Journal of Medicinal Chemistry

ARTICLE

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Optimization of (Arylpiperazinylbutyl)oxindoles Exhibiting Selective 5-HT$_7$ Receptor Antagonist Activity

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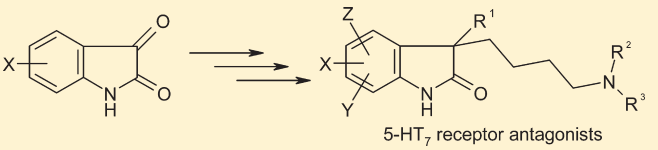
Supporting Information

ABSTRACT: A series of (arylpiperazinylbutyl)oxindoles as highly potent 5-HT$_7$ receptor antagonists has been studied for their selectivity toward the 5-HT$_{1A}$ receptor and α_1-adrenoceptor. Several derivatives exhibited high 5-HT$_7$/5-HT$_{1A}$ selectivity, and the key structural factors for reducing undesired α_1-adrenergic receptor binding have also been identified. Rapid metabolism, a common problem within this family of compounds, could be circumvented with appropriate substitution patterns on the oxindole carbocycle. Contrary to expectations, none of the compounds produced an antidepressant-like action in the forced swimming test in mice despite sufficiently high brain concentrations. On the other hand, certain analogues showed significant anxiolytic activity in two different animal models: the Vogel conflict drinking test in rats and the light−dark test in mice.



5-HT$_7$ receptor antagonists

INTRODUCTION

In recent decades, an increasing demand has emerged for the discovery of new types of anxiolytics and antidepressants. The therapy of these psychiatric diseases is dominated by benzodiazepine-type anxiolytics and selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) antidepressants. However, both classes of drugs possess several drawbacks. Benzodiazepines have sedative, hypnotic, muscle relaxant, and amnesic effects, and their abuse and dependence potential is high, while 5-HT uptake inhibitors produce a therapeutic effect only after several weeks of treatment.

One of the possible alternatives to benzodiazepines and SSRIs is to apply 5-HT$_7$ receptor ligands for the treatment of anxiety, stress, and depression. The 5-HT$_7$ receptor was the last addition to the mammalian 5-HT receptor family.[1,2] This receptor belongs to the group of G-protein coupled receptors that are positively coupled to adenylate cyclase. Several pieces of experimental evidence support the role of the 5-HT$_7$ receptor in the pathomechanism of depression,[3] sleep disorders,[3d,4] stress, and anxiety,[3e,5] and furthermore in learning and memory deficiencies.[4a,6] Continuous interest in 5-HT$_7$ receptor ligands is best demonstrated by the high number of papers and reviews[4a,7] dealing with this topic.

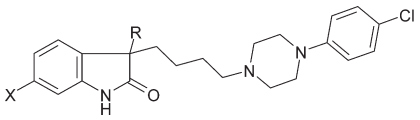
In a recent publication[8] we have demonstrated the pharmacological efficacy of (arylpiperazinylbutyl)oxindoles as 5-HT$_7$ ligands. The structure−activity relationship studies performed led us to compounds that exhibited subnanomolar binding to the 5-HT$_7$ receptor, combined with good selectivity toward the structurally related[9] 5-HT$_{1A}$ receptor. All compounds tested proved to be 5-HT$_7$ receptor antagonists. The selectivity of some compounds with high 5-HT$_7$ receptor affinity was evaluated toward other 5-HT receptor subtypes and a series of other receptors. A representative of the family 3-{4-[4-(4-chlorophenyl)-piperazin-1-yl]butyl}-3-ethyl-6-fluoro-1,3-dihydro-2*H*-indol-2-one (**1a**, Table 1) exhibited selective 5-HT$_7$ receptor antagonist activity. Nevertheless, this derivative was not effective in two different anxiolytic tests: the conflict drinking (Vogel) test[10] and the light−dark test.[11] On the other hand, we have demonstrated that **2a** (Table 1) showed outstanding anxiolytic efficacy in both tests, although it had lower 5-HT$_7$ receptor affinity and it was less selective toward the 5-HT$_{2A}$ receptor than **1a**. As the microsomal metabolic stability of the two compounds is poor, in case of **2a** one can not exclude the formation of an active metabolite, which is responsible for the anxiolytic effect. Thus, in the present study we aimed to evaluate also the structure−metabolic stability relationship of the synthesized compounds. Furthermore, we also assayed the affinity of our new chemical entities (NCEs) for the α_1-adrenoceptor (α_1-AR) as a potential source of serious cardiovascular side effects, because compounds with an arylpiperazine moiety can likely bind to this receptor.[12] Lack of α_1-AR affinity is also important, as blockade of this receptor can diminish the efficacy of antidepressant drugs.[13,14]

CHEMISTRY

Synthesis of 3,3-disubstituted oxindole derivatives was carried out in most cases via the synthetic route described in our previous paper (**1**, Scheme 1, route A).[8] Isatin (**3**, X = H) is commercially available, and the substituted isatins (**3**, X = 5-F, 6-F) were

Received: May 3, 2011
Published: August 22, 2011

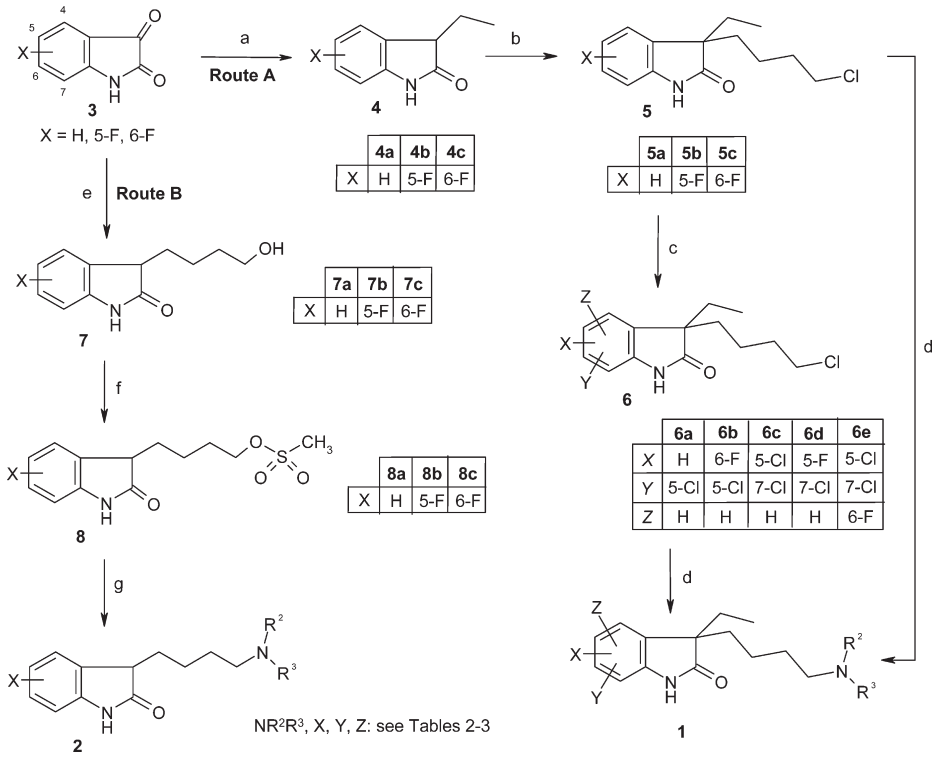
Table 1. Representative Receptor Affinity Data and Anxiolytic Activity of Two Oxindole-Type 5-HT$_7$ Receptor Antagonists



compound	R	X	5-HT$_7$[a] K_i (nM)	5-HT$_{1A}$[a] K_i (nM)	5-HT$_{2A}$[a] K_i (nM)	conflict drinking test[b] MED (mg/kg ip)	light−dark test[c] MED (mg/kg ip)
1a	Et	F	0.79	1610	19.4	>20	>10
2a	H	H	7.0	1800	17.5	<2.5	<1

[a] Receptors and radioligands used in binding assays and data analysis: see Experimental Section. [b] A modification of the Vogel[10] method was used for the conflict drinking test in rats, drugs were administered intraperitoneally. For details, see Experimental Section. [c] Testing of drugs in the light−dark test was carried out in mice as described by Costall[11] after intraperitoneal administration of the drugs. For details, see Experimental Section.

Scheme 1[a]



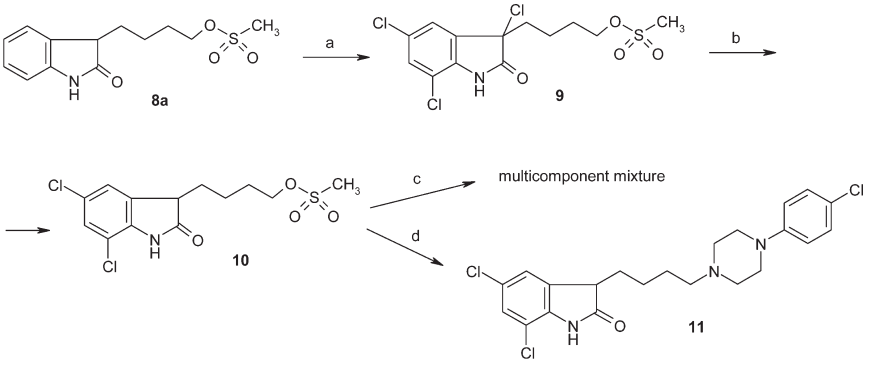
[a] Reagents and conditions: (a) EtOH, Ra−Ni, 15 bar H_2, 180−210 °C, 3−5 h, 71−91%; (b) BuLi, Br-$(CH_2)_4$-Cl, THF, −78 °C to rt, 4 h, 86−95%; (c) chlorination at the 5-position: SO_2Cl_2, glacial AcOH, 16−18 °C, 2 h, 86−93%; 7-chlorination or 5,7-dichlorination: SO_2Cl_2, glacial AcOH, 60 °C, 3 h, 65−79%; (d) HNR^2R^3, Na_2CO_3, in melt, 180 °C, 1−2 h, 39−81%; (e) HO-$(CH_2)_4$-OH, Ra−Ni, 15 bar H_2, 190 °C, 4−5 h, 75−81%; (f) CH_3SO_2Cl, Et_3N, THF, −78 °C to rt, 1−2 h, 81−93%; (g) HNR^2R^3, Na_2CO_3, in melt, 120 °C, 1 h, 41−82%.

synthesized, starting from anilines, by the classical Sandmeyer procedure.[15] The one-pot reductive alkylation of these starting materials (with alcohols, in the presence of Raney nickel) to the corresponding 3-alkyloxindoles (**4**) was performed by the method elaborated in our laboratory.[16] Selective C(3)-alkylation of compounds **4** was carried out using 1-bromo-4-chlorobutane after deprotonation with BuLi,[17] resulting in 3-ethyl-3-(4-chlorobutyl)oxindole intermediates **5**. Chlorination of compounds **5** afforded the corresponding mono- and dichloro derivatives **6**. Reaction of **5a** and **5c** with sulfuryl chloride (3 equiv) in glacial acetic acid at 16−18 °C yielded selectively the 5-chloro derivatives (**6a** and **6b**), while at 60 °C the 7-position was also affected; thus, 7-chloro-5-fluoro (**5b** → **6d**), 5,7-dichloro (**5a** → **6c**), or 5,7-dichloro-6-fluoro (**5c** → **6e**) analogues were obtained. Finally, replacement of the side-chain chlorine atom with various secondary amines in melt led to the target compounds **1**. When necessary, the product was purified by column chromatography. In cases where it did not result in a solid compound, the oily products were converted into their hydrochloride salts.

A different route was applied for the synthesis of the 3-monosubstituted derivatives (**2**, Scheme 1, route B). Isatins (**3**) were reductively hydroxybutylated with butane-1,4-diol in the presence of Ra−Ni to afford 3-(4-hydroxybutyl)oxindoles (**7**).[16] Mesylation of compounds **7** with methanesulfonyl chloride to derivatives **8**,

dx.doi.org/10.1021/jm200547z |J. Med. Chem. 2011, 54, 6657–6669

Scheme 2[a]



[a] Reagents and conditions: (a) SO_2Cl_2, reflux, 4 h, 80%; (b) Ra−Ni, 20 bar H_2, rt, 18 h, 79%; (c) 1-(4-chlorophenyl)piperazine, Na_2CO_3, in melt, 120−180 °C, 1−2 h; (d) 1-(4-chlorophenyl)piperazine, acetonitrile, evaporation, then rt, 2 h, 47%.

followed by treatment with the appropriate secondary amines, led to the desired compounds **2**. Finally, the crude products were purified by column chromatography, and, if necessary, they were converted into their hydrochloride or oxalate salts.

A different pathway had now to be elaborated for the preparation of 3-monosubstituted 5,7-dichloro derivative **11** (Scheme 2). It is known from the literature[18] that chlorination (SO_2Cl_2) or bromination (Br_2, NBS) of 3-unsubstituted or 3-monosubstituted oxindoles leads primarily to halogenation at position 3, while aromatic substitution at C(5) and especially at C(7) necessitates harsher conditions. Thus, selective direct 5,7-dichlorination of **8a** to **10** could not be expected. Therefore, mesyl ester **8a** was 3,5,7-trichlorinated, resulting in compound **9**. Since selective removal of the 3-chloro substituent using catalytic hydrogenation could only be expected under mild conditions, Raney nickel was used as catalyst in THF. Preparation of **10** was accomplished in 79% yield. Coupling of mesylate **10** with 1-(4-chlorophenyl)piperazine was first attempted in melt, in the presence of Na_2CO_3. Although we have synthesized all other derivatives using this methodology, we surprisingly found that in this particular case, a multicomponent reaction mixture was obtained. HPLC-MS analysis of the mixture showed that the desired compound **11** was only a minor component. Finally, a modified version of the coupling reaction led to **11.** The acetonitrile solution of **10** and 1-(4-chlorophenyl)piperazine was evaporated, and the thus obtained intimate mixture was stirred at ambient temperature for 2 h. After chromatographic purification, **11** was obtained in 47% yield.

RESULTS AND DISCUSSION

The above results encouraged us to synthesize further derivatives and to study the structure−activity relationship of this family of compounds more in detail. In the course of our previous work,[8,19] compounds unsubstituted at the oxindole nitrogen atom and containing a tetramethylene spacer between the oxindole skeleton and the basic nitrogen atom proved to be the most potent and most selective 5-HT_7 receptor ligands, so further investigations, disclosed in the present paper, were performed with such derivatives. Starting from the simplest representative of this family, 3-[4-(4-phenylpiperazin-1-yl)butyl]-1,3-dihydro-2*H*-indol-2-one (**2b**, Table 2), systematic modifications were performed: (i) variation of the basic group, (ii) introduction of an alkyl substituent at position 3 of the oxindole ring, (iii) introduction of one or more halogen atoms. The effect of halogenation was studied both on the oxindole carbocycle and the aromatic ring of the basic group, so that we could determine how electronegative halogen atoms affect the receptor binding affinities. Binding of the compounds was evaluated to a series of 5-HT and other receptors. Undesired 5-HT_{1A} receptor affinity had already been a crucial element in our previous study for the evaluation of the receptor profile of the compound family. Because blockade or activation of α_{1A}-ARs can cause cardiovascular side effects, α_{1A}-AR affinity was also measured for all compounds.

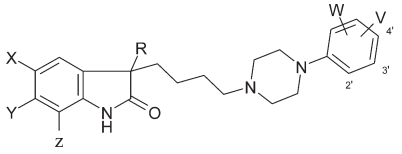
The pharmacological action of compounds demonstrating high 5-HT_7 receptor affinity and an acceptable selectivity profile was evaluated in two anxiolytic tests, the conflict drinking test in rats[10] and light−dark test in mice,[11] and in a model assessing the depression-like behavior, the forced swimming test in mice.[20−22] Oxindoles with piperazine basic group are summarized in Table 2, and derivatives with different basic groups are shown in Table 3.

5-HT_7 Receptor Binding. Data show that most of the compounds synthesized were very potent 5-HT_7 receptor ligands (Tables 2 and 3). Not surprisingly, receptor affinities could be influenced by various substitution patterns of the phenylpiperazine moiety. Compounds having a CF_3, OMe, F, or Cl substituent on the phenyl group showed 5-HT_7 receptor K_i values ranging from 0.38 to 107 nM. The position of substituents also affected the 5-HT_7 receptor affinity. As demonstrated in our previous publication,[8] among methoxyphenyl analogues, the position of the substituent changed the binding significantly, in an order of meta > ortho ≫ para. These results were in accordance with the ones published recently for a structurally related family of compounds.[23] However, in the case of fluoro compounds, the above postulated trend failed, the para-substituted compound (**1o**) proved to be a more potent 5-HT_7 receptor ligand than the meta-substituted analogue (**1n**). Concerning the chloro-substituted analogues, both 3-chloro- (**2d,e**, **1d**−**g**) and 4-chlorophenylpiperazines (**1h**−**m**, **2a**, **2f,g**) show high affinity for the 5-HT_7 receptor. In our hands, the 3,4-dichloro analogue (**1r**) was similarly potent, contrary to the expectations based on literature data.[24]

A halogen substituent on the oxindole carbocycle did not substantially change the 5-HT_7 receptor affinities, as 5-chloro, 5-fluoro,[25] and 6-fluoro derivatives had similar K_i values to the unsubstituted derivatives. Na et al. drew the same conclusion regarding the effect of fluorine substitution on 5-HT_7 receptor

dx.doi.org/10.1021/jm200547z |*J. Med. Chem.* 2011, 54, 6657–6669

Table 2. Oxindoles with Phenylpiperazine-Type Basic Groups



compound	V	W	X	Y	Z	R	5-HT$_7$[a] K_i (nM)	5-HT$_{1A}$[a] K_i (nM) or % at 10^{-7} M	α_1-AR[a] K_i (nM)
2b	H	H	H	H	H	H	16	243 nM	22
2c	H	H	F	H	H	H	47	154 nM	20
1b	2′-MeO	H	H	H	H	Et	5.4	9.8 nM	27
1c	2′-Cl	H	H	H	H	Et	53	30 nM	64
2d	3′-Cl	H	H	H	H	H	0.49	66 nM	26
1d	3′-Cl	H	H	H	H	Et	0.40	46 nM	31
2e	3′-Cl	H	H	F	H	H	6.7	118 nM	12.5
1e	3′-Cl	H	F	H	H	Et	2.1	111 nM	46
1f	3′-Cl	H	Cl	F	H	Et	9.07	16%	117
1g	3′-Cl	H	Cl	F	Cl	Et	72	680 nM	97
2a	4′-Cl	H	H	H	H	H	7.0	1800 nM	42
2f	4′-Cl	H	F	H	H	H	40	0%	116
2g	4′-Cl	H	H	F	H	H	25	6%	55
1h	4′-Cl	H	H	H	H	Et	0.38	11%	93
1i	4′-Cl	H	F	H	H	Et	2.81	9%	190
1j	4′-Cl	H	Cl	H	H	Et	1.1	0%	175
1a	4′-Cl	H	H	F	H	Et	0.79	9%	215
1k	4′-Cl	H	Cl	F	H	Et	10	0%	191
1l	4′-Cl	H	Cl	H	Cl	Et	9.5	5%	110
1i	4′-Cl	H	Cl	H	Cl	H	107	ND[b]	757
1m	4′-Cl	H	Cl	F	Cl	Et	96	10%	380
1n	3′-F	H	H	H	H	Et	11	73 nM	ND
2h	4′-F	H	H	H	H	H	5.0	10%	14
1o	4′-F	H	H	H	H	Et	0.43	10%	39
1p	3′-CF_3	H	H	H	H	Et	5.1	36 nM	ND
1q	2′-Cl	4′-Cl	H	H	H	Et	139	11%	380
1r	3′-Cl	4′-Cl	H	H	H	Et	0.60	11%	125

[a] Receptors and radioligands used in binding assays and data analysis: see Experimental Section. [b] Not determined.

affinity in quinazolinone-type agents.[24] However, when studying compounds di- (e.g., **1f**, **1l**) or especially trihalogenated (**1g**, **1m**) on the aromatic ring of the oxindole skeleton, a clear tendency of diminished 5-HT$_7$ receptor potency could be observed.

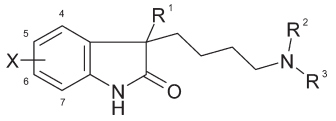
Selectivity toward the 5-HT$_{1A}$ Receptor. The examination performed by Wilcox et al. demonstrated close similarities between the 5-HT$_7$ and 5-HT$_{1A}$ receptor binding sites that can explain difficulties in developing selective ligands for either of the above receptors.[9] This finding is well exemplified by "long-chain arylpiperazines",[26] an intensively investigated family of serotonergic compounds, which, besides being 5-HT$_7$ receptor agents, have long been known to be strong 5-HT$_{1A}$ receptor ligands as well, as reported by Leopoldo et al. and other research groups.[7a,27] We now report our efforts in identifying a new series of 5-HT$_7$ receptor ligands of arylpiperazine-type with good selectivity toward the 5-HT$_{1A}$ receptor.

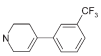
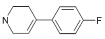
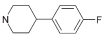
Selectivity toward the 5-HT$_{1A}$ receptor was required, although stimulation of this serotonin receptor may elicit an anxiolytic action as exemplified by buspirone, a marketed 5-HT$_{1A}$ receptor partial agonist.[28] On the contrary, inhibition of the 5-HT$_{1A}$ receptor is anxiogenic in animal experiments, and anxiolytic effects of some compounds, depending on the mechanism of action, can be blocked by WAY 100635, a 5-HT$_{1A}$ receptor antagonist.[29] The picture is further complicated by results demonstrating that the anxiolytic effect of 5-HT$_{1A}$ receptor ligand anxiolytic drugs was modified by acute stress in mice, and adrenalectomy turned the anxiolytic effect of 8-OH-DPAT to an anxiogenic effect in rats.[30] Therefore, the results produced by 5-HT$_{1A}$ receptor ligands in animal experiments may not be easily extrapolated to humans.

Selectivity of various 3-(arylpiperazinylalkyl)oxindoles (type **1**, **2**) toward the 5-HT$_{1A}$ receptor was investigated in detail in our recent paper.[8] Previous studies revealed that the substitution pattern of the phenylpiperazine ring strongly influenced the 5-HT$_{1A}$ receptor affinity.[31] Therefore, we also aimed at varying the substituents on the aromatic ring of the basic group.

dx.doi.org/10.1021/jm200547z |J. Med. Chem. 2011, 54, 6657–6669

Table 3. Oxindoles with Basic Groups Other Than Phenylpiperazines



Compound	X	R^1	NR^2R^3	5-HT_7[a] K_i (nM)	5-HT_{1A}[a] K_i (nM) or % at 10^{-7} M	α_1-AR[a] K_i (nM) or % at 10^{-7} M
2i	H	H		10.9	56 nM	130 nM
1s	H	Et		4.0	55 nM	150 nM
2j	6-F	H		16.8	99 nM	33 nM
2k	H	H		11.1	19 nM	310 nM
2l	5-F	H		40	55 nM	11%
1t	H	Et		1.2	62%	46 nM
1u	H	Et		6.9	11%	29 nM
1v	5-F	Et		52	123 nM	140 nM
2m	6-F	H		13	72%	ND[b]

[a] Receptors and radioligands used in binding assays and data analysis: see Experimental Section. [b] Not determined.

According to the data in Tables 2 and 3, selectivity toward the 5-HT_{1A} receptor was acceptable in most cases. Compounds **2b** and **2c** containing an unsubstituted phenylpiperazine moiety showed some selectivity, in accordance with the results of other research groups.[32] Derivative **1b** exhibited a rather strong 5-HT_{1A} receptor binding, due to the presence of the 2-methoxyphenylpiperazine moiety, which is a key fragment in many 5-HT_{1A} receptor ligands.[32a,33] In our previous paper[8] we demonstrated that both the 3- and 4-methoxyphenyl derivatives had an affinity for the 5-HT_{1A} receptor weaker than that of the 2-methoxy congener.[34] Concerning the 3-trifluoromethyl moiety on the phenylpiperazine basic group, the literature data are contradictory: this substituent favors 5-HT_{1A} receptor binding in certain families of compounds,[35] while one paper reports low 5-HT_{1A} receptor affinity of these derivatives.[31a] Our observations supported the former finding, i.e., strong 5-HT_{1A} receptor affinity and poor 5-HT_7/5-HT_{1A} selectivity, independently of whether the 3-CF_3 substituent was on a phenylpiperazine (**1p**) or on an aryl-1,2,3,6-tetrahydropyridine (**2k**,**l**) moiety.

Bojarski et al. found that 3-chlorophenylpiperazine derivatives had strong affinity for the 5-HT_{1A} receptor in two different compound families.[32b,35b] According to our measurements, 5-HT_{1A} receptor binding of 3-chlorophenylpiperazine compounds unsubstituted on the oxindole skeleton was moderate (**1d**, **2d**), and it could be further reduced with appropriate substitution patterns (**1e**−**g**). When changing the position of the chlorine atom to position 4, 5-HT_{1A} receptor affinity decreased dramatically, while the 5-HT_7 receptor binding was maintained. The 4-chloro and 4-fluoro substituents on the phenylpiperazine ring positively influenced both 5-HT_7 receptor binding and selectivity toward the 5-HT_{1A} receptor (cp., e.g., **2a**−**2b**, **2b**−**h**), contrary to the literature data.[31a] 2,4-Dichloro (**1q**) and 3,4-dichloro (**1r**) derivatives maintained the high selectivity of the 4-monochloro analogue (**1h**) toward the 5-HT_{1A} receptor, although in the former case with lower 5-HT_7 receptor affinity. A similarly low 5-HT_{1A} receptor binding caused by 2,3-dichlorination of the phenylpiperazine ring was also observed by Wustrow et al.[31a]

Alterations in receptor bindings caused by replacement of the phenylpiperazine moiety with other basic groups were also studied. Although 2-pyridylpiperazine derivatives were found by other researchers to exhibit strong 5-HT_{1A} receptor binding,[31,36] our results demonstrated that the 5-HT_7 and 5-HT_{1A} receptor affinities of the 2-pyridylpiperazine analogues were very similar to those of the corresponding phenylpiperazine analogues (cp., **2b**−**2i**). When the piperazine moiety was replaced with the analogously substituted 1,2,3,6-tetrahydropyridine, the 5-HT_{1A} receptor affinity did not decrease and the 5-HT_7/5-HT_{1A} selectivity did not improve either (cp., **1o**−**1t**, **1e**−**1v**).

Selectivity toward the α_1-Adrenoceptor. Inhibitors of the α_1-AR play an important role in the treatment of benign prostatic hyperplasia (BPH)[37] and hypertension.[38] Arylpiperazines are one of the most studied classes of molecules exhibiting affinity for the α_1-AR.[12] However, it was our aim to synthesize compounds that exhibit good selectivity not only toward the 5-HT_{1A} receptor but also toward the α_1-AR, thereby decreasing the risk of cardiovascular side effects. Low affinity for the α_1-AR is important to avoid the potential hypotensive or hypertensive effects of compounds due to blockade or activation of the α_1-AR in the vascular bed, respectively. Another reason to avoid α_1-AR affinity of our NCEs was to prevent interference with the assumed antidepressant-like effect of 5-HT_7 receptor antagonists via inhibition of central α_1-ARs. It has been shown for some compounds such as lamotrigine[13] and chlorpheniramine[14] that their antidepressant-like effect was antagonized by α_1-AR inhibitors or potentiated by α_1-AR activation in the tail suspension or forced swimming tests in mice. On the other hand, the antidepressant-like effect of imipramine was not altered by the α_1-AR antagonist prazosin in the same animal experiments.[14] Therefore, we set a minimum of 50-fold selectivity ratio between the 5-HT_7 receptor and the α_1-AR affinities for selecting compounds for further development.

Data in Tables 2 and 3 demonstrate that the majority of our derivatives, as expected, are also α_1-AR ligands. The unsubstituted phenylpiperazine derivatives (**2b**,**c**) and the 4-fluorophenyl compounds (**1o**, **1t**,**u**, **2h**) possessed high α_1-AR binding. Similarly, 3-chlorophenylpiperazine derivatives (**1d**−**g**, **2d**,**e**), which otherwise had strong 5-HT_7 receptor affinity, and displayed partial selectivity toward the 5-HT_{1A} receptor, had stronger α_1-AR than 5-HT_{1A} receptor binding.

Although literature results showed that 2-methoxy substitution on the phenylpiperazine aromatic ring decreased the α_1-AR binding of compounds,[32a] other studies of Perrone et al.[31b,39] and other research groups[36] demonstrated, in accordance with our results with **1b**, that these derivatives had high affinity not only for the 5-HT_7 and 5-HT_{1A} receptors but also for the α_1-AR. 2-Pyridylpiperazine derivatives (**1s**, **2i**,**j**), known from the literature as moderate α_1-AR ligands,[36] also exhibited strong α_1-AR affinity in our hands.

In terms of low α_1-AR binding, the 3-trifluoromethylphenyl-1,2,3,6-tetrahydropyridine basic group in **2k** and **2l** proved to be advantageous.[40,41] However, these derivatives showed very strong affinity for the 5-HT_{1A} receptor, as discussed above. On the other

dx.doi.org/10.1021/jm200547z |J. Med. Chem. 2011, 54, 6657–6669

Table 4. Microsomal Metabolic Stability and in Vivo Anxiolytic and Antidepressant Activity of Some Selective (upper portion of table) and Nonselective (lower portion of table) Compounds

compound	5-HT$_7$[a] K_i (nM)	5-HT$_{1A}$[a] K_i (nM) or % at 10^{-7} M	ratio of 5-HT$_{1A}$ vs 5-HT$_7$	α_1-AR[a] K_i (nM)	ratio of α_1 vs 5-HT$_7$	MF%[b] rat	MF%[b] human	conflict drinking test[c] MED (mg/kg) ip	light−dark test[d] MED (mg/kg) ip	forced swimming test[e] MED (mg/kg) ip
1a	0.79	9%	≫100	215	272	9	29	ND[f]	>10	>10
1d	0.40	46 nM	115	31	78	4	31	>10	>20	>10
1h	0.38	11%	≫100	93	245	9	34	10	>10	>10
1i	2.81	9%	>100	190	68	17	36	ND	>10	>10
1j	1.1	0%	≫100	175	159	21	23	>20	ND	>10
1o	0.43	10%	≫100	39	91	8	34	10	<1	>10
1r	0.60	11%	≫100	125	208	13	29	>20	ND	>10
2d	0.49	66 nM	135	26	53	9	39	<5	>10	>10
1f	9.07	16%	>100	117	13	16	46	ND	ND	ND
1k	10	0%	>100	191	19	18	10	ND	ND	ND
1l	9.5	5%	>100	110	12	76	53	5	1	>30
1m	96	10%	ND	380	4	72	76	>20	>10	>30
1q	139	11%	ND	380	3	17	28	ND	ND	ND
2a	7.0	1800 nM	257	42	6	10	52	<2.5	<1	ND
2f	40	0%	>100	116	3	28	44	ND	ND	ND

[a] Receptors and radioligands used in binding assays and data analysis: see Experimental Section. [b] In vitro microsomal metabolic stability. For the protocol of its determination, see Experimental Section. [c] A modified version of the Vogel[10] method was used for the conflict drinking test in rats, drugs were administered intraperitoneally. For details, see Experimental Section. [d] Testing of drugs in the light−dark test was carried out in mice as described by Costall[11] after intraperitoneal administration of the drugs. For details, see Experimental Section. [e] The forced swimming test of compounds administered intraperitoneally was carried out in male NMRI mice according to the method of Lucki et al.[20] For details, see Experimental Section. [f] Not determined.

hand, 4-chlorophenylpiperazine derivatives exhibited substantially lower α_1-AR binding than the corresponding 3-chloro analogues (cp., **1e**−**1i**, **1d**−**1h**). When the 3-unsubstituted and the 3-ethyl series are compared, it can be stated that the presence of the 3-ethyl substituent is favorable (cp., e.g., **1h**−**2a**). Halogen substituents at positions 5, 6, or 7 of the oxindole carbocycle did not significantly influence the α_1-AR affinity. Taking into consideration all the compounds in Tables 2 and 3, there are only a few, namely 4-chlorophenylpiperazine compounds **1a**, **1h**, and **1r**, that were more than 100-fold selective toward both the 5-HT$_{1A}$ receptor and α_1-AR.

Lead Compound Selection and Further Optimization. For a better understanding of the structure−metabolic stability relationship, eight selective 5-HT$_7$ ligands (Table 4, upper portion) and seven compounds not selective for the α_1-AR (Table 4, lower portion) were then tested for their metabolic stability in rat and human liver microsomes, in vitro, for prediction of their oral bioavailability. The anxiolytic activity of most compounds was determined in the conflict drinking (Vogel) test[10] in rats and the light−dark test[11] in mice. The antidepressant-like effect of the 5-HT$_7$ receptor ligands was tested using the forced swimming test (Porsolt test or FST) in mice.[3e,22] It was found that 5 out of 15 compounds showed anxiolytic activity in the Vogel test. The most active compound, **2a**, produced an effect at 2.5 mg/kg ip, while **1l** and **2d** as well as **1h** and **1o** were effective at 5 mg/kg ip and at 10 mg/kg ip, respectively. Among these, **1l**, **1o** and **2a** were also effective at low dose (1 mg/kg ip or lower) in the light−dark test in mice. Except for **1l**, the compounds exhibited poor metabolic stability prediction for humans; thus, these compounds were not suitable for further development, despite two of them (**1h**, **1o**) having good receptor selectivity.

In terms of bioavailability, serious problems were described in the literature also for tetrahydrobenzindolinones, a structurally related family of compounds.[42] Halogen substitution somewhat increased the in vitro metabolic stability; however, the measured value of bioavailability (18% in rats for compound DR4485) was still quite low.[43] We encountered the same difficulty when investigating the oxindole family. Microsomal metabolic stability tests, in vitro, demonstrated that the bioavailability prediction of most oxindole derivatives for rats and humans was below 20% and 40%, respectively, making these compounds hardly appropriate for further development (Table 4). Interestingly, the 3-unsubstituted derivatives were more stable than the corresponding 3-ethyl analogues (cp., e.g., **1h**−**2a**, **1i**−**2f**). In an attempt to improve metabolic stability we introduced halogen substituents at different positions of the molecule. It became obvious that halogenation of the aromatic ring of the arylpiperazine or aryltetrahydropyridine moieties did not result in stable derivatives, not even in the case of compounds bearing dichlorinated aromatic rings (**1q**, **1r**). As a next step, the same approach was applied to the oxindole carbocycle. The introduction of a fluorine atom at position 5 or 6, or a chlorine atom at position 5 of the carbocycle, did not improve the stability. The presence of a substituent at both positions simultaneously (**1f**, **1k**) also failed to increase metabolic stability, in vitro. On the other hand, 5,7-dichloro (**1l**) and 5,7-dichloro-6-fluoro (**1m**) substitutions led to metabolically stable compounds, suggesting that blocking position 7 of the oxindole ring was the key step for achieving metabolic stability. These two compounds were selective toward the 5-HT$_{1A}$ receptor, but only a low (4 to 12-fold) selectivity was observed toward the α_1-AR (Table 4).

Table 5. Plasma and Brain Concentrations of 5-HT$_7$ Selective Compounds after ip Administration at Various Doses in Male NMRI Mice (n = 3/dose)

dose (mg/kg ip)	plasma concentration (nM)	brain concentration (nM)	BBBP (%)
	1a		
1	46 ± 9	33 ± 3	77 ± 11
3	130 ± 11	99 ± 3	77 ± 5
10	177 ± 95	135 ± 67	79 ± 4
	1d		
1	22 ± 13	65 ± 47	244 ± 50
3	168 ± 10	613 ± 60	364 ± 14
10	272 ± 144	824 ± 523	266 ± 35
	1h		
1	70 ± 39	42 ± 7	89 ± 26
3	245 ± 133	141 ± 15	87 ± 28
10	399 ± 82	379 ± 78	95 ± 4
	1i		
1	44 ± 31	51 ± 19	188 ± 53
3	211 ± 157	186 ± 49	188 ± 69
10	271 ± 1	342 ± 79	177 ± 22
	1j		
1	10 ± 2	17 ± 1	172 ± 24
3	14 ± 4	23 ± 1	191 ± 53
10	8 ± 4	27 ± 19	271 ± 62
	1o		
1	34 ± 23	62 ± 34	226 ± 37
3	178 ± 14	431 ± 21	245 ± 22
10	168 ± 101	364 ± 191	233 ± 27
	1r		
1	31 ± 19	43 ± 29	128 ± 11
3	174 ± 126	223 ± 170	117 ± 10
10	861 ± 390	1154 ± 537	125 ± 10
	2d		
1	21 ± 18	216 ± 173	1310 ± 171
3	114 ± 97	827 ± 668	854 ± 77
10	744 ± 339	4311 ± 1924	615 ± 40

Published data showed that the majority of 5-HT$_7$ receptor antagonists with various chemical structures possessed an antidepressant effect.[44] Surprisingly, despite their high 5-HT$_7$ receptor affinity, none of the oxindole derivatives tested demonstrated a significant antidepressant activity. Even compounds with subnanomolar affinity for the 5-HT$_7$ receptor failed to change the immobility time in the mouse FST.

In order to check further if poor metabolic stability explains the lack of antidepressant effect of the eight selective high-affinity 5-HT$_7$ receptor ligands (**1a**, **1d**, **1h**−**j**, **1o**, **1r**, **2d**), their plasma and brain concentrations were measured 30 min after ip administrations at the doses used also in the FST (see results in Table 5). The plasma and brain concentrations increased dose-dependently in most cases and varied considerably among the eight compounds. Poor microsomal bioavailability and relatively high plasma and brain concentrations of some compounds are not necessarily in contradiction, as these compounds can have better microsomal metabolic stability in mice than in rats and humans. Blood−brain barrier permeability (BBBP), expressed as the percentage of brain to plasma concentrations, ranged from about 0.7 to about 8, proving good brain penetration of the compounds. Molar brain concentrations were above the 5-HT$_7$ K_i values for all compounds even at the low dose tested (1 mg/kg ip), and, except for **1j**, they were a minimum of 100-fold higher than the individual K_i values at the high dose administered (10 mg/kg ip). On the basis of the above results, it can be stated that free brain concentrations were likely above the level necessary to produce sufficient 5-HT$_7$ receptor occupancy. Therefore, the lack of antidepressant effect of our oxindole derivatives may not be explained with poor metabolic stability, inappropriate pharmacokinetics, or insufficient blood−brain barrier penetration.

CONCLUSION

In our earlier study we reported on a new compound family, (arylpiperazinylbutyl)oxindoles, which proved to be highly potent 5-HT$_7$ receptor antagonists. The compounds were tested routinely for their 5-HT$_{1A}$ receptor binding, and several selective 5-HT$_7$ receptor agents were identified. Certain analogues (e.g., **2a**) also showed anxiolytic activity in in vivo animal models.

In the present paper we aimed at studying also the selectivity toward the α_1-AR and metabolic stability, by involving new, structurally optimized derivatives, as well. Moreover, antidepressant activity of selected compounds was now also tested in the forced swimming test in mice.

Our lead compound **2a** was not selective toward the α_1-AR, and its metabolic stability was also insufficient. In the present study, several compounds exhibiting strong affinity for the 5-HT$_7$ receptor were selective toward the 5-HT$_{1A}$ receptor and in some cases toward the α_1-AR. We also successfully identified structural key elements of metabolic stability within this family. Some of the selective 5-HT$_7$ receptor ligands were active in one or two anxiolytic tests, the Vogel conflict drinking test in rats and the light−dark test in mice. Surprisingly, none of the tested compounds produced an antidepressant-like action in the forced swimming test in mice.

The current study greatly advanced the understanding of the receptor binding and metabolic stability SAR in the (arylpiperazinylbutyl)oxindole family. As all compounds are chiral, enantiomer separation can be a further step in the hope of improving the receptor selectivity and anxiolytic efficacy of our compounds.

EXPERIMENTAL SECTION

Chemistry. All melting points were determined on a Büchi 535 capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Bruker Vector 22 FT spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Unity Inova 400 (400 and 100 MHz for ^{1}H and ^{13}C NMR spectra, respectively) or 500 (500 and 125 MHz for ^{1}H and ^{13}C NMR spectra, respectively) spectrometer using TMS as internal standard. Chemical shifts (δ) and coupling constants (J) are given in ppm and in hertz (Hz), respectively. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer. Measured elemental analysis data were in the range of calculated value ± 0.4%, except where the measured value is given specifically. All reactions were followed by analytical thin layer chromatography (TLC) on silica gel 60 F_{254}. Hydrogenation reactions were carried out in an autoclave (volume: 250 mL), which was equipped with a temperature

controller, a manometer (60 bar), a valve for a gas inlet, and a magnetic stirrer. Degussa's activated Raney nickel catalyst in water was used as hydrogenation catalyst. Synthetic procedures, yields, melting point, spectral data, and elemental analyses of all new compounds are described below. Purity of new compounds was above 95%, based on ^{1}H NMR.Compounds **1a**,**b**, **1d**−**f**, **1h**−**j**, **1o**, **1r**, **1t**,**u**, **2a**, and **2c**,**d** were already described in our earlier paper,[8] although with a smaller set of biological data.

*General Procedure A: Coupling Reaction of the 3-Ethyl-3-(4-chlorobutyl)oxindole Intermediates (**5** or **6**) with the Appropriate Secondary Amines.* The melt of the secondary amine (12 mmol) was heated to 180 °C under slow stirring. The appropriate 3-ethyl-3-(4-chlorobutyl)-oxindole (**5** or **6**, 12 mmol) and sodium carbonate (1.36 g, 12 mmol) were added. After 1 h reaction time, the brown melt was cooled to ambient temperature. Ethyl acetate and water were added, and the layers were separated. The organic layer was dried over $MgSO_4$ and evaporated. The residual oil or solid was purified by column chromatography using ethyl acetate as eluent.

General Procedure A/1. In case the product of the chromatographic purification crystallized upon treatment with diethyl ether, it was triturated in this solvent and filtered, and the solid was recrystallized from the solvent indicated below and dried in vacuo to give a white solid.

General Procedure A/2. In case the product of the chromatographic purification did not crystallize upon treatment with diethyl ether, it was dissolved in diethyl ether (200 mL), the solid residue was removed by filtration, and a calculated amount (1 equiv) of hydrogen chloride (saturated solution of HCl gas in diethyl ether) was added dropwise, under vigorous stirring. The white precipitate was filtered, washed with diethyl ether and hexane, and dried in vacuo. Where indicated, the obtained hydrochloric salt was recrystallized from the solvent indicated to give a white solid.

*5,7-Dichloro-3-{4-[4-(3-chlorophenyl)piperazin-1-yl]butyl}-3-ethyl-6-fluoro-1,3-dihydro-2H-indol-2-one Hydrochloride (**1g**).* The title compound was prepared according to the general procedure A/2, starting from 5,7-dichloro-3-(4-chlorobutyl)-3-ethyl-6-fluorooxindole (**6e**) and 1-(3-chlorophenyl)piperazine. Yield: 39%, mp 238−240 °C (isopropyl alcohol). IR (KBr): 3426, 1723 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.53 (3H, t, J = 7.3 Hz), 0.90−0.75 (1H, m), 1.00−0.90 (1H, m), 1.70−1.55 (2H, m), 1.90−1.70 (4H, m), 2.98 (4H, br s), 3.16 (2H, t, J = 12.0 Hz), 3.43 (2H, br s), 3.84 (2H, d, J = 12.6 Hz), 6.86 (1H, dd, J = 1.7, 7.9 Hz), 6.94 (1H, dd, J = 2.3, 8.4 Hz), 7.03 (1H, t, J = 2.0 Hz), 7.25 (1H, t, J = 8.2 Hz), 7.58 (1H, d, J = 6.8 Hz), 10.9 (1H, br s), 11.3 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 8.5, 21.3, 23.2, 30.2, 36.4, 45.0, 50.4, 54.6, 55.0, 103.2 (d, J = 22.5 Hz), 113.0 (d, J = 18.3 Hz), 114.3, 115.4, 119.4, 123.6, 129.6 (d, J = 3.8 Hz), 130.8, 134.1, 141.1, 151.0, 152.8 (d, J = 244.5 Hz), 180.6. Anal. ($C_{24}H_{28}Cl_4FN_3O$) C, H, N, Cl.

*5,7-Dichloro-3-{4-[4-(4-chlorophenyl)piperazin-1-yl]butyl}-3-ethyl-6-fluoro-1,3-dihydro-2H-indol-2-one (**1m**).* The title compound was prepared according to the general procedure A/1, starting from 5,7-dichloro-3-(4-chlorobutyl)-3-ethyl-6-fluorooxindole (**5c**) and 1-(4-chlorophenyl)piperazine. Yield: 61%, mp 102−103 °C (hexane−ethyl acetate). IR (KBr): 2961, 1736 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 0.52 (3H, t, J = 7.4 Hz), 0.86 (1H, m), 0.95 (1H, m), 1.18 (1H, m), 1.29 (1H, m), 1.74 (2H, m), 1.81 (2H, m), 2.17 (2H, m), 2.38 (t, 4H, J = 4.4 Hz), 3.04 (t, 4H, J = 4.4 Hz), 6.90 (d, 2H, J = 9.1 Hz), 7.21 (d, 2H, J = 9.0 Hz), 7.53 (d, 1H, J = 6.7 Hz), 11.22 (1H, s). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 8.5, 21.7, 26.3, 30.4, 36.7, 48.1, 52.6, 54.8, 57.2, 103.1 (d, J = 22.5 Hz), 112.8 (d, J = 18.1 Hz), 116.9, 122.4, 123.5, 128.7, 129.9 (d, J = 3.9 Hz), 141.1 (d, J = 2.4 Hz), 150.0, 152.7 (d, J = 244.1 Hz), 180.8. Anal. ($C_{22}H_{27}Cl_3FN_3O$) C, H, N, Cl.

*General Procedure B: Coupling Reaction of the Methanesulfonyloxybutyl Derivative (**8**) with the Appropriate Secondary Amines.* The melt of the secondary amine (12 mmol) was heated to 120 °C under slow stirring. The appropriate methanesulfonyloxybutyl derivative **8** (12 mmol) and sodium carbonate (1.36 g, 12 mmol) were added. After 1 h reaction time, the brown melt was cooled to ambient temperature. Ethyl acetate and water were added, and the layers were separated. The organic layer was dried over $MgSO_4$ and evaporated. The residual oil or solid was purified by column chromatography using ethyl acetate as eluent.

General Procedure B/1. In case the product of the chromatographic purification crystallized upon treatment with diethyl ether, it was triturated in this solvent and filtered, and the solid was recrystallized from the solvent indicated below and dried in vacuo to give a white solid.

General Procedure B/2. In case the product of the chromatographic purification did not crystallize upon treatment with diethyl ether, it was dissolved in diethyl ether (200 mL), the solid residue was removed by filtration, and a calculated amount (1 equiv) of hydrogen chloride (saturated solution of HCl gas in diethyl ether) was added dropwise, under vigorous stirring. The white precipitate was filtered, washed with diethyl ether and hexane, and dried in vacuo to give a white solid.

General Procedure B/3. In case the product of the chromatographic purification did not crystallize upon treatment with diethyl ether and it did not give a crystalline hydrochloric acid salt in diethyl ether, it was dissolved in ethyl acetate (100 mL) at 60−65 °C and the solution of oxalic acid dihydrate (1 equiv) in ethyl acetate (50 mL) was added dropwise, keeping the temperature at 60−65 °C during the addition. After the addition, the suspension was cooled to ambient temperature, and the white oxalic acid salt was filtered, washed with ethyl acetate, and dried in vacuo.

*3-[4-(4-Phenylpiperazin-1-yl)butyl]-1,3-dihydro-2H-indol-2-one (**2b**).* The title compound was prepared according to the general procedure B/1, starting from **8a** and 1-phenylpiperazine. Yield: 61%, mp 111−113 °C (hexane−ethyl acetate). IR (KBr): 3191, 1705 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.31−1.24 (2H, m), 1.43 (2H, quintet, J = 7.2 Hz), 1.82−1.79 (1H, m), 1.91−1.81 (1H, m), 2.25 (2H, t, J = 7.3 Hz), 2.43 (4H, t, J = 5.0 Hz), 3.07 (4H, t, J = 4.9 Hz), 3.42 (1H, t, J = 4.9 Hz), 6.76 (1H, t, J = 7.2 Hz), 6.82 (1H, d, J = 7.7 Hz), 6.90 (2H, dd, J = 1.0, 7.8 Hz), 6.94 (1H, dt, J = 1.0, 7.6 Hz), 7.21−7.14 (3H, m), 7.24 (1H, d, J = 7.3 Hz), 10.35 (1H, s). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 23.3, 26.4, 29.9, 45.3, 48.3, 52.9, 57.8, 109.3, 115.4, 118.8, 121.3, 124.1, 127.7, 129.0, 129.9, 142.9, 151.2, 179.1. Anal. ($C_{22}H_{27}N_3O$) C, H, N.

*3-{4-[(3-Chlorophenyl)piperazin-1-yl]butyl}-6-fluoro-1,3-dihydro-2H-indol-2-one Oxalate (**2e**).* The title compound was prepared according to the general procedure B/3, starting from **8c** and 1-(3-chlorophenyl)piperazine. Yield: 41%, mp 215−218 °C. IR (KBr): 3256, 1712 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.29−1.26 (2H, m), 1.65−1.58 (2H, m), 1.89−1.80 (2H, m), 2.88 (2H, t, J = 7.9 Hz), 3.08 (4H, br s), 3.38 (4H, br s), 3.44 (1H, t, J = 5.4 Hz), 4.67 (2H, br s), 6.65 (1H, dd, J = 2.4, 9.1 Hz), 6.75 (1H, dt, J = 2.4, 9.1 Hz), 6.89 (1H, dd, J = 1.3, 7.8 Hz), 6.94 (1H, dd, J = 1.8, 8.4 Hz), 7.01 (1H, t, J = 2.1 Hz), 7.24 (1H, t, J = 8.2 Hz), 7.29−7.22 (1H, m), 10.5 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 22.7, 24.0, 29.6, 44.6, 45.7, 51.0, 55.8, 97.6 (d, J = 27.1 Hz), 107.4 (d, J = 21.7 Hz), 114.2, 115.2, 119.1, 125.4 (d, J = 15.3 Hz), 125.5 (d, J = 7.4 Hz), 130.7, 134.1, 144.5 (d, J = 12.2 Hz), 151.4, 162.1 (d, J = 240.7 Hz), 164.4, 179.4. Anal. ($C_{27}H_{27}ClFN_3O_5$) C, H, N, Cl.

*3-{4-[4-(4-Chlorophenyl)piperazin-1-yl]butyl}-5-fluoro-1,3-dihydro-2H-indol-2-one Hydrochloride (**2f**).* The title compound was prepared according to the general procedure B/2, starting from **8b** and 1-(4-chlorophenyl)piperazine. Yield: 49%, mp 195−196 °C (ethyl acetate−ethanol). IR (KBr): 3145, 1712 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.32−1.22 (2H, m), 1.95−1.71 (4H, m), 3.20−3.06 (6H, m), 3.53−3.49 (3H, m), 3.80−3.76 (2H, m), 6.82 (1H, dd, J = 4.5, 8.5 Hz), 7.04−6.98 (1H, m), 7.01 (2H, d, J = 9.1 Hz), 7.21 (1H, dd, J = 2.1, 8.6 Hz), 7.28 (2H, d, J = 9.1 Hz), 10.5 (1H, s), 11.1 (1H, br s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 22.5, 23.1, 29.3, 42.6, 45.3, 45.4, 45.6 (d, J = 1.9 Hz), 50.5, 55.1, 110.0 (d, J = 8.4 Hz), 112.1 (d, J = 24.4 Hz), 114.0 (d, J = 23.3 Hz), 117.6, 117.7, 123.7, 129.0, 131.6 (d, J = 8.4 Hz), 139.2 (d, J = 1.9 Hz), 148.7, 149.1, 158.1 (d, J = 235.8 Hz), 178.8. Anal. ($C_{22}H_{26}Cl_2FN_3O$) C: calcd, 60.28; found, 59.35; H, N, Cl.

*5,7-Dichloro-3-(4-chlorobutyl)-3-ethyl-6-fluorooxindole (**6e**).* 3-(4-Chlorobutyl)-3-ethyl-6-fluorooxindole (**5c**, 5.39 g, 20 mmol)[8] was dissolved in glacial acetic acid (100 mL), and sulfuryl chloride (8 mL, 100 mmol) was added dropwise. The reaction mixture was heated to 60 °C and stirred at this temperature for 2 h. The reaction mixture was poured on ice (100 g). At room temperature, it was extracted with diethyl ether (100 mL). The organic layer was extracted with water (3 × 50 mL), dried over $MgSO_4$, and evaporated. The residue (7.80 g, 115%) was triturated in hexane (30 mL) and filtered to give the title compound as a pale yellow solid (4.40 g, 65%). The product was used without further purification. An analytical sample was recrystallized from a mixture of hexane and ethyl acetate. Mp 132−134 °C. IR (KBr): 3143, 1718 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.66 (3H, t, J = 7.4 Hz), 1.14−1.00 (1H, m), 1.30−1.16 (1H, m), 1.80−1.60 (4H, m), 2.00−1.86 (2H, m), 3.43 (2H, t, J = 6.6 Hz), 7.05 (1H, dd, J = 0.5, 6.3 Hz), 8.22 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 8.5, 21.7, 31.0, 32.4, 36.7, 44.3, 55.4, 104.6 (d, J = 22.9 Hz), 115.3 (d, J = 18.7 Hz), 122.8, 128.5 (d, J = 4.2 Hz), 139.0, 153.6 (d, J = 249.1 Hz), 180.4. Anal. ($C_{14}H_{15}Cl_3FNO$) C, H, N, Cl.

*4-(6-Fluoro-2-oxo-2,3-dihydro-1H-indol-3-yl)butyl Methanesulfonate (**8c**).* 6-Fluoro-3-(4-hydroxybutyl)-1,3-dihydro-2*H*-indol-2-one (**7c**, 1.11 g, 5 mmol)[16] and triethylamine (0.83 mL, 6 mmol) were dissolved in dichloromethane (25 mL). The reaction mixture was cooled to ca. −10 °C, and methanesulfonyl chloride (0.46 mL, 6 mmol), dissolved in dichloromethane (10 mL), was added dropwise over a period of 30 min. The cooling bath was removed, and the reaction was stirred for 1 h at ambient temperature. The solvent was removed in vacuo at room temperature, and the residue was taken up in ethyl acetate (30 mL) and extracted with water (30 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residual oil was triturated in diisopropyl ether (20 mL) and filtered to give the title compound as a pale yellow solid (1.26 g, 85%). The product was used without further purification. An analytical sample was recrystallized from a mixture of hexane and ethyl acetate to give a white solid. Mp 106−108 °C. IR (KBr): 3161, 1705 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.51−1.46 (2H, m), 1.78 (2H, quintet, J = 6.7 Hz), 2.00 (2H, q, J = 8.1 Hz), 2.99 (3H, s), 3.46 (1H, t, J = 5.9 Hz), 4.21 (2H, dt, J = 1.5, 6.5 Hz), 6.68 (1H, dd, J = 2.3, 8.8 Hz), 6.72 (1H, dt, J = 2.3, 8.9 Hz), 7.15 (1H, dd, J = 5.4, 8.1 Hz), 9.15 (1H, br s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 21.6, 28.9, 29.7, 37.3, 45.3, 69.5, 98.6 (d, J = 27.4 Hz), 108.7 (d, J = 22.5 Hz), 124.5 (d, J = 3.0 Hz), 124.9 (d, J = 9.5 Hz), 142.8 (d, J = 11.8 Hz), 162.6 (d, J = 244.6 Hz), 180.7. Anal. ($C_{13}H_{16}FNO_4S$) C, H, N, S.

*4-(3,5,7-Trichloro-2-oxo-2,3-dihydro-1H-indol-3-yl)butyl Methanesulfonate (**9**).* 4-(2-Oxo-2,3-dihydro-1*H*-indol-3-yl)butyl methanesulfonate (**8a**, 5.0 g, 17.7 mmol) was added in portions into sulfuryl chloride (20 mL, 0.248 mol) at room temperature, over a period of 10 min. The reaction mixture was heated to reflux temperature. After 1 h reaction time, sulfuryl chloride (10 mL, 0.124 mol) was added again, and this procedure was repeated after 2 h reaction time. After 4 h reaction time, the mixture was poured on ice (50 g) and extracted with ethyl acetate. The organic layer was extracted with saturated $NaHCO_3$ solution (2 × 100 mL), dried over $MgSO_4$, and evaporated. The residual oil was triturated in diisopropyl ether (30 mL) to give the title compound as a yellow solid (5.48 g, 80%). The compound was used without further purification. An analytical sample was recrystallized from a mixture of hexane and ethyl acetate to give a white solid. Mp 108−110 °C. IR (KBr): 3274, 1739 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.37 (2H, m), 1.77 (2H, m), 2.29 (2H, m), 3.00 (3H, s), 4.19 (2H, t, J = 6.4 Hz), 7.28 (1H, d, J = 1.8 Hz), 7.33 (d, 1H, J = 1.8 Hz), 8.95 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 20.3, 28.5, 37.3, 38.1, 64.8, 68.9, 116.4, 123.3, 129.1, 129.9, 131.8, 136.5, 174.4. Anal. ($C_{13}H_{14}Cl_3NO_4S$) C, H, N, Cl, S.

*4-(5,7-Dichloro-2-oxo-2,3-dihydro-1H-indol-3-yl)butyl Methanesulfonate (**10**).* 4-(3,5,7-Trichloro-2-oxo-2,3-dihydro-1*H*-indol-3-yl)butyl methanesulfonate (**9**, 10.96 g, 28 mmol), THF (100 mL), and Raney nickel (5 g, ca. 0.085 mol) were placed into an autoclave (volume 250 mL). It was flushed with nitrogen, charged with hydrogen (20 bar) and stirred at room temperature. After 6 h reaction time, Raney nickel (5 g, ca. 0.085 mol) was added again and the stirring (under 20 bar H_2) was continued for further 12 h. The mixture was filtered, and the filtrate was evaporated. The residue was triturated in cold (ca. 5 °C) diethyl ether to give the title compound as a white solid (7.86 g, 79%). The compound was used without further purification. An analytical sample was recrystallized from a mixture of hexane and ethyl acetate. Mp 126−127 °C. IR (KBr): 3389, 1714 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ: 1.31 (2H, m), 1.66 (2H, m), 1.90 (2H, m), 3.14 (3H, s), 3.65 (1H, t, J = 5.6 Hz), 4.17 (2H, t, J = 6.4 Hz), 7.36 (1H, s), 7.40 (1H, s), 10.95 (1H, s). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 19.8, 27.2, 27.6, 35.4, 44.9, 69.0, 112.7, 121.9, 124.7, 125.8, 131.8, 138.4, 177.1. Anal. ($C_{13}H_{15}Cl_2NO_4S$) C, H, N, Cl, S.

*5,7-Dichloro-3-{4-[4-(4-chlorophenyl)piperazin-1-yl]butyl}-1,3-dihydro-2H-indol-2-one (**11**).* 4-(5,7-Dichloro-2-oxo-2,3-dihydro-1*H*-indol-3-yl)butyl methanesulfonate (**10**, 1.23 g, 3.5 mmol) and 1-(4-chlorophenyl)piperazine (2.07 g, 10.5 mmol) were dissolved in acetonitrile (20 mL), and the solvent was evaporated. The residual oil was stirred for 2 h at ambient temperature. The crude product was purified by column chromatography using toluene−ethyl acetate (gradient from 50:1 to 5:1) as eluent. Yield: 47%, mp 173−175 °C. IR (KBr): 3148, 1714 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ: 1.24 (2H, m), 1.42 (2H, m), 1.86 (1H, m), 1.93 (1H, m), 2.26 (2H, m), 2.43 (4H, t, J = 4.8 Hz), 3.08 (4H, m), 3.63 (1H, t, J = 5.5 Hz), 6.91 (2H, d, J = 9.2 Hz), 7.21 (2H, d, J = 9.2 Hz), 7.33 (1H, d, J = 1.8 Hz), 7.39 (1H, d, J = 1.8 Hz), 10.91 (1H, s). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 22.9, 26.1, 29.2, 46.3, 48.1, 52.6, 57.4, 114.0, 116.8, 122.3, 123.2, 126.0, 127.1, 128.7, 133.3, 139.8, 150.0, 178.5. Anal. ($C_{22}H_{24}Cl_3N_3O$) C, H, N, Cl.

Pharmacology. Cell Culture. CHO cells stably expressing the 5-HT_{7A} receptor were cultured in 1:1 F12:DMEM (Sigma) supplemented with 1% (v/v) penicillin−streptomycin (Sigma), 5% fetal calf serum (Gibco BRL), and 2 mM L-glutamine (Sigma) at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded at 30 000 cells per well in a 96-well plate 24 h before treatment with compounds.

Radioligand Binding Assay and Data Analysis. In the case of 5-HT_7 assays, we used cloned human serotonin receptor subtype 7 (h5-HT_7) produced in CHO cells (PerkinElmer), following the recommended assay conditions using [^{3}H]CT (PerkinElmer) as radioligand. Nonspecific binding was determined in the presence of 25 μM clozapine (Sigma). Binding assays were terminated by filtration over Whatman GF/C glass-fiber filter (presoaked in 0.3% polyethylenimine for 30 min) using a Brandel cell harvester, and bound radioactivity was measured with a Packard TopCount scintillation spectrometer.

5-HT_{1A} receptor binding assays were performed according to the method of Peroutka and Snyder[45] with minor modifications. 5-HT_{1A} membrane was prepared from adult rat frontal cortex obtained immediately following decapitation. The wet weight was measured and the tissue was homogenized (Potter−Elvehjem) in 50 volumes of 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The tissue homogenate was centrifuged at 40 000 g for 15 min at 4 °C, and the pellet was resuspended in the same volume of the above buffer. The homogenate was incubated in a shaking water bath for 10 min at 37 °C. After a quick chilling on ice, the homogenate was centrifuged (40 000 g, 15 min, 4 °C), resuspended, and centrifuged again (40 000 g, 15 min, 4 °C). The membrane was finally resuspended in 30-fold volume of 50 mM Tris-HCl buffer (pH 7.7). The incubation buffer contained 50 mM Tris-HCl (pH 7.7), 6.66 mM $CaCl_2$, 16.66 μM pargyline, and 0.166% ascorbic acid. 5-HT_{1A} sites were labeled with 1.5 nM [^{3}H]8-OH-DPAT (Amersham). Nonspecific binding was determined in the presence of 10 μM 5-hydroxytryptamine−creatinine sulfate complex (5-HT, Sigma). The samples were incubated for 45 min at 25 °C. Following the incubation, the samples were rapidly filtered over Whatman GF/B glass fiber filter (presoaked in 0.05% polyethylenimine for 30 min) and washed. Individual filters were inserted into vials,

equilibrated for 6 h with Opti-Fluor scintillation fluid (PerkinElmer), and counted with a Packard TopCount scintillation spectrometer.

α_1-AR binding assays were performed in membrane prepared from adult rat frontal cortex. The tissue was immediately frozen in dry ice and then weighed. The tissue was homogenized (Potter−Elvehjem) in 50-fold volume of 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The tissue homogenate was centrifuged at 40 000 g for 15 min at 4 °C, and the pellet was twice resuspended in the same volume of buffer and centrifuged. The membrane was finally resuspended in 20-fold volume of 50 mM Tris-HCl buffer (pH 7.7). α_1-AR sites were labeled with 0.6 nM [^{3}H]prazosin (Amersham). Nonspecific binding was determined in the presence of 10 μM prazosin (RBI). The samples were incubated for 45 min at 25 °C. Following the incubation, the samples were rapidly filtered over Whatman GF/B glass fiber filter (presoaked in 0.05% polyethylenimine for 2−3 h) and washed. Individual filters were inserted into vials, equilibrated for 4 h with scintillation fluid (Fisons Chemicals), and counted with a Packard TopCount scintillation spectrometer.

Membranes, radioligands, and varying concentrations of competitive ligands were incubated in triplicates. Competition binding data were fit to a single-site binding model using Prism GraphPad for K_i determination. K_i values were calculated from two displacement curves (nine concentrations) in triplicate.

Prediction Measurement of Microsomal Metabolic Stability. The compounds were incubated for 0, 5, 15, 30, and 60 min with rat and human liver microsomes at 37 °C. Incubation mixtures (the final volume was 1 mL) consisted of 814 μL of 0.1 mM Tris-HCl buffer (pH 7.4), 100 μL of 5 mM Mg^{2+}, 36 μL of microsomes (0.33 mg/mL), and 50 μL of 2 μM substrate (final concentration 100 nM). The test item was added after dilution of a 1 mM stock solution (DMSO) to 2 μM in 0.1% BSA solution. Reactions were started by the addition of 100 μL of 20 mM NADPH and were terminated with ice-cold MeOH. The samples were centrifuged, and the supernatants were transferred to autosampler vials. Calibration solutions were prepared in the range of 0.5−50 nM by diluting the 100 nM substrate solution with different volumes of BSA/Tris and MeOH. The assay was performed using a HPLC-MS/MS system consisting of a Waters Alliance 2795 HPLC and a Quattro Ultima Platinum mass spectrometer. Appropriate assay methods were developed for all compounds tested. Ionization was performed in the positive ion mode, and the drug was monitored in the multiple-reaction monitoring (MRM) mode. The orifice and ring potentials were optimized to the highest signal for all analytes. Clearance of the compound in the test system was calculated by linear regression and was expressed as (mL/min/g protein). Prediction of metabolic stability was calculated, taking into account of the liver blood flow, liver weight, and body weight of the species using our own formula.

Measurement of Plasma and Brain Concentrations in Mice. Male NMRI mice weighing 25−30 g were treated ip with four compounds simultaneously. Each dose of the cassette mixtures were administered as a methyl cellulose (0.4%) suspension (n = 3/dose). 30 min after treatment, blood was taken from the vena cava under isoflurane anesthesia into Sarstedt (LH/1.3) tubes and centrifuged by a Heraeus Megafuge 1.0 R centrifuge (Kendro Laboratory Products GmbH, Osterode, Germany) for 10 min (RCF: 1625 × g) at 4 °C. After decapitation, the brain was removed and weighed. The plasma and brain tissue was stored at about −70 °C until analysis. Upon thawing, the brain tissue was homogenized with 4-fold volume of distilled water in a potter and mixed. Calibration curves were prepared by adding variable amounts of test compound mixtures into drug-free plasma and homogenate of brain tissue. Both the plasma and homogenized brain tissue were extracted by mixing in a vortex for 30 min using an acetonitrile−distilled water mixture (3:1 by volume). The mixtures were centrifuged for 15 min at 15 000 rpm, at 25 °C. After centrifugation, the supernatants were mixed with an equal amount of the mobile phase. The HPLC-MS/MS system consisted of a Waters Alliance 2795 HPLC and a Quattro Ultima Platinum mass spectrometer. Gradient chromatographic separation of test compounds was achieved with 10 mM ammonium formate, 5% acetonitrile, and 0.1% trifluoracetic acid in distilled water (mobile phase A) and acetonitrile and 0.1% trifluoracetic acid (mobile phase B) on a Purospher STAR RP-8 column (125 mm × 4 mm, 3 μm particle size). A linear gradient from 100% A to 100% B was programmed between 0 and 10 min. Appropriate retention windows were set for each test compound. Ionization was performed in the positive ion mode. The dwell time was set to 200 ms, and the drugs were monitored in the multiple-reaction monitoring (MRM) mode. The most abundant fragmentation paths, m/z 384→146 and 384→188, were set for detection of **2d**, m/z 412→160 and 412→114 for **1h**, m/z 430→178 and 430→192 for **1i**, m/z 446→160 and 446→174 for **1r**, m/z 396→160 and 396→174 for **1o**, m/z 412→160 and 412→174 for **1d**, m/z 430→178 and 430→192 for **1a** and m/z 446→194 and 446→208 for **1j**, respectively. The orifice and ring potentials were optimized to the highest signal for the analytes. Calibration curves were calculated from the analyte peak area ratios of 2−2 parallels of the calibration standards plotted against the nominal concentration ratios of calibrator sample standard. Quadratic curve fitting was done using $1/x$ as weighing factor.

Conflict Drinking (Vogel) Test in Rats. Experiments were performed in a computer-operated system (LIIKOSYS, Experimetria, Hungary) consisting of eight test chambers (20 cm ×20 cm ×20 cm Plexiglas boxes), each of which was equipped with a drinking spout mounted at appropriate height on the wall of the chamber and a metal grid floor for delivering electric shocks. Male Wistar rats, weighing 160−180 g, were deprived of drinking water for 48 h and fasted for 24 h prior to test. Test and reference compounds or vehicle were administered ip 30 min prior to test. All procedures were carried out in a quiet, air-conditioned room between 07:30 and 13:00 h at an ambient temperature of 23 °C. At the beginning of the experiment, the animals were placed in the test chamber where they had free access to drinking water for a 30 s grace period. After that, electric shocks (600 μA, 0.6 s) were applied through the drinking spout following every 20 licks during a 5-min test period.[10] The number of punished licks was recorded and stored in a computer. Means ± SEM of tolerated shocks were calculated in each group, and statistical analysis of the results was performed by one-way ANOVA, followed by Duncan's test.

Light−Dark Test in Mice. Test was performed in a room illuminated with a 2-lx light source. An animal activity monitor equipped with six two-compartment automated test chambers (Omnitech, Digiscan, Model RXYZCM16) was used for all experiments. Each box consisted of one dark and one lit compartment. Both areas measured 39 cm × 20 cm × 29 cm. Access between the two compartments was provided by an 8 cm × 8 cm passage way. A 60 W white tungsten light bulb was used to illuminate the lit area. Interruptions of the 32 infrared beams (16 at 2 cm and 16 at 8 cm height above the box floor) in both compartments were automatically recorded by the Digiscan analyzer and transmitted to a computer. Male NMRI mice, weighing 25−33 g, were used for the test. Mice were kept in a dark room, treated ip 30 min prior to test and were placed individually in the center of the lit area. Behavioral activity was detected for 5 min. Time spent in each area, number of transitions, and horizontal and vertical activities were recorded.[11] Means ± SEM values were calculated, and the statistical analysis of data was performed by one-way ANOVA, followed by Duncan's test.

Forced Swimming Test (Porsolt) in Mice. Adult male NMRI mice (n = 10−12/group, weight range: 23−30 g, Charles River, Hungary) were used. The animals were introduced into the testing room 1 h before the test. The compounds were dissolved in 0.9% physiological saline or were suspended in 0.4% methylcellulose. All drugs were administered ip 30 min prior to the test. Control animals received vehicle only. The test was carried out according to the method of Lucki et al.[20] The animals were put into 17.7 cm high and 12 cm diameter transparent glass cylinders filled up with 25 °C water up to 12.5 cm. Four mice were tested simultaneously. The duration of immobility was measured during the

last 4 min of the 6 min testing period. Each mouse was judged immobile when it ceased to struggle and floated motionless in the water, making only those movements necessary to keep its head above the water surface. Mean and SEM values and the statistical significance between groups were determined with one-way ANOVA followed by Dunnett's posthoc test using GraphPad Software GraphPad Prism 4.0 programs.

■ ASSOCIATED CONTENT

Supporting Information. Preparation and characterization of compounds **1c**, **1n**, **1p**, **1s**, **1v**, **2g**−**m**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors are grateful to Dr. Rita Kapiller-Dezsöfi for NMR measurements and helpful discussions. We also thank Ms. Krisztina Horváth and Mr. Károly Lozsi for technical assistance.

■ ABBREVIATIONS USED

5-HT, 5-hydroxytryptamine; SSRI, selective serotonin reuptake inhibitor; NCE, new chemical entity; AR, adrenoceptor; MED, minimum effective dose; BuLi, *n*-butyllithium; equiv, equivalent; Ra−Ni, Raney nickel; THF, tetrahydrofuran; AcOH, acetic acid; rt, room temperature; NBS, *N*-bromosuccinimide; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; BPH, benign prostatic hyperplasia; MF, in vitro microsomal metabolic stability; ip, intraperitoneal(ly); FST, forced swimming test; BBBP, blood−brain barrier permeability; SAR, structure−activity relationship; DMSO, dimethyl sulfoxide; TMS, tetramethylsilane; CHO, Chinese hamster ovary; F12:DMEM, Dulbecco's Modified Eagle's Medium with Ham's F-12; 5-CT, 5-carboxytryptamine; cAMP, cyclic adenosine monophosphate; Tris, tris(hydroxymethyl)aminomethane; BSA, bovine serum albumin; NADPH, nicotinamide adenine dinucleotide phosphate; SEM, standard error of the mean; SNRI, serotonin−norepinephrine reuptake inhibitor

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