–	–	–
mesulergine			–	–	1.2 ± 0.2	–	–

dopamine D-2 receptors [^{3}H]spiroperidol, rat strial membranes; (f) α_1 adrenergic receptors [^{3}H]prazosin, rat brain cortex membranes; (g) α_2 adrenergic receptors [^{3}H]yohimbine, rat brain cortex membranes. Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined through 2–4 independent experiments with samples in triplicate using 7–9 different concentrations of the title compounds. Specific binding,

Table III. Binding affinities and selectivities for D-1, D-2, α_1 and α_2 receptors in compounds **1h**, **2c**, **3c**, **3e** and **4c**.

Compound	X	n	IC_{50} nM (± SEM)			
			D-1 [^{3}H] SCH-23390	D-2 [^{3}H] spiroperidol	α_1 [^{3}H] prazosin	α_2 [^{3}H] yohimbine
1h	*p*-F	2	$> 10^5$	$> 10^5$	7 200 ± 610	24 000 ± 1 800
2c	*m*-Cl	2	$> 10^5$	$> 10^5$	$> 10^5$	24 000 ± 1 700
3c	*m*-Cl	3	11 000 ± 1 100	10 000 ± 1 500	$> 10^5$	220 ± 20
3e	*o*-OCH_3	3	7 300 ± 620	1 500 ± 210	1 700 ± 250	43 ± 7
4c	*m*-Cl	3	1 900 ± 15	1 800 ± 270	120 ± 15	260 ± 28
spiroperidol			3.6 ± 0.5	4.6 ± 0.7		
prazosin					1.3 ± 0.7	
yohimbine						22 ± 2

defined as described in the experimental section, represented more than 75% of total binding in all three assays. The obtained IC_{50} values are listed in *tables II* and *III*.

4. Results and discussion

In this work we examined affinity and selectivity of 4 series of 4-benzoyl-1,2,3-triazole derivatives (**1–4**) towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors by a preliminary binding screen. As mentioned, these compounds can be regarded as open chain analogues of previously reported benzotriazole derivatives **I** [8].

The IC_{50} nM values listed in *table II* reveal that several compounds exhibit nanomolar affinity for the 5-HT_{1A} receptor, whereas generally only a few bind to 5-HT_{2A} and 5-HT_{2C} receptors with comparable affinity.

As far as 5-HT_{1A} receptor affinity is concerned, the results reported in *table II* indicate that the structures endowed with highest 5-HT_{1A} affinity are **1b**, **2a–c**, **2e**, **3a, 3c** and **3e**. With respect to the length of the alkyl chain between the arylpiperazine moiety and the terminal 4-benzoyl-1,2,3-triazole nucleus, the spacer effect is different for 1- or 2-substituted compounds. For the analogues having an ethyl [$(CH_2)_2$] connecting group between the triazole and piperazine rings, several 2-substituted compounds have lower IC_{50} values than the corresponding 1-substituted derivatives. For example, compare **2a** vs. **1a** (IC_{50} nM = 73 and 3 200, respectively); **2c** vs. **1c** (IC_{50} nM = 2 and 240, respectively) and **2e** vs. **1e** (IC_{50} nM = 62 and 480, respectively). The only partial exceptions are compounds **2b** and **2d** whose affinities are in fact lower than those of **1b** and **1d** respectively.

By contrast, for the analogues having a propyl connecting group [$(CH_2)_3$] between the triazole and the piperazine rings, several 1-substituted compounds have lower IC_{50} values than the corresponding 2-substituted derivatives. For example, compare **3a** vs. **4a** (IC_{50} nM = 20 and 91, respectively); **3c** vs. **4c** (IC_{50} nM = 7.2 and 710, respectively); **3e** vs. **4e** (IC_{50} nM = 2.6 and 93, respectively). Only *ortho* chloro (**3b**) and *ortho* and *para* fluoro substituted compounds (**3g** and **3h)** exhibited IC_{50} values higher than 2-substituted analogues. It seems that in these series the alkyl chain is not interacting directly with the receptor on the basis of its hydrophobicity but is more likely acting as a spacer.

The nature and the position of the substituent on the phenyl ring also has a significant influence on affinity. For all series, *p*-substitution (by Cl, F, or OCH_3) of the piperazinyl-aromatic ring led to compounds with supra nM affinity for the 5-HT_{1A} receptor (but note, compound **1a** with no substituent did not bind with high affinity). For compounds of structure **2** and **3**, *m*-Cl substituents afforded analogues with high 5-HT_{1A} receptor affinity, whereas, in the benzotriazole series it had little effect on affinity for the same receptor [8]. An *o*-OCH_3 substituent also appeared to support 5-HT_{1A} receptor binding for these compounds.

Lòpez-Rodrìguez et al. [13] reported that the *o*-methoxy group on the phenyl ring in various hydantoin-phenylpiperazine homologues exerted effects on 5-HT_{1A} affinity strongly related to the overall ligand structure. Taken together, these findings suggest that there are different possible orientations within the 5-HT_{1A} binding site accessible to the X substituent on the phenyl ring. Such orientations are dictated by the global conformational arrangement of the bound ligand. As a result, contribution of X to affinity depends on the properties of X as well as on the nature of the receptor subsite into which such a substituent is docked.

Among compounds evaluated in *table II*, **2c** (*m*-Cl) and **3e** (*o*-OCH_3) had the highest binding affinity and show the most favourable affinity-selectivity profiles for the 5-HT_{1A} receptor (5-HT_{2A}/5-HT_{1A} IC_{50} ratios are about

10 000 and 3 000, respectively, while 5-HT_{2C}/5-HT_{1A} IC_{50} ratios are about 1 000 and 300, respectively). While fluorine substitution generally led to compounds with low binding affinity to all 5-HT receptors, paradoxically, compound **4g** (*o*-fluoro) had IC_{50} values of 95 nM and 11 nM in the 5-HT_{1A} and 5-HT_{2A} binding assays respectively.

The other two highly potent ligands of the 5-HT_{1A} receptor, **1b** and **3c**, are moderately selective as they retain appreciable affinities for 5-HT_{2C} receptors. A comparison between affinity profiles of benzoyltriazole derivatives **2c** and **3e** versus those of the above mentioned benzotriazole counterparts [8] (IC_{50} ratios always less than 50) clearly show that the 5-HT_{1A}/5-HT_{2A} and 5-HT_{1A}/5-HT_{2C} selectivity ratios of the former compounds are significantly superior. This result might be partly ascribed to poor steric and/or electronic complementarity between the benzoyltriazole residue and the 5-HT_{2A} and 5-HT_{2C} binding sites compared to the benzotriazole moiety in compounds **I**.

The results of this study indicate that the affinity for the 5-HT_{2A} receptor is usually lower than the affinity for 5-HT_{1A} receptors. The introduction of the electron-withdrawing substituents in the *ortho* and *para* position, such as the fluoro group, increased the binding affinity for the 5-HT_{2A} receptor in all series except **3**. Only **4g** exhibits nanomolar affinity (IC_{50} = 11 nM), but this compound binds also to the 5-HT_{1A} receptors with appreciable potency. A remarkable selectivity (1 000-fold) for 5-HT_{2A} versus 5-HT_{1A} and 5-HT_{2C} receptors was achieved with **1h,** although the affinity of this compound for the 5-HT_{2A} receptor fell within the submicromolar range (IC_{50} = 89 nM).

As far as the 5-HT_{2C} receptor is concerned, nanomolar potency was observed for compounds **1b**, **3a** and **4c** (IC_{50}s < 50 nM). Binding of the former two ligands to the 5-HT_{1A} receptor was, however, slightly stronger with respect to the 5-HT_{2C} receptor. The compound **4c** was the only one showing appreciable selectivity towards the 5-HT_{2C} receptor (5-HT_{1A}/5-HT_{2C} and 5-HT_{2A}/5-HT_{2C} IC_{50} ratios were about 40 and 500, respectively).

As mentioned in the introduction section, in order to evaluate potential therapeutic uses, it is crucial to check whether high potency is flanked by undesirable affinity for other receptors, e.g., adrenergic receptors [14, 15]. Thus the most active compounds on 5-HT_{1A} (**2c**, **3c**, and **3e),** 5-HT_{2A} (**1h**) and 5-HT_{2C} (**4c**) were further evaluated for their affinity at dopaminergic and adrenergic receptors. Results are summarized in *table III*. As far as the dopaminergic system was concerned, the D-1 and D-2 receptor affinity consistently showed IC_{50} values above 10^{-6} M. As regards the affinity for α_1 adrenergic receptors, the IC_{50} values were high for all selected compounds, with the exception of **4c** (IC_{50} = 120 nM). The affinity for α_2 receptors were in some cases quite considerable (**3c**, **3e** and **4c**). In particular, *o*-OCH_3-Ph derivative **3e** showed affinity for the α_2 receptor which was only one order of magnitude lower than the affinity toward the 5-HT_{1A} receptor.

However, compound **2c,** the most potent 5-HT_{1A} ligand showed the best selectivity profile versus all considered receptors.

In conclusion, some of the newly investigated benzoyltriazole derivatives were endowed with high affinity and selectivity for the 5-HT_{1A} receptor. Particularly, the IC_{50} values of **2c** and **3e** measured on this receptor are 2 and 2.6 nM, and in terms of selectivity, these two ligands represent a substantial improvement over previously described benzotriazole analogues [8].

These observations suggest that both the arylpiperazine pharmacophore and the terminal benzoyltriazole system contribute to the 5-HT_{1A} interaction of the compounds.

Further synthesis and biological in vivo evaluation of derivatives with new substituents are currently in progress, and the results will be reported in due course.

5. Experimental protocols

5.1. Chemistry

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Kieselgel 60 was used for column chromatography and kieselgel 60 F_{254} plates from Merck were used for TLC. Where analyses are indicated only by the symbols of the elements, results obtained are within ± 0.4% of the theoretical values. The purity of compounds were carefully assessed using analytical TLC and the structure verified spectroscopically by proton NMR spectra recorded on a Bruker AMX-500 instrument. Chemical shifts in ppm are referenced to the DMSO signal at 2.50 ppm. Elemental analyses (C, H, N, Cl, F) were determined within 0.4% of the theoretical values.

The following compounds were synthesized by published procedures: aromatically substituted 1-(2-chloroethyl)-4-phenylpiperazine and aromatically substituted 1-(3-chloropropyl)-4-phenylpiperazine derivatives [8].

5.1.1. Phenyl ethynyl ketone (**6**)

A solution of chromium trioxide (0.10 mol) in water (30 mL) and concentrated sulphuric acid (8.5 mL) was slowly added to a stirred and cooled solution of propylethynylcarbinol **5** (0.15 mol) in acetone (50 mL). The reaction mixture was carried out at 4 °C in an atmosphere

of nitrogen. After stirring for 7 h, water was added to dissolve the precipitated chromium salts and the product was extracted with chloroform. Evaporation of the organic solution gave a yellow solid which was recrystallized from aqueous methanol to give 16.6 g (85%) of **6** as pale yellow needles. The physical data are in agreement with those given in reference [9].

5.1.2. 4-benzoyl-1H-1,2,3-triazole (**7**)

To a stirred and heated solution of NaN_3 (0.10 mol) in anhydrous dimethylacetamide (80 mL) was slowly added phenyl ethynyl ketone **6** (0.10 mol) dissolved in dimethylacetamide, anhydrous (80 mL). The reaction mixture was kept at 100 °C for 2 h. After stirring for a further 12 h at room temperature, evaporation of the solvent under reduced pressure gave a liquid residue which was diluted with water. The aqueous layer was acidified to pH = 5 with 10% HCl and extracted with ether (3 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After evaporation of the solvent, the solid residue was purified by crystallization from ethyl alcohol to give 16.4 g (95%) of **7**. The physical data are in agreement with those given in reference [10].

5.1.3. General procedure for the preparation of 1- and 2-[2-[4-(X-Phenyl)piperazinyl]ethyl](4-benzoyl)-1,2,3-triazole (**1a–h** *and* **2a–h**)

To a stirred solution of 4-benzoyl-1,2,3-triazole (0.1 mol) and appropriate aromatically substituted 1-(2-chloroethyl)-4-phenylpiperazines (0.15 mol) in 80 mL of butan-2-one was added anhydrous K_2CO_3 (0.3 mol). The mixture was refluxed for 20 h and the solvent was removed under reduced pressure. The residue was diluted with H_2O and extracted with $CHCl_3$ (3 × 200 mL). The combined organic layers were washed with water and dried over Na_2SO_4. Evaporation of the solvent in vacuo provided the crude residue consisting of the expected triazole isomers. The mixture of isomers was separated and purified using a silica-gel column chromatography (diethyl ether/*n*-hexane 95:5 as eluent) to give 1-isomers **1** and 2-isomers **2** in the relative yields provided in *table I*. Spectral data of the title compounds refer to the hydrochloride salts.

5.1.3.1. 1-[2-[4-(Phenyl)piperazinyl]ethyl](4-benzoyl)-1,2,3-triazole·HCL (**1a**)

M.p. 201–202 °C; ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 4H, $2CH_2$-pip), 3.71 (t, *J* = 7.5 Hz, 2H, CH_2N-pip), 3.94 (m, 4H, CH_2-pip), 5.22 (t, *J* = 7.5 Hz, 2H, CH_2N-triaz), 6.97–8.35 (mm, 10H, ArH), 9.17 (s, 1H, H-triaz).

5.1.3.2. 2-[2-[4-(Phenyl)piperazinyl]ethyl](4-benzoyl)-1,2,3-triazole·HCL (**2a**)

M.p. 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 4H, $2CH_2$-pip), 3.71 (t, *J* = 7.5 Hz, 2H, CH_2N-pip), 3.96 (m, 4H, 2-CH_2-pip), 5.32 (t, *J* = 7.5 Hz, 2H, CH_2N-triaz), 6.97–8.30 (mm, 10H, ArH), 8.60 (s, 1H, H-triaz).

5.1.4. General procedure for the preparation of 1- and 2-[3-[4-(X-Phenyl)piperazinyl]propyl](4-benzoyl)-1,2,3-triazole (**3a–h** *and* **4a–h**)

These compounds were prepared in a manner similar to that mentioned above, starting from 4-benzoyl-1,2,3-triazole (0.1 mol) and aromatically substituted 1-(3-chloropropyl)-4-phenyl-piperazines (0.1 mol). The crude mixture of isomers was chromatographed on a silica-gel column (diethyl ether/*n*-hexane 95:5 as eluent) to yield pure **3** and **4** in the relative yields shown in *table I*. Spectral data of title compounds refer to the hydrochloride salts.

5.1.4.1. 1-[3-[4-(Phenyl)piperazinyl]propyl](4-benzoyl)-1,2,3-triazole·HCL (**3a**)

M.p. 183–184 °C; ^{1}H NMR (DMSO-d_6) δ 2.58 (m, 2H, $CH_2CH_2CH_2$), 3.27 (m, 4H, $2CH_2$-pip), 3.32 (t, *J* = 7.5 Hz, 2H, CH_2N-pip), 3.67 (m, 4H, $2CH_2$-pip), 4.76 (t, *J* = 7.5 Hz, 2H, CH_2N-triaz), 6.95–8.37 (mm, 10H, ArH), 9.31 (s, 1H, H-triaz).

5.1.4.2. 2-[3-[4-(Phenyl)piperazinyl]propyl](4-benzoyl)-1,2,3-triazole·HCL (**4a**)

M.p. 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 2H, $CH_2CH_2CH_2$), 3.26 (m, 4H, $2CH_2$-pip), 3.45 (t, *J* = 7.5 Hz, 2H, CH_2N-pip), 3.67–3.88 (m, 4H, $2CH_2$-pip), 4.81 (t, *J* = 7.5 Hz, 2H, CH_2N-triaz), 6.84–8.28 (mm, 10H, ArH), 8.56 (s, 1H, H-triaz).

5.1.5. Hydrochloride salts: general procedure

The hydrochloride salts were prepared by adding an HCl ethereal solution to an ethanolic solution of free bases. Recrystallization solvent, formulae and melting points are reported in *table I*. They were obtained as white crystals.

5.2. Biological methods

5.2.1. 5-HT_{1A} binding assay

Radioligand binding assays were performed following a published procedure [16]. Cerebral cortex from male Sprague-Dawley rats (180–220 g) was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 22 °C) with a Brinkmann Polytron (setting 5 for 15 s), and the homogenate was centrifuged at 50 000 *g* for 10 min. The resulting pellet was then resuspended in the

same buffer, incubated for 10 min at 37 °C, and centrifuged at 50 000 *g* for 10 min. The final pellet was resuspended in 80 volumes of the Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. To each assay tube was added the following: 0.1 mL of the drug dilution (0.1 mL of distilled water if no competing drug was added), 0.1 mL of [^{3}H]-8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]-8-OH-DPAT) in buffer (containing Tris, $CaCl_2$, pargyline, and ascorbate) to achieve a final assay concentration of 0.1 nM, and 0.8 mL of resuspended membranes. The tubes were incubated for 30 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters. The filters were washed twice with 5 mL of ice-cold Tris-HCl buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 5-HT (10 μM).

5.2.2. 5-HT$_{2A}$ and 5-HT$_{2C}$ binding assays

Radioligand binding assays were performed as previously reported by Herndon et al. [17]. Briefly, frontal cortical regions of male Sprague-Dawley rats (200–250 g, Charles River) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4) and centrifuged at 3 000 *g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37 °C for 15 min and then centrifuged twice more at 3 000 *g* for 10 min (with resuspension between centrifugations). The final pellet was resuspended in buffer that also contained 0.1% ascorbate and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0 mL volume containing 5 mg wet weight of tissue and 0.4 nM [^{3}H] ketanserin (hyphen) (76 Ci/mmol; New England Nuclear) for 5-HT$_{2A}$ receptor assays, and 10 mg wet weight of tissue and 1 nM [^{3}H]mesulergine (75.8 Ci/mmol; Amersham) for 5-HT$_{2C}$ receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT$_{2A}$ assay. In the 5-HT$_{2C}$ assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block binding to 5-HT$_{2A}$ receptors. Tubes were incubated for 15 min at 37 °C, filtered on Schliecher and Schuell (Keene, NH) glass fibre filters presoaked in poly(ethylene imine), and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

5.2.3. D-1 dopaminergic binding assay

The binding assay for D-1 dopaminergic receptors was that described by Billard et al. [18]. Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL [^{3}H]SCH-23390 to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]SCH-23390 binding was defined as the difference between binding in the absence or in the presence of 0.1 μM piflutixol.

5.2.4. D-2 dopaminergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Creese et al. [19]. Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL [^{3}H]spiroperidol to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]spiroperidol binding was defined as the difference between binding in the absence or in the presence of 1 μM (+)-butaclamol.

5.2.5. α_1 adrenergic binding assay

The procedure used in the radioligand binding assay has been reported in detail by Greengrass and Bremner [20]. Brain cortex was homogenized in 30 vol. (w/v) ice-cold 50 mM Tris-HCl buffer, (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000 *g* with resuspension of the pellet in fresh buffer. The final pellet was

resuspended in 50mM ice-cold Tris-HCl, (pH 7.4 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]prazosin to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

5.2.6. α_2 adrenergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Perry and U'Prichard [21]. Brain cortex was homogenized in 30 vol. (w/v) ice-cold 5mM tris-HCl, 5mM EDTA buffer (pH 7.3 at 25 °C) using a polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50 000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl, 0.5 mM EDTA (pH 7.5 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]yohimbine to achieve a final concentration of 1 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, 0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]yohimbine binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

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