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Published on: 05 May 2016 - ACS Chemical Neuroscience (American Chemical Society)

Topics: Inverse agonist, 5-HT6 receptor and Serotonin Antagonists

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Novel 1*H*-Pyrrolo[3,2-*c*]quinoline Based 5-HT$_6$ Receptor Antagonists with Potential Application for the Treatment of Cognitive Disorders Associated with Alzheimer's Disease

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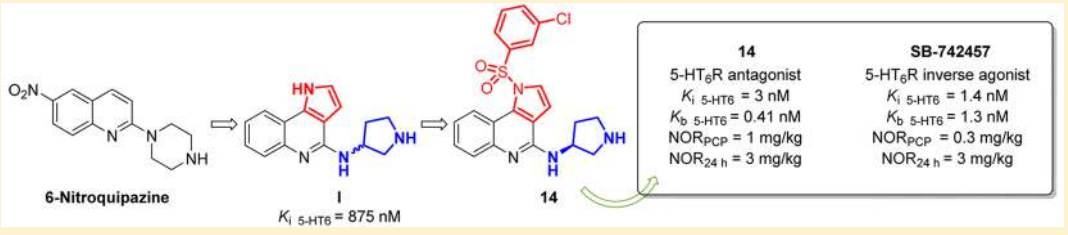


ABSTRACT: Modulators of the serotonin 5-HT$_6$ receptor (5-HT$_6$R) offer a promising strategy for the treatment of the cognitive deficits that are associated with dementia and Alzheimer's disease. Herein, we report the design, synthesis, and characterization of a novel class of 5-HT$_6$R antagonists that is based on the 1*H*-pyrrolo[3,2-*c*]quinoline core. The most active compounds exhibited comparable binding affinity to the reference compound, SB-742457, and markedly improved selectivity. Lead optimization led to the identification of (*S*)-1-[(3-chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1*H*-pyrrolo[3,2-*c*]quinoline (**14**) (K_i = 3 nM and K_b = 0.41 nM). Pharmacological characterization of the 5-HT$_6$R's constitutive activity at Gs signaling revealed that **14** behaved as a neutral antagonist, while SB-742457 was classified as an inverse agonist. Both compounds **14** and SB-742457 reversed phencyclidine-induced memory deficits and displayed distinct procognitive properties in cognitively unimpaired animals (3 mg/kg) in NOR tasks. Compounds **14** and SB-742457 were also active in the Vogel test, yet the anxiolytic effect of **14** was 2-fold higher (MED = 3 mg/kg). Moreover, **14** produced, in a 3-fold higher dose (MED = 10 mg/kg), antidepressant-like effects that were similar to those produced by SB-742457 (MED = 3 mg/kg). Together, these data suggest that the 4-(pyrrolidine-3-yl-amino)-1*H*-pyrrolo[3,2-*c*]quinoline scaffold is an attractive molecular framework for the development of procognitive agents. The results are promising enough to warrant further detailed mechanistic studies on the therapeutic potential of 5-HT$_6$R antagonists and inverse agonists for the treatment of cognitive decline and depression/anxiety symptoms that are comorbidities of Alzheimer's disease.

KEYWORDS: *5-HT$_6$R antagonist, 5-HT$_6$R inverse agonist, pyrrolo[3,2-c]quinoline, SB-742457, cognition, memory, novel object recognition test, forced swim test, Vogel test, Alzheimer's disease*

Alzheimer's disease (AD), an irreversible neurodegenerative disorder, is the most common form of dementia worldwide. AD is characterized by the progressive deterioration of various cognitive domains, including a decline in memory and thinking. More than 35 million people are suffering from AD. Currently available therapeutic options for the treatment of AD, based on acetylcholinesterase inhibitors and NMDA receptor antagonist, provide modest efficacy and symptomatic treatment without affecting the neurodegeneration process.[1]

In recent years, the serotonin 5-HT$_6$ receptor (5-HT$_6$R) has emerged as a promising molecular target for the treatment of

cognitive deficits in AD.[2] The 5-HT$_6$R belongs to the family of G-protein coupled receptors, which are positively coupled with adenylyl cyclase. Additionally, 5-HT$_6$Rs engage extracellular signal-regulated kinase (ERK)1/2 pathway via the Src family tyrosine kinase Fyn,[3] as well as cyclin-dependent kinase (Cdk)5,[4] and mammalian Target Of Rapamycin (mTOR) pathways.[5] Moreover, nonphysiological mTOR activation in prefrontal cortex, under the control of 5-HT$_6$R, underlies deficits in social cognition and episodic memory in two neurodevelopmental models of schizophrenia in the rat, neonatal administration of the NMDA receptor antagonist phencyclidine and rearing in social isolation after the weaning.[5] 5-HT$_6$R displays high constitutive activity at various signaling cascades, including Gs adenylyl cyclase,[6] and Cdk5 pathways.[4] Accordingly, the deleterious influence of 5-HT$_6$R in AD may reflect an alteration in receptor expression as well as a deregulation of its activity. The 5-HT$_6$R is almost exclusively expressed in the CNS, with high levels in the prefrontal cortex, hippocampus, and striatum, the brain regions that are predominantly involved in the learning and memory processes.[7] It has also been demonstrated that 5-HT$_6$R antagonists modify the transmission of acetylcholine, glutamate, dopamine, and norepinephrine.[8,9]

The progress in development of 5-HT$_6$R modulators has recently been comprehensively reviewed by Holenz et al.,[10] Glennon et al.,[11] and Benhamu et al.[12] Important research in the field led to the discovery of several classes of indole and indole-like derivatives (e.g., benzofurans, indazoles, pyrazolopyrimidines, indolines) that preferentially bind to the 5-HT$_6$R and behave as antagonists at these sites (Figure 1).[13]

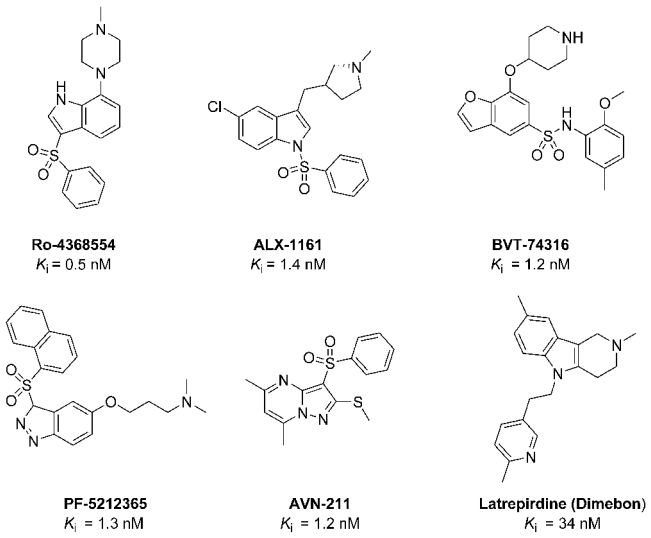


Figure 1. Representatives of 5-HT$_6$R antagonists from various chemotypes.

As a consequence, the structural requirements for 5-HT$_6$R antagonists have been described, and pharmacophore models for 5-HT$_6$R antagonists have been developed.[14]

It is worth noting that 5-HT$_6$R antagonists displayed procognitive effects in several learning and memory in vivo paradigms in rodents, and their efficacy was confirmed in phase II clinical trials when used in combination with donepezil (AChE inhibitor).[15,16] Specifically, SB-742457 and idalopirdine (Lu-AE58054) have been evaluated in phase III clinical trials as an adjunctive therapy to donepezil in patients with mild to moderate AD (Figure 2).[17] Furthermore, 5-HT$_6$R antagonists

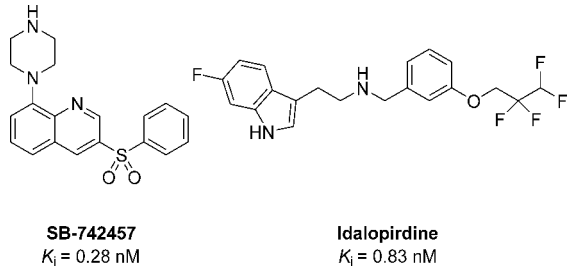


Figure 2. Structures of 5-HT$_6$R antagonists evaluated in phase 3 clinical trials.

produced procognitive and/or antiamnesic effects in other neuropsychiatric disorders, i.e. schizophrenia, and Parkinson's disease, and might open therapeutic opportunity for autism treatment.[5,18]

Continuing our efforts to generate selective 5-HT$_6$R ligands, we applied a scaffold-hopping approach to fuse a pyrrole ring onto the *c* edge of the quinoline fragment of 6-nitroquipazine (selective serotonin-reuptake inhibitor) and then replaced the piperazine moiety with its 3-aminopyrrolidine bioisostere. Although the resulting *N*-4-(pyrrolidin-3-yl-amino)-1*H*-pyrrolo-[3,2-*c*]quinoline **I**, displayed a low affinity for the 5-HT$_6$R, further functionalization of **I**, introducing the phenylsulfonyl moiety on the nitrogen atom of pyrrole (**II**), fulfilled the structural requirements of a 5-HT$_6$R antagonist and significantly increased the compound's affinity for the 5-HT$_6$R. This result prompted us to further investigate the substitution pattern of the arylsulfonyl fragment of derivative **II** to develop potent 5-HT$_6$R antagonists (Figure 3).

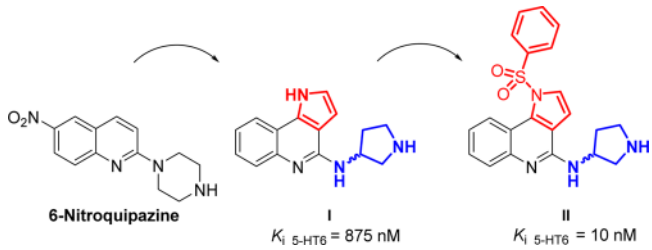


Figure 3. Strategy to develop a new group of 5-HT$_6$R antagonists.

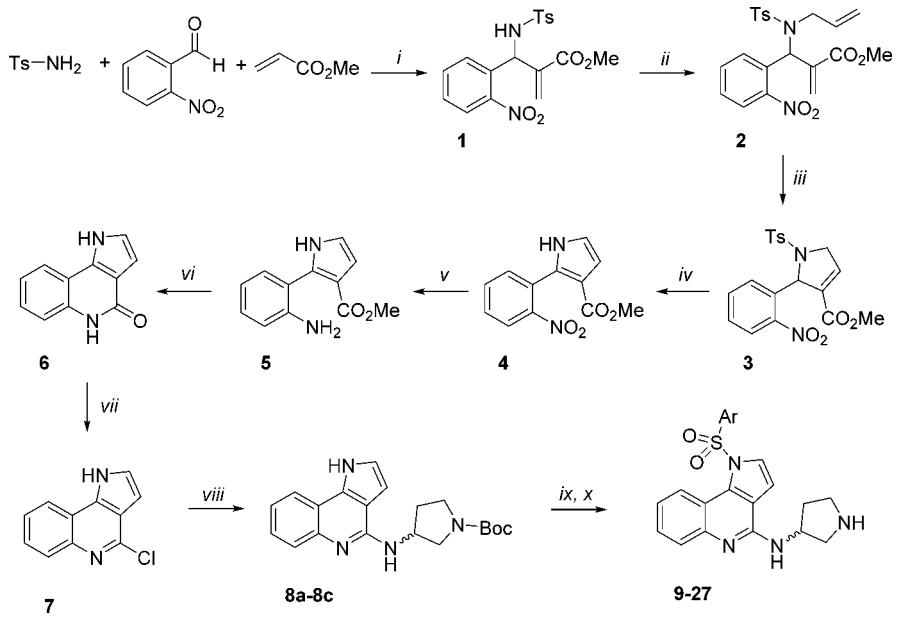
Herein, we report on a small series of arylsulfonyl derivatives of a novel molecular framework that is based on *N*-4-(pyrrolidin-3-yl-amino)-1*H*-pyrrolo[3,2-*c*]quinoline as potent and selective 5-HT$_6$R antagonists. The most promising derivatives were evaluated for their ability to reverse drug-induced memory deficits and for their procognitive properties in novel object recognition test in rats. Because noncognitive traits are often associated with dementia and Alzheimer's disease, the most promising derivatives were also evaluated in animal models of anxiety and depression.

CHEMISTRY

The designed compounds were synthesized according to a multi-step procedure (Scheme 1).

The starting nitrobenzaldehyde, tosylamine, and methyl acrylate reacted in the presence of DABCO and titanium isopropoxide, in isopropanol, under the *aza*-Bayliss–Hillman reaction conditions, to yield the unsaturated *β*-aminoester **1**.[19] Subsequently, compound **1** was treated with allyl bromide in a biphasic system to give diene **2**. The latter was submitted to a

Scheme 1[a]



[a]Reagents and conditions: (i) DABCO, Ti(*i*OPr)$_4$, molecular sieves, isopropanol, RT, o/n; (ii) allyl bromide, K_2CO_3, DMF, RT, 6 h; (iii) Grubbs second-generation catalyst (3 mmol %), CH_2Cl_2, 36 °C MW, 30 min; (iv) NaO*t*-Bu, DMF, RT, 2 h; (v) H_2, Pd/C, MeOH, RT, 2 h; (vi) AcOH, *sec*-BuOH, 60 °C, 3 h; (vii) $POCl_3$, 105 °C, 4 h; (viii) 1-Boc-3-aminopyrrolidine, MeCN, 140 °C, MW, 5 h; (ix) arylsulfonyl chloride, BTPP, CH_2Cl_2, 0 °C 3 h; (x) 4 N, HCl/dioxane.

ring-closing metathesis reaction, using Grubbs second-generation catalyst, in refluxing dichloromethane for 30 min, to yield pyrroline **3**. This process was promoted by microwave irradiation in the open-vessel mode. Next, the tosyl group of **3** was eliminated in the presence of sodium *tert*-butoxide (NaO*t*-Bu).[20] Such an approach allowed for the simultaneous aromatization of the pyrroline moiety and generation of the pyrrole derivative **4**. Compound **4** was then reduced using palladium on activated charcoal, in methanol, under hydrogen atmosphere. Intramolecular cyclization of aromatic amine **5**, upon treatment with acetic acid, provided the lactam derivative **6**, which was subsequently submitted to oxidative chlorination to yield 4-chloro-1*H*-pyrrolo[3,2-*c*]quinoline (**7**). Nucleophilic substitution with 1-Boc-3-aminopyrrolidine (either a racemic mixture or pure enantiomers) was carried out under microwave-assisted conditions and gave compounds **8a−8c**. Coupling **8a−8c** with selected arylsulfonyl chlorides in the presence of a strong phosphazene base, P$_1$-*t*-Bu-tris(tetramethylene) (BTPP), provided sulfonyl derivatives of Boc-protected *N*-4-(pyrrolidin-3-yl-amino)-1*H*-pyrrolo[3,2-*c*]quinolines.[21] These compounds furnished the final products **9−27**, as hydrochloride salts, upon removal of the Boc group in acidic conditions.

RESULTS AND DISCUSSION

Pharmacological in Vitro Evaluation and Structure−Activity Relationship Studies. Pyrroloquinolines have been previously explored as privileged structures for the synthesis of biologically active compounds with potential anticancer, antimalarial, antiobesity, and analgesic properties.[22−25] In the present study, the 1*H*-pyrrolo[3,2-*c*]quinoline scaffold was originally proposed as a promising core motif for the development of a new class of 5-HT$_6$R antagonists.

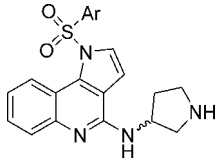
The synthesized compounds **9−27** displayed high-to-moderate affinity for the 5-HT$_6$R (K_i = 3−101 nM) and generally did not bind to serotonergic 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$, and dopaminergic D$_2$ receptors (Table 1). The initially identified compound **9**, with the phenylsulfonyl group, displayed high affinity for the 5-HT$_6$R (K_i = 10 nM). First, the lead optimization focused on the arylsulfonyl moiety.

Introduction of electron-withdrawing substituents, such as fluorine, chlorine (**10**, **13**) or the bulky trifluoromethyl group (**16**), in the *meta* position of the phenyl ring slightly increased the affinity for the 5-HT$_6$R. A shift of the fluorine atom from the *meta* to the *para* position of the phenylsulfonyl fragment induced a 2-fold decrease in the affinity (**19** with K_i = 18 nM vs **10** with K_i = 9 nM). Introduction of an additional fluorine atom in the *ortho* position of the phenyl ring further decreased the affinity, as compared with the monofluorinated analogue (**20** vs **10**). Interestingly, replacement of the electron-withdrawing substituent in the *meta* position of the phenyl ring with electron-donating methoxy (**21**, K_i = 7 nM) or methyl (**22**, K_i = 8 nM) groups maintained the high affinity for the 5-HT$_6$R.

Conversely, introduction of sterically hindered *tert*-butyl or isopropyl groups in the *para* position (**23**, **24**) significantly decreased the affinity, suggesting that bulky substituents at this position were unfavorable for binding to the 5-HT$_6$R (**23** vs **9**, **24** vs **9**). Compounds **23** and **24**, with bulky *t*-Bu and *i*-Pr substituents, were the least potent ligands among the evaluated series, with K_i of 101 nM and 99 nM, respectively.

To further reveal the impact of the size of substituents in the arylsulfonyl fragment on 5-HT$_6$R affinity, bicyclic aromatic systems were introduced. Replacement of the phenyl moiety with 1-naphthyl (**25**, K_i = 19 nM) slightly reduced affinity for the 5-HT$_6$R, whereas the 8-quinolinyl fragment (**26**, K_i = 4 nM) was better accommodated. Furthermore, it was found that the sterically encumbered 5-Me-benzothien-2-yl moiety caused a 10-fold decrease in the affinity for 5-HT$_6$ sites (**27** with K_i = 94 nM vs **9** with K_i = 10 nM) and, thus, showed the limitations of the binding pocket.

Table 1. Binding Data of Compounds 9−27 for the 5-HT$_6$R and Their Functional Activity for 5-HT$_6$R, and Binding Data for 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$, and D$_2$Rs for the Selected Compounds



			K_i [nM][a]			K_i [nM][b]			
compd	Ar	enantiomer	5-HT$_6$	agonist effect[c]	antagonist effect[d]	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_7$	D$_2$
9	phenyl	*R/S*	10 ± 2	6.5	68	9148	1984	7890	1133
10	3-F-phenyl	*R/S*	9 ± 1	9.6	81	3006	2262	9594	900
11	3-F-phenyl	*S*	7 ± 1	9.8	83	3198	2349	9770	873
12	3-F-phenyl	*R*	12 ± 3	0.3	72	2898	2190	9367	907
13	3-Cl-phenyl	*R/S*	5 ± 1	2	90	3026	2768	2133	1437
14	3-Cl-phenyl	*S*	3 ± 1	9.6	93	2351	1281	4989	1012
15	3-Cl-phenyl	*R*	7 ± 2	0.8	86	2675	4192	3943	754
16	3-CF$_3$-phenyl	*R/S*	7 ± 1	10.8	80	5876	2778	3816	1282
17	3-CF$_3$-phenyl	*S*	6 ± 1	9.6	81	5291	4566	2083	1553
18	3-CF$_3$-phenyl	*R*	9 ± 1	6.7	78	6233	1345	699	942
19	4-F-phenyl	*R/S*	18 ± 2	4.8	52	5832	6754	6877	664
20	2,5-diF-phenyl	*R/S*	94 ± 15	NT[e]	NT	NT	NT	NT	NT
21	3-MeO-phenyl	*R/S*	7 ± 2	6.5	69	NT	2387	3816	1282
22	3-Me-phenyl	*R/S*	8 ± 2	5.2	67	NT	3421	3256	1765
23	4-*tert*-Bu-phenyl	*R/S*	101 ± 17	NT	NT	NT	NT	NT	NT
24	4-iso-Pr-phenyl	*R/S*	99 ± 16	NT	NT	NT	NT	NT	NT
25	1-naphthyl	*R/S*	19 ± 2	10.6	60	5330	5789	1421	2260
26	quinolin-8-yl	*R/S*	4 ± 1	16.4	70	15230	8765	18470	6400
27	5-Me-benzothien-2-yl	*R/S*	94 ± 14	NT	NT	NT	NT	NT	NT

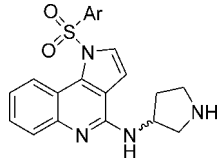
[a]Mean K_i values, based on three independent binding experiments. [b]Mean K_i values (SEM ≤ 36%), based on at least two independent binding experiments. [c]Percent activity of agonist control response at 10^{-6} M; performed at Eurofins Cerep. [d]Percent inhibition of control agonist response at 10^{-6} M; performed at Eurofins Cerep [e]NT: not tested.

Because the stereochemical properties of molecules might influence their binding in the receptor pocket, three pairs of enantiomers were investigated. A slight preference for the *S* enantiomers (**11**, **14**, **17**) over their *R* counterparts (**12**, **15**, **18**) was observed with respect to their 5-HT$_6$ receptor affinity and selectivity. Compound **14**, an (*S*)-enantiomer bearing a chloro substituent in the *meta* position of the phenyl ring, displayed the highest affinity for the 5-HT$_6$R (K_i = 3 nM).

The most potent compounds (K_i < 20 nM) were further evaluated for their functional activity at the 5-HT$_6$R in cAMP cellular assays. In the screening procedure carried out in Cerep, compounds were initially classified as 5-HT$_6$R antagonists (Table 1). Subsequently, two pairs of enantiomers, **11**, **12**, **14**, and **15**, displaying the highest affinity for the 5-HT$_6$R and the highest receptor-antagonist activity (% inhibition of control agonist), were evaluated in the cellular functional assay to inhibit 5-CT-induced cAMP production (Table 2). Compounds bearing a chlorine atom in the *meta* position of the arylsulfonyl displayed stronger antagonist properties than their fluoro counterparts. Among the tested enantiomers, the *S* isomers (**11**, **14**) were the most potent antagonists and showed a greater antagonistic effect at the 5-HT$_6$ sites than those exhibited by SB-742457 with K_b values 0.83 nM and 0.41 nM for **11** and **14**, respectively.

Furthermore, to look for potential inverse agonist activity, two 5-HT$_6$R antagonists, **14** and **15**, and model 5-HT$_6$R antagonist SB-742457, were evaluated in an NG108−15 neuroblastoma cell line that was transiently expressing the 5-HT$_6$R. The antagonists' effects on the receptor's constitutive activity at Gs signaling was assessed by BRET using the cAMP sensor CAMYEL. As previously

Table 2. Antagonist Activity of the Selected Compounds 11, 12, 14, 15, and SB-742457 for the 5-HT$_6$R



			5-HT$_6$	
compd	Ar	enantiomer	K_i [nM][a]	K_b [nM][b]
11	3-F-phenyl	*S*	7 ± 1	0.83
12	3-F-phenyl	*R*	12 ± 3	12
14	3-Cl-phenyl	*S*	3 ± 1	0.41
15	3-Cl-phenyl	*R*	7 ± 2	6.1
SB-742457[c]			1.4 ± 0.2	1.3

[a]Mean K_i values, based on three independent binding experiments. [b]Mean K_b values (SEM ≤ 22%), based on three independent binding experiments. [c]For selectivity panel see ref 13.

observed,[6] expressing the 5-HT$_6$R strongly activates cAMP production in NG108−15 cells, and the level of constitutive activity represented approximately 60% of the activity of a maximally effective concentration of the full agonist WAY181187 (Figure 4A). The constitutive activity was reduced in a concentration-dependent manner by the 5-HT$_6$R antagonist SB-742457, which behaved as an inverse agonist in this model, with pEC$_{50}$ of 7.67 (Figure 4A and 4B). In contrast, neither **14** nor **15** significantly affected receptor-elicited cAMP production (Figure 4B), indicating that these compounds behaved as neutral antagonists.

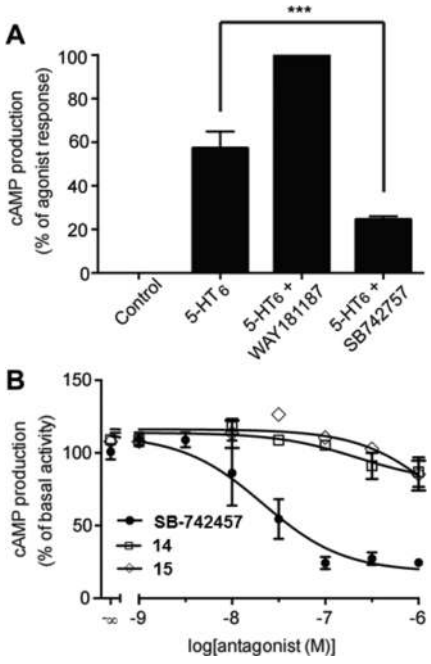


Figure 4. Influence of compounds **14**, **15**, and SB-742457 on the 5-HT$_6$R constitutive activity at Gs signaling in NG108−15 cells. (A) The 5-HT$_6$ receptor constitutively activates Gs-cAMP signaling in NG108−15 cells. Cells were transfected with an empty vector (control) or the plasmid encoding the 5-HT$_6$ receptor and were treated with vehicle (5-HT$_6$), WAY181187, or SB-742457 (1 μM each) for 5 min. (B) The NG108-15 cells expressing the 5-HT$_6$ receptor were exposed to incremental concentrations of either SB-742457 or **14** or **15** for 5 min. Cyclic AMP levels were estimated by BRET using the cAMP sensor CAMYEL. Data are the means ± SEM of the values obtained in three independent experiments that were performed in quadruplicate using different sets of cultured cells. ***p < 0.001 vs vehicle (ANOVA followed by Student−Newman−Keuls test).

It was further demonstrated that the selected compounds **14** and **15** did not bind to adrenergic α_{1A} (<18% at 1 μM), histaminergic H$_1$ (<17% at 1 μM), muscarinic M$_1$ (<9% at 1 μM), and serotonin 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors (<21% at 1 μM), and they did not show affinity for the serotonin transporter (SERT) (<12% at 1 μM). Of note, this distinguishes the reported compounds **14** and **15** from the well-known 5-HT$_6$R agents; for example, SB-742457 displays a high affinity for 5-HT$_{2A}$ (99% at 1 μM) and 5-HT$_{2B}$R (99% at 1 μM),[13] and idalopirdine binds at α_{1A}R (K_i = 21 nM) and displays moderate affinity for the 5-HT$_{2A}$R (K_i = 83 nM).[26]

Pharmacological in Vivo Evaluation. Several preclinical studies have shown that 5-HT$_6$R antagonists reverse drug-induced memory decline or time-dependent natural forgetting in rats in a variety of preclinical behavioral paradigms (e.g., NOR, ASST, 5CCT).[26−29] The precise impact of 5-HT$_6$R antagonists on learning and memory depends on the age of animals and the task used.[30−33]

Based on rodents' natural preference for novelty, the NOR test is regarded as an etiologically relevant paradigm for studying visual episodic memory.[34] This paradigm is based on the spontaneous exploration of novel and familiar objects, and the NOR test has been used for investigating drug-induced (e.g., phencyclidine, scopolamine) cognitive deficits in psychiatric disorders, including schizophrenia and Alzheimer's disease.[35,36] Furthermore, as the use of long intertrial intervals (24 h) in cognitively unimpaired animals abolishes object discrimination, the NOR test is also a screening method for evaluating potential cognitive enhancers. In such conditions requiring longer retention of information, animals display poor memory that closely resembles natural forgetting. This test allows for determination of the procognitive properties of the evaluated compounds.

The procognitive effects of compounds **14** and **15** were assessed in the NOR task in rats. Following a single administration, **14** and **15** significantly reversed phencyclidine-induced episodic memory decline at doses of 1−3 mg/kg (ip) (Figure 5). The effects were comparable to that produced by SB-742457, which was used as an active comparator. It is worth noting that **15**, which is the *R* enantiomer, produced a slightly stronger effect than its *S* congener.

The same compounds were further evaluated in the NOR test to determine their memory-enhancing effects 24 h after their administration in cognitively unimpaired rats (Figure 6). In contrast to PCP-impaired conditions, compounds **14** and

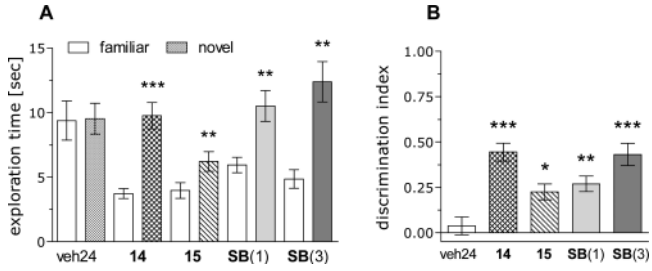


Figure 6. Effects of the selected compounds **14** (3 mg/kg, ip), **15** (3 mg/kg, ip), and SB-742457 (1 mg/kg and 3 mg/kg, ip) in the novel-object recognition test performed in rats in cognitively unimpaired conditions. Data are presented as the mean ± standard error of the mean of N = 9−10 animals per group. (A) T2, **p < 0.01 vs familiar, ***p < 0.001 vs familiar (paired Student's t test). (B) Discrimination index, *p < 0.05 vs vehicle, ***p < 0.001 vs vehicle (one-way ANOVA followed by Dunnett's post hoc test).

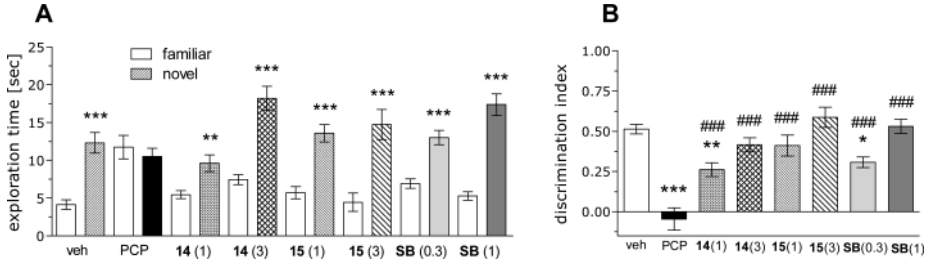


Figure 5. Effects of the selected compounds, **14**, **15**, and SB-742457, in the novel-object recognition test in rats performed in PCP-impaired conditions. Data are presented as the mean ± standard error of the mean of N = 7−10 animals per group. (A) T2, **p < 0.01 vs familiar, ***p < 0.001 vs familiar (paired Student's t test). (B) Discrimination index, *p < 0.05 vs vehicle, **p < 0.01 vs vehicle, ***p < 0.001 vs vehicle, ###p < 0.001 vs PCP (one-way ANOVA followed by Newman−Keuls post hoc test).

SB-742457 (3 mg/kg, ip) strongly affected the ability of rats to distinguish between novel and familiar objects, indicating distinct procognitive effects.

Because anxiety and depression are common comorbidities of AD, the improvement of these behavioral symptoms may be considered a valuable therapeutic attribute in the treatment of patients with AD. Thus, to further extend the pharmacological evaluation of the newly designed compounds, their anxiolytic and antidepressant properties were evaluated in Wistar rats in commonly used screening procedures: the Vogel conflict drinking test[37] and the modified forced swim test.[38,39] SB-742457 was used as a reference. Compound **14**, given at a dose of 3 mg/kg (ip), demonstrated anticonflict activity in Vogel test by significantly increasing the number of shocks that were accepted by rats. This effect was almost 2-fold higher than that observed for SB-742457 (340% vs 178%) (Figure 7). Moreover, compounds **14** and **15**

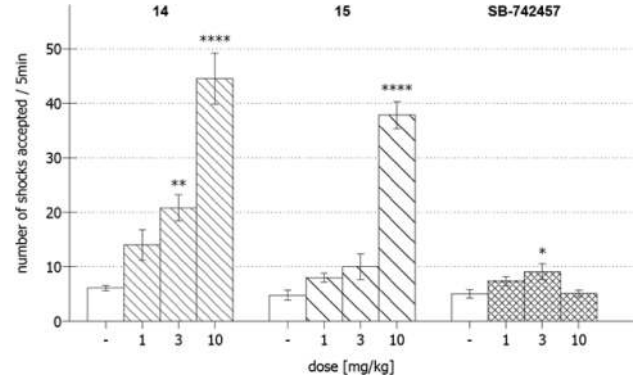


Figure 7. Anxiolytic-like effects of **14**, **15**, and SB-742457 in the Vogel conflict drinking test in rats. Values represent the mean ± standard error of the mean. *p < 0.05 vs vehicle, **p < 0.01 vs vehicle, ****p < 0.0001 vs vehicle (one-way ANOVA followed by Bonferroni's post hoc test); N = 7−8.

were both active at doses of 10 mg/kg (ip), a dose of this level increased the number of shocks that rats accepted by 787% and 729%, respectively.

In the forced swim test, the derivatives **14** (10 mg/kg, ip) and **15** (3 mg/kg, ip) and SB-742457 (3 mg/kg, ip) exerted significant antidepressant-like properties, with similar potencies; the effects were assessed according to the decreased immobility time and increased climbing time of the animals (Figure 8).

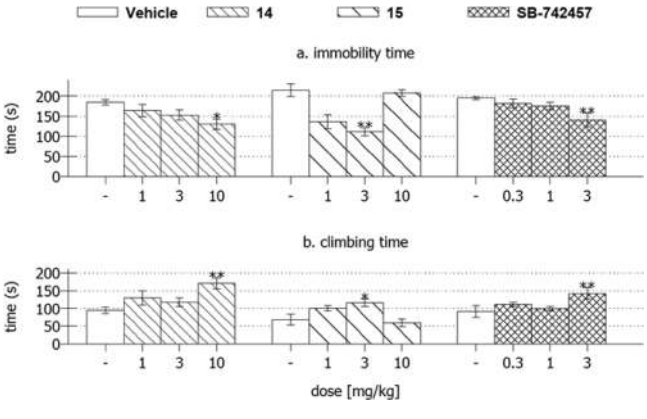


Figure 8. Antidepressant-like properties of **14, 15** and SB-742457 in the modified forced swim test in rats. Values represent the mean ± standard error of the mean. *p < 0.05 vs vehicle, **p < 0.01 vs vehicle (ANOVA is followed by the Bonferroni's post hoc test); N = 7−8.

The results suggest that the mechanism of antidepressant action of the tested compounds is associated with noradrenergic, rather than serotonergic, neurotransmission,[39,40] which is in agreement with literature data for 5-HT$_6$ receptor antagonists.[41] The antidepressant-like activity of compounds **14**, **15**, and SB-742457 seems to be specific because they did not affect the rats' locomotor activity in the open-field paradigm (data not shown).

CONCLUSION

As part of ongoing efforts to develop novel procognitive therapies, we demonstrated that the scaffold-hopping approach could be efficiently applied to identify a structurally novel class of 5-HT$_6$R antagonists in a group of 1*H*-pyrrolo[3,2-*c*]quinolines. Initial lead optimization around the arylsulfonyl fragment afforded compound **14**, a more selective and potent 5-HT$_6$R antagonist than the reference compound SB-742457. Further evaluation of the 5-HT$_6$R's constitutive activity showed that **14** was classified as a neutral antagonist, while SB-742457 behaved as an inverse agonist. The high potency and different functional properties of **14** and SB-742457 that were demonstrated in in vitro tests translated well into in vivo procognitive properties either in PCP-induced memory decline (MED = 3 mg/kg) or in the natural forgetting model in NOR tasks (MED = 3 mg/kg). Compound **14** has additionally demonstrated a higher anxiolytic effect than SB-742457 in Vogel test (MED for **14** = 3 mg/kg) and showed similar antidepressant-like properties in 3-fold higher dose than that observed for SB-742457 in the forced swim test. The study evidenced comparable behavioral effects of **14** (5-HT$_6$R antagonist) and SB-742457 (5-HT$_6$R inverse agonist). These results support the therapeutic potential of 5-HT$_6$R antagonists and/or inverse agonists for the treatment of cognitive decline and other symptoms associated with Alzheimer's disease. It seems that more detailed biochemical studies would provide further information about the action of 5-HT$_6$R ligands. Likewise, their therapeutic potential in other neuropsychiatric disorders like autism or Parkinson's disease certainly warrants further exploration.

METHODS

General Methods. The synthesis was carried out at ambient temperature, unless indicated otherwise. Organic solvents (from Aldrich and Chempur) were of reagent grade and were used without purification. The reagents were purchased from Sigma-Aldrich, Apeiron Synthesis, Chembridge, and Fluorochem.

^{1}H NMR and ^{13}C NMR spectra were obtained in a Varian BB 200 spectrometer and were recorded at 300 and 75 MHz, respectively; J values are in hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), and m (multiplet).

UPLC/MS were carried out on a system consisting of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer. All the analyses were carried out using an Acquity UPLC BEH C18, 100 × 2.1 mm column, at 40 °C. A flow rate of 0.3 mL/min and a gradient of (0−100)% B over 10 min was used. Eluent A, water/0.1% HCO2H; eluent B, acetonitrile/0.1% HCO2H. Retention times t_R were given in minutes. The UPLC/MS purity of all the test compounds and key intermediates was determined to be >99%.

HRMS analyses were performed at The Laboratory for Physical Measurements of the Université de Montpellier, on a Synapt G2-S (Waters) with ESI ionization mode.

Melting points were determined with a Büchi apparatus and are uncorrected.

Optical rotation was measured on a Jasco polarimeter (model P-2000, Eastan) using a sodium lamp, emitting light at a wavelength of 589 nm and 10 cm polarimetric tube. Measurements were performed in thermostatic conditions at 20 °C.

Elemental analyses for C, H, and N were carried out using the elemental Vario EI III elemental analyzer (Hanau, Germany). Elemental analyses were found within ±0.4% of the theoretical values.

Synthetic Procedures. *Methyl 2-[(2-Nitrophenyl)(p-toluenesulfonylamino)-methyl]acrylate (**1**).* In a dried flask, *p*-toluenesulfonamide (18 g, 105 mmol, 1 equiv) and 1,4-diazabicyclo[2.2.2]octane (DABCO) (1.78 g, 15.7 mmol, 0.15 equiv) were mixed together with the previously activated molecular sieves (4 Å, 21 g). The mixture was suspended in isopropanol (300 mL), followed by addition of nitrobenzaldehyde (15.8 g, 105 mmol, 1 equiv) and methyl acrylate (10.7 mL, 115 mmol, 1.1 equiv). Subsequently, titanium isopropoxide ($Ti(iOPr)_4$) was added as a freshly prepared solution in isopropanol (0.6 mL, 2.1 mmol, 0.02 equiv). The flask was filled with nitrogen and the mixture was stirred at room temperature overnight. Then, a mixture was filtered through Celite, which was rinsed with CH_2Cl_2. The solvent was evaporated, and the remaining crude was dissolved in AcOEt (400 mL), washed three times with 1 M $KHSO_4$, once with saturated $NaHCO_3$, water, and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a yellow oil which was subsequently dissolved in AcOEt (300 mL) and precipitated upon portionwise addition of *n*-hexane (300 mL). The appearing white precipitate was filtered, rinsed with diethyl ether and dried under vacuum.

White solid, 63% yield, t_R = 6.30, Mp 109−111 °C, $C_{18}H_{18}N_2O_6S$, MW 390.41. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.40 (s, 3H), 3.57 (s, 3H), 5.68 (d, *J* = 0.77 Hz, 1H), 5.84 (d, *J* = 8.72 Hz, 1H), 6.08 (d, *J* = 8.46 Hz, 1H), 6.21 (s, 1H), 7.20−7.26 (m, 2H), 7.40 (m, *J* = 7.76, 1.41 Hz, 1H), 7.54 (m, *J* = 7.69, 1.28 Hz, 1H), 7.66−7.71 (m, 3H), 7.82 (dd, *J* = 8.08, 1.41 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 21.1, 52.1, 54.4, 124.5, 127.2, 128.8, 129.1, 129.6, 130.1, 133.1, 133.4, 137.1, 137.4, 143.6, 148.0, 165.4. Monoisotopic mass 390.09, $[M + H]^+$ 391.4.

*Methyl 2-[(N-Allyl-N-tosylamino)(2-nitrophenyl)-methyl]acrylate (**2**).* β-Aminoester **1** (10 g, 25.6 mmol, 1 equiv) was dissolved in DMF (100 mL), followed by addition of K_2CO_3 (10.5 g, 76.5 mmol, 3 equiv). Subsequently, allyl bromide (4.42 mL, 51.2 mmol, 2 equiv) was added dropwise. The reaction was stirred at room temperature for 6 h. The mixture was diluted with ethyl acetate (300 mL) and washed 5× with water and brine, and dried over Na_2SO_4. The organic phase was filtered and hexane was added to precipitate a white solid, which was filtered and dried under vacuum.

White solid, 95% yield, t_R = 7.48, Mp 83−85 °C, $C_{21}H_{22}N_2O_6S$, MW 430.47. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.41 (s, 3H), 3.49−3.55 (m, 3H), 3.92−4.01 (m, 1H), 4.15−4.25 (m, 1H), 4.92−5.03 (m, 2H), 5.43−5.59 (m, 2H), 6.44 (s, 1H), 6.79 (s, 1H), 7.23−7.27 (m, 2H), 7.42−7.49 (m, 1H), 7.61−7.68 (m, 3H), 7.78 (d, *J* = 7.69 Hz, 1H), 7.93 (dd, *J* = 8.21 Hz, 1.28 Hz, 1H). Monoisotopic mass 430.12, $[M + H]^+$ 430.8. HRMS calcd for $C_{21}H_{23}N_2O_6S$, 431.1277; found, 431.1277.

*Methyl 2,5-Dihydro-2-(2-nitrophenyl)-1-tosyl-1H-pyrrole-3-carboxylate (**3**).* The β-aminoester **2** (5 g, 11.6 mmol, 1 equiv) was dissolved in CH_2Cl_2 (30 mL) in a round-bottom flask, and the Grubbs II catalyst (297 mg, 0.35 mmol, 3 mol %) was added. The reaction was stirred at 36 °C for 30 min under microwave irradiation in the open vessel mode. DMSO (1.22 mL, 17.4 mmol, 1.5 equiv) was added and the mixture stirred at room temperature for 20 h. After this time, silica gel was added into the solution and it was stirred for 5 min. The mixture was diluted with CH_2Cl_2 and filtered through a layer of silica gel. The filtrate was evaporated and the obtained residue was treated with diethyl ether giving a white precipitate which was filtered and dried under vacuum.

White solid, 80% yield, Mp 125−127 °C, t_R = 6.83, $C_{19}H_{18}N_2O_6S$, MW 402.42.^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.36 (s, 3H), 3.45 (s, 3H), 4.29 (m, 1H), 4.54 (m, 1H), 6.61−6.64 (m, 1H), 6.68 (t, *J* = 2.0 Hz, 1H), 7.27 (d, *J* = 8.27 Hz, 2H), 7.31−7.36 (m, 1H), 7.48−7.50 (m, 2H), 7.74 (d, *J* = 8.27 Hz, 2H), 7.83 (d, *J* = 8.22 Hz 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 21.56, 52.03, 55.73, 62.29, 124.54, 127.75, 128.62, 129.64, 130.04, 133.16, 133.38, 134.91, 135.68, 136.76, 144.25, 148.70, 161.57. Monoisotopic mass 402.09, $[M + H]^+$ 403.3. HRMS calcd for $C_{19}H_{19}N_2O_6S$, 403.0964; found, 403.0967.

*Methyl 2-(2-Nitrophenyl)-1H-pyrrole-3-carboxylate (**4**).* To a solution of 2,5-dihydropyrrole **3** (2.5 g, 6.2 mmol, 1 equiv) in DMF (40 mL), sodium *tert*-butoxide (2.09 g, 18.7 mmol, 3 equiv) was added. The reaction was stirred for 2 h and monitored by TLC. The mixture was diluted with ethyl acetate, neutralized with 1 M $KHSO_4$, and washed with a saturated solution of $NaHCO_3$, three times with water, and once with brine. The organic layer was dried over Na_2SO_4 and evaporated. The obtained crude product was purified on silica gel with AcOEt/Hex (4/6) as a developing solvent.

Yellow oil, 85% yield, t_R = 5.72, $C_{12}H_{10}N_2O_4$, MW 246.22. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.62 (s, 3H), 6.64 (t, *J* = 2.80 Hz, 1H), 6.72 (t, *J* = 2.95 Hz, 1H) 6.82−6.85 (m, 1H) 7.44−7.48 (m, 1H) 7.51−7.58 (m, 1H) 7.60−7.66 (m, 1H) 8.02 (dd, *J* = 8.21, 1.28 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 51.02, 111.45, 113.60, 118.61, 124.37, 127.29, 129.49, 132.54, 149.57, 164.67. Monoisotopic mass 246.06, $[M + H]^+$ 247.3.

*Methyl 2-(2-Aminophenyl)-1H-pyrrole-3-carboxylate (**5**).* The nitro derivative **4** (2.5 g, 10.2 mmol, 1 equiv) was dissolved in methanol, and 10% Pd/C (180 mg, 7 weigh%) and acetic acid (0.26 mL, 4.6 mmol, 0.45 equiv) were subsequently added. The mixture was stirred under a hydrogen atmosphere at room temperature for 2 h. Then, the mixture was filtered though Celite, which was rinsed with methanol. The subsequent evaporation yielded an orange oil.

Orange oil, 95% yield, t_R = 4.84, $C_{12}H_{12}N_2O_2$, MW 216.24. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 3.60−3.62 (m, 3H) 6.59 (d, *J* = 3.08 Hz, 1H) 6.67−6.70 (m, 2H) 6.71−6.73 (m, 1H) 7.04−7.11 (m, 2H). Monoisotopic mass 216.09, $[M + H]^+$ 217.0.

*1H-Pyrrolo[3,2-c]quinolin-4(5H)-one (**6**).* Compound **5** (2.5 g, 11.6 mmol, 1 equiv) was suspended in *sec*-butanol (50 mL), and acetic acid (0.5 mL, 8.7 mmol, 0.75 equiv) was added. The reaction mixture was stirred at 70 °C for 3 h. The solvent was evaporated under vacuum giving an orange oil.

Orange oil, 99% yield, t_R = 2.97, $C_{11}H_8N_2O$, MW 184.19. ^{1}H NMR (300 MHz, methanol-d_4) δ (ppm) 6.81 (d, *J* = 3.08 Hz, 1H), 7.19 (d, *J* = 3.08 Hz, 1H), 7.23−7.30 (m, 1H), 7.36−7.45 (m, 2H), 7.97 (d, *J* = 7.69 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 105.53, 113.45, 115.20, 116.25, 121.81, 123.02, 127.51, 134.83, 136.53, 160.20, 172.54. Monoisotopic mass 184.06, $[M + H]^+$ 185.0. HRMS calcd for $C_{11}H_9N_2O$,185.0715; found, 185.0714.

*4-Chloro-1H-pyrrolo[3,2-c]quinoline (**7**).* 80 mL of $POCl_3$ was added to the lactam **6** (2 g, 10.8 mmol, 1 equiv), and the mixture was heated at 105 °C for 4 h. Then, the mixture was evaporated under vacuum and 50 g of ice was added. After melting, the solution was neutralized with ammonia solution. The product was extracted three times with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and evaporated, yielding a white solid.

White solid, 85% yield, Mp 253−255 °C, t_R = 5.12, $C_{11}H_7ClN_2$, MW 202.64. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 6.71−6.73 (m, 1H), 7.60−7.66 (m, 1H), 7.93−7.98 (m, 3H), 8.36−8.41 (m, 1H), 12.82 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) ppm 102.72, 118.01, 119.18, 121.70, 126.05, 126.67, 127.63, 128.77, 136.45, 142.94, 144.47. Monoisotopic mass 202.03, $[M + H]^+$ 203.1, 205.1. HRMS calcd for $C_{11}H_8ClN_2$, 203.0376; found, 203.0377.

General Procedure for Preparation of Compounds 8a−8c. Compound **7** (0.35 g, 1.7 mmol, 1 equiv) was suspended in 12 mL of MeCN followed by addition of (*R,S*)-1-Boc-3-aminopyrrolidine (**8a**), (*R*)-1-Boc-3-aminopyrrolidine (**8b**) or (*S*)-1-Boc-3-aminopyrrolidine (**8c**) (1.3 g, 6.9 mmol, 4 equiv). The reaction was performed in a Biotage Microwave and stirred at 140 °C for 5h. The solvent was subsequently evaporated and the mixture was purified on silica gel with CH_2Cl_2/MeOH 9/1.5 as a developing solvent.

*(R,S)-4-(1-tert-Butoxycarbonyl-pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline (**8a**).* Orange oil, 60% yield, t_R = 4.38, $C_{20}H_{24}N_4O_2$, MW 352.43. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 1.40 (s, 9H), 1.98 (bs, 1H), 2.30 (bs, 1 H), 3.27−3.56 (m, 4H), 3.71−3.81 (m, 1 H), 4.93 (bs, 1H), 6.55 (d, *J* = 3.08 Hz, 1H), 7.13 (d, *J* = 3.08 Hz, 1H), 7.16−7.23 (m, 1H), 7.25−7.27 (m, 1H), 7.33−7.34 (t, *J* = 7.31 Hz, 1H), 7.71−7.81 (d, *J* = 8.46 Hz, 1H), 7.86 (dd, *J* = 7.95, 1.28 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 28.65, 44.59, 44.75, 50.56, 52.05, 78.65, 102.14, 111.10, 115.93, 120.63, 121.24, 122.72, 126.41, 134.96, 144.33, 152.01, 154.11. Monoisotopic mass 352.19, $[M + H]^+$ 353.2. HRMS calcd for $C_{20}H_{25}N_4O_2$, 353.1978; found, 353.1978.

*(R)-4-(1-tert-Butoxycarbonyl-pyrrolidine-3-yl-amino)-1H-pyrrolo-[3,2-c]quinoline (**8b**).* Orange oil, 62% yield, t_R = 4.38, $C_{20}H_{24}N_4O_2$,

MW 352.43. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 1.37−1.50 (m, 9H), 2.00 (bs, 1H), 2.30 (bs, 1H), 3.28−3.56 (m, 4H), 3.70−3.85 (m, 1H), 4.92 (bs, 1H), 6.50−5.58 (d, J = 3.08 Hz, 1H), 7.10−7.15 (d, J = 3.08 Hz, 1H), 7.17−7.24 (m, 1H), 7.25−7.28 (m, 1H), 7.34−7.43 (t, J = 7.18 Hz, 1H), 7.72−7.82 (d, J = 7.95 Hz, 1H), 7.83−7.90 (dd, J = 7.95, J = 1.03 Hz, 1H). Monoisotopic mass 352.19, $[M + H]^+$ 353.2. HRMS calcd for $C_{20}H_{25}N_4O_2$, 353.1978; found, 353.1978.

*(S)-4-(1-tert-Butoxycarbonyl-pyrrolidine-3-yl-amino)-1H-pyrrolo-[3,2-c]quinoline (**8c**).* Orange oil, 60% yield, t_R = 4.38, $C_{20}H_{24}N_4O_2$, MW 352.43. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 1.35−1.49 (m, 9H) 1.98 (bs, 1H) 2.30 (bs, 1H) 3.27−3.56 (m, 4H) 3.71−3.81 (m, 1H) 4.93 (bs, 1H) 6.55 (d, J = 3.08 Hz, 1H) 7.13 (d, J = 3.08 Hz, 1H) 7.16−7.23 (m, 1H) 7.25−7.27 (m, 1H) 7.33−7.34 (t, J = 7.31 Hz, 1H,) 7.71−7.81 (d, J = 8.46 Hz, 1H) 7.86 (dd, J = 7.95, 1.28 Hz, 1H). Monoisotopic mass 352.19, $[M + H]^+$ 353.2. HRMS calcd for $C_{20}H_{25}N_4O_2$, 353.1978, found, 353.1978.

General Procedure for Preparation of Final Compounds 9−27. Compounds **8a−8c** (100 mg, 0.28 mmol, 1 equiv) were dissolved in CH_2Cl_2 (5 mL), and BTPP (171 μL, 0.56 mmol, 2 equiv) was added. The mixture was placed in an ice-bath, sulfonyl chloride (1.8 equiv) was added, and the reaction mixture was stirred for 3 h. Subsequently, the mixture was evaporated and the remaining yellow crude product was purified on silica gel. The Boc-protected derivatives were treated with 4 N HCl solution in dioxane to give the final products as HCl salts of secondary amines.

*(R,S)-1-(Phenylsulfonyl)-N-(pyrrolidin-3-yl)-1H-pyrrolo[3,2-c]-quinolin-4-amine dihydrochloride (**9**).* White solid, 80% yield, t_R = 4.78, Mp 220−222 °C, Anal. ($C_{21}H_{22}Cl_2N_4O_2S{\cdot}H_2O$) C, H, N. MW 483.41. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.21 (d, J = 4.98 Hz, 1H), 2.31−2.45 (m, 1H), 3.56 (d, J = 4.98 Hz, 5H), 5.28 (bs, 1H), 7.44 (d, J = 7.04 Hz, 1H), 7.55−7.65 (m, 3H), 7.68−7.77 (m, 1H), 7.92 (d, J = 7.62 Hz, 2H), 8.19 (d, J = 3.52 Hz, 1H), 8.65 (d, J = 7.92 Hz, 1H), 9.50 (bs, 2H). Monoisotopic mass: 392.13, $[M + H]^+$ 393.1. HRMS calcd for $C_{21}H_{21}N_4O_2S$, 393.1385, found, 393.1389.

*(R,S)-1-[(3-Fluorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**10**).* White solid, 87% yield, t_R = 3.96, Mp 207−209 °C, Anal. ($C_{21}H_{21}Cl_2FN_4O_2S{\cdot}H_2O$) C, H, N. MW 501.40. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.15−2.26 (m, 1 H), 2.25−2.40 (m, 1H), 3.12−3.18 (m, 1H), 3.20−3.35 (m, 1H), 3.45−3.70 (m, 3H), 5.29 (bs, 1H), 7.41−7.46 (m, 1H), 7.58−7.71 (m, 3H), 7.78 (d, J = 7.06 Hz, 1H), 7.93 (d, J = 7.92 Hz, 1H), 8.06 (bs, 1H), 8.19 (d, J = 3.52 Hz, 1H), 8.39 (bs, 1H), 8.63 (d, J = 8.21 Hz, 1 H), 9.52 (bs, 2H). Monoisotopic mass 410.12, $[M + H]^+$ 411.3. HRMS calcd for $C_{21}H_{20}FN_4O_2S$, 411.1291; found, 411.1291.

*(S)-1-[(3-Fluorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**11**).* White solid, 85% yield, t_R = 3.95, Mp 197−199 °C, Anal. ($C_{21}H_{21}Cl_2FN_4O_2S{\cdot}H_2O$) C, H, N, MW 501.40. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.21 (d, J = 4.69 Hz, 1H), 2.37−2.42 (m, 1H), 3.23−3.34 (m, 2 H), 3.52−3.59 (m, 3H), 5.28 (bs, 1H), 7.43−7.47 (m, 1H), 7.58−7.69 (m, 2H), 7.77 (d, J = 7.04 Hz, 1H), 7.94 (d, J = 7.92 Hz, 1H), 8.05 (bs, 1H), 8.18 (d, J = 3.52 Hz, 1H), 8.39 (bs, 1H), 8.63 (d, J = 8.21 Hz, 1 H), 9.51 (bs, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 31.07, 44.22, 49.18, 52.49, 108.61, 113.01, 114.85, 115.20, 115.89, 120.49, 123.73, 124.02, 125.46, 130.41, 130.58, 133.41, 134.47, 138.65, 148.62, 160.56, 163.88. Monoisotopic mass 410.12, $[M + H]^+$ 411.3. HRMS calcd for $C_{21}H_{20}FN_4O_2S$, 411.1291; found, 411.1292.

*(R)-1-[(3-Fluorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**12**).* White solid, 90% yield, t_R = 3.76, Mp 220−221 °C, Anal. ($C_{21}H_{21}Cl_2FN_4O_2S{\cdot}H_2O$) C, H, N. MW 501.40. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.14−2.27 (m, 1H), 2.33−2.45 (m, 1H), 3.20−3.35 (m, 2H), 3.50−3.67 (m, 3H), 5.30 (bs, 1H), 7.42−7.46 (m, 1H), 7.56−7.70 (m, 3H), 7.73−7.80 (m, 1H), 7.94 (d, J = 7.92 Hz, 1H), 8.07 (bs, 1H), 8.19 (d, J = 3.81 Hz, 1H), 8.40 (bs, 1H), 8.63 (dd, J = 8.50, 1.17 Hz, 1H), 9.52 (bs, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 31.18, 44.19, 49.10, 52.73, 108.80, 112.94, 114.86 115.20, 115.89, 120.26, 123.73, 124.03, 125.56, 130.16, 130.63, 133.42, 134.44, 138.63, 148.47, 160.53, 163.86. Monoisotopic mass 410.12, $[M + H]^+$ 411.3. HRMS calcd for $C_{21}H_{20}FN_4O_2S$, 411.1291; found, 411.1291.

*(R,S)-1-[(3-Chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**13**).* White solid, 90% yield, t_R = 4.13, Mp 220−221 °C, Anal. ($C_{21}H_{21}Cl_3N_4O_2S{\cdot}H_2O$) C, H, N. MW 517.85. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.20−2.25 (m, 1H), 2.36−2.43 (m, 1H), 3.25−3.36 (m, 1H), 3.49−3.57 (m, 4H), 5.24 (s, 1H), 7.36−7.46 (m, 1H), 7.63 (t, J = 7.96 Hz, 2H), 7.81−7.83 (m, 1H), 7.88−8.08 (m, 2H), 8.19−8.20 (m, 1H), 8.30−8.33 (m, 1H), 8.63−8.66 (m, 1H), 9.46 (s, 2H). Monoisotopic mass 426.09, $[M + H]^+$ 427.2. HRMS calcd for $C_{21}H_{20}ClN_4O_2S$, 427.0995; found, 427.0996.

*(S)-1-[(3-Chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**14**).* White solid, 92% yield, t_R = 4.18, Mp 219−221 °C, Anal. ($C_{21}H_{21}Cl_3N_4O_2S{\cdot}H_2O$) C, H, N. MW 517.85. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.25−2.38 (m, 1H), 2.54−2.68 (m, 1H), 3.23−3.35 (m, 1H), 3.57−3.76 (m, 4H), 5.50−5.64 (m, 1H), 7.35−7.45 (m, 2H), 7.48−7.61 (m, 3H), 7.68−7.72 (m, 1H), 7.84−7.95 (m, 2H), 8.46 (d, J = 8.46 Hz, 1H), 8.75 (dd, J = 8.59, 1.15 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 31.16, 44.21, 49.11, 52.70, 108.85, 112.93, 115.91, 120.30, 123.69, 125.56, 126.41, 127.18, 130.19, 130.67, 132.77, 134.42, 135.29, 136.14, 138.49, 148.45. $[\alpha]_D^{20}$ = +2.93° (0.40, EtOH/H_2O 9/1). Monoisotopic mass 426.09, $[M + H]^+$ 427.2. HRMS calcd for $C_{21}H_{20}ClN_4O_2S$, 427.0995; found, 427.0992.

*(R)-1-[(3-Chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**15**).* White solid, 91% yield, t_R = 4.18, Mp 221−223 °C, Anal. ($C_{21}H_{21}Cl_3N_4O_2S{\cdot}H_2O$) C, H, N. MW 517.85. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.24−2.40 (m, 1H), 2.52−2.69 (m, 1H), 3.27−3.44 (m, 2H), 3.56−3.79 (m, 3H), 5.46−5.68 (m, 1H), 7.35−7.46 (m, 1H), 7.52−7.65 (m, 2H), 7.75−7.85 (m, 2H), 7.92−8.02 (m, 2H), 8.05−8.20 (bs, 1H), 8.48 (d, J = 8.25 Hz, 1H), 8.76 (dd, J = 8.53, J = 1.10 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 31.16, 44.21, 49.11, 52.72, 108.85, 112.92, 115.90, 120.33, 123.69, 125.57, 126.41, 127.18, 130.20, 130.67, 132.77, 134.42, 135.29, 136.14, 138.48, 148.44. $[\alpha]_D^{20}$ = −5.65° (0.48, EtOH/H_2O 9/1). Monoisotopic mass 426.09, $[M + H]^+$ 427.2. HRMS calcd for $C_{21}H_{20}ClN_4O_2S$, 427.0995; found, 427.0998.

*(R,S)-1-{[3-(Trifluoromethyl)phenyl]sulfonyl}-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**16**).* White solid, 90% yield, t_R = 4.68, Mp 223−225 °C, Anal. ($C_{22}H_{21}Cl_2F_3N_4O_2S{\cdot}H_2O$) C, H, N. MW 551.41. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.22−2.44 (m, 1H), 2.53−2.74 (m, 1H), 3.32 (s, 2H), 3.66 (d, J = 10.18 Hz, 3H), 5.50−5.71 (m, 1H), 7.43 (d, J = 7.70 Hz, 1H), 7.51−7.68 (m, 2H), 7.81 (d, J = 7.70 Hz, 2H), 7.90−8.03 (m, 2H), 8.12 (s, 1H), 8.49 (d, J = 8.25 Hz, 1H), 8.79 (dd, J = 8.53, J = 0.83 Hz, 1H). Monoisotopic mass 460.12, $[M + H]^+$ 461.2. HRMS calcd for $C_{22}H_{20}N_4OF_3S$, 461.1259, found, 461.1259.

*(S)-1-{[3-(Trifluoromethyl)phenyl]sulfonyl}-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**17**).* White solid, 91% yield, t_R = 4.67, Mp 219−221 °C, Anal. ($C_{22}H_{21}Cl_2F_3N_4O_2S{\cdot}H_2O$) C, H, N, MW 551.41. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.21 (d, J = 5.28 Hz, 1H), 2.33−2.44 (m, 1H), 3.48 (dd, J = 11.58, 5.72 Hz, 2H), 3.62−3.68 (m, 3H), 5.29 (bs, 1H), 7.46 (t, J = 7.48 Hz, 1H), 7.64 (t, J = 7.77 Hz, 1H), 7.79−7.89 (m, 1H), 8.01−8.20 (m, 2H), 8.25 (d, J = 3.52 Hz, 1H), 8.39 (bs, 2H), 8.67 (d, J = 7.92 Hz, 1H), 9.51 (bs, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 31.08, 44.22, 49.17, 52.47, 108.66, 113.03, 114.85, 115.20, 115.91, 120.49, 123.34, 123.73, 124.01, 125.46, 130.14, 130.58, 133.41, 134.47, 138.66, 148.62, 160.54, 163.87. $[\alpha]_D^{20}$ = +3.50° (0.49, EtOH/H_2O 9/1). Monoisotopic mass 460.12, $[M + H]^+$ 461.2. HRMS calcd for $C_{22}H_{20}N_4OF_3S$, 461.1259, found, 461.1258.

*(R)-1-{[3-(Trifluoromethyl)phenyl]sulfonyl}-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**18**).* White solid, 88% yield, t_R = 4.67, Mp 230−232 °C, Anal. ($C_{22}H_{21}Cl_2F_3N_4O_2S{\cdot}H_2O$) C, H, N. MW 551.41. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.11−2.27 (m, 1H), 2.30−2.45 (m, 1H), 3.20−3.35 (m, 1H), 3.40−3.52 (m, 1H), 3.60−3.82 (m, 3H), 5.27 (bs, 1H), 7.42−7.46 (m, 1H), 7.63 (t, J = 7.04 Hz, 1H), 7.79−7.87 (m, 1H), 8.01−8.20 (m, 2H), 8.25 (d, J = 2.93 Hz, 1H), 8.39 (bs, 2H), 8.67 (d, J = 8.50 Hz, 1H), 9.47 (bs, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 31.18, 44.19, 49.10, 52.70, 108.80, 112.94, 114.86, 115.20, 115.89, 120.26, 123.35, 123.73, 124.02, 125.56, 130.16, 130.63, 133.42, 134.44, 138.61, 148.47, 160.53, 163.86.

$[\alpha]_D^{20} = -2.57°$ (0.48, EtOH/H_2O 9/1). Monoisotopic mass 460.12. $[M + H]^+$ 461.3. HRMS calcd for $C_{22}H_{20}N_4OF_3S$, 461.1259; found, 461.1263.

*(R,S)-1-[(4-Fluorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**19**).* White solid, 90% yield, t_R = 3.76, Mp 220−221 °C, Anal. ($C_{21}H_{21}Cl_2FN_4O_2S·H_2O$) C, H, N. MW 501.40. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.07−2.33 (m, 1H), 2.10−2.29 (m, 1H), 2.41−2.58 (m, 1H), 3.21−3.35 (m, 1H), 3.43−3.68 (m, 3H), 5.36 (bs, 1H), 7.03 (t, J = 7.95 Hz, 2H), 7.27−7.38 (m, 1H), 7.47 (t, J = 7.82 Hz, 1H), 7.59−7.72 (m, 2H), 7.72−7.80 (m, 1H), 7.80−7.88 (m, 1H), 8.27 (d, J = 8.46 Hz, 1H), 8.70 (d, J = 8.72 Hz, 1H). Monoisotopic mass 410.12, $[M + H]^+$ 411.3. HRMS calcd for $C_{21}H_{20}FN_4O_2S$, 411.1291; found, 411.1292.

*(R,S)-1-[(2,5-Difluorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**20**).* White solid, 79% yield, t_R = 4.63, Mp 191−193 °C, Anal. ($C_{21}H_{20}Cl_2F_2N_4O_2S·H_2O$) C, H, N. MW 519.39. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.22 (d, J = 5.28 Hz, 1H), 2.34−2.45 (m, 1H), 3.12−3.35 (m, 2H), 3.50−3.68 (m, 3H), 5.27 (bs, 1H), 7.45 (bs, 1H), 7.51−7.66 (m, 3H), 7.70−7.79 (m, 1H), 7.90−8.07 (m, 1H), 8.14 (bs, 1H), 8.35 (bs, 1H), 8.54 (d, J = 8.80 Hz, 1H), 9.49 (bs, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 30.75, 44.33, 49.11, 51.88, 106.91, 112.82, 114.60, 116.63, 116.99, 119.47, 119.58, 119.89, 123.56, 125.61, 130.28, 130.62, 134.99, 147.82. Monoisotopic mass: 428.11, $[M + H]^+$ 429.0. HRMS calcd for $C_{21}H_{19}F_2N_4O_2S$, 429.1197; found, 429.1201.

*(R,S)-1-[(3-Methoxyphenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**21**).* White solid, 95% yield, t_R = 3.92, Mp 210−212 °C, Anal. ($C_{22}H_{24}Cl_2N_4O_3S·H_2O$) C, H, N. MW 513.43. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.41 (s, 3H), 2.62−2.72 (m, 3H), 3.67−3.78 (m, 4H), 6.89 (d, J = 3.85 Hz, 1H), 7.07 (m, 1H), 7.29−7.44 (m, 2H), 7.57−7.75 (m, 3H), 7.81 (dd, J = 8.46, 0.77 Hz, 1H), 7.87−7.93 (m, 1H), 8.48 (dd, J = 8.46, J = 0.77 Hz, 1H), 8.56−8.63 (m, 1H). Monoisotopic mass 422.14. $[M + H]^+$ 423.2. HRMS calcd for $C_{22}H_{23}N_4O_3S$, 423.1491; found, 423.1494.

*(R,S)-1-[(3-Methylphenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**22**).* White solid, 90% yield, t_R = 3.88, Mp 200−202 °C, Anal. ($C_{22}H_{24}Cl_2N_4O_2S·H_2O$) C, H, N. MW 497.44. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.47 (s, 3H), 2.25−2.38 (m, 1H), 2.57−2.67(m, 1H), 3.26−3.35 (m, 1H), 3.40−3.47 (m, 1H), 3.59−3.75 (m, 3H), 5.52−5.64 (m, 1H), 7.29−7.61 (m, 5H), 7.70 (t, J = 1.80 Hz, 1H), 7.84−7.99 (m, 2H), 8.49 (d, J = 8.46 Hz, 1H), 8.78 (dd, J = 8.59, J = 1.15 Hz, 1H). Monoisotopic mass 406.15. $[M + H]^+$ = 407.2. HRMS calcd for $C_{22}H_{23}N_4O_2S$, 407.1541; found, 407.1542.

*(R,S)-1-{[4-(tert-Butyl)phenyl]sulfonyl}-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**23**).* White solid, 78% yield, t_R = 5.02, Mp 196−198 °C, Anal. ($C_{25}H_{30}Cl_2N_4O_2S·H_2O$) C, H, N. MW 539.52. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 1.17 (s, 9H) 2.23−2.39 (m, 1H), 2.53−2.70 (m, 1H), 3.20−3.32 (m, 2H), 3.55−3.65 (m, 3H), 5.49−5.64 (m, 1H), 7.32−7.46 (m, 3H), 7.49−7.58 (m, 1H), 7.58−7.77 (m, 2H), 7.78−7.92 (m, 2H), 8.36−8.49 (m, 1H), 8.84 (d, J = 8.53 Hz, 1H). Monoisotopic mass 448.19, $[M + H]^+$ 449.3. HRMS calcd for $C_{25}H_{29}N_4O_2S$, 449.2011; found, 449.2007.

*(R,S)-1-{[4-(iso-Propyl)phenyl]sulfonyl}-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**24**).* White solid, 75% yield, t_R = 5.00, Mp 190−192 °C, Anal. ($C_{24}H_{28}Cl_2N_4O_2S·H_2O$) C, H, N. MW 525.49. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 1.12 (s, 6H) 2.20−2.35 (m, 1H), 2.50−2.69 (m, 1H), 3.18−3.30 (m, 2H), 3.55−3.66 (m, 3H), 5.51−5.66 (m, 1H), 7.27 (s, 1H), 7.32−7.45 (m, 2H), 7.46−7.70 (m, 3H), 7.78−7.90 (m, 2H), 8.36−8.50 (m, 2H), 8.84 (d, J = 8.53 Hz, 1H). Monoisotopic mass 434.18, $[M + H]^+$ 435.2. HRMS calcd for $C_{24}H_{27}N_4O_2S$, 435.1854; found, 435.1855.

*(R,S)-1-(Naphthalen-1-ylsulfonyl)-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**25**).* White solid, 85% yield, t_R = 4.30, Mp 223−225 °C, Anal. ($C_{25}H_{25}Cl_2N_4O_2S·H_2O$) C, H, N. MW 533.47. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.14−2.27 (m, 1H), 2.32−2.45 (m, 1H), 3.20−3.33 (m, 2H), 3.54−3.63 (m, 3H), 5.28 (bs, 1H), 7.33,(t, J = 7.33 Hz, 1H), 7.53−7.81 (m, 4H), 8.05−8.10 (m, 2H), 8.10−8.48 (d, J = 8.50 Hz, 4H), 8.49−8.52 (m, 1H), 9.51 (bs, 2H). Monoisotopic mass 442.15, $[M + H]^+$ 443.2. HRMS calcd for $C_{25}H_{23}N_4O_2S$, 443.1542; found, 443.1541.

*(R,S)-1-(Quinolin-8-ylsulfonyl)-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**26**).* White solid, 86% yield, t_R = 5.11, Mp 209−211 °C, Anal. ($C_{24}H_{23}Cl_2N_5O_2S·H_2O$) C, H, N. MW 534.46. ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4) δ (ppm) 2.17−2.33 (m, 1H), 2.48−2.63 (m, 1H), 3.27−3.40 (m, 2H), 3.50−3.57 (m, 3H), 5.39−5.55 (m, 1H), 7.25−7.40 (m, 1H), 7.55−7.70 (m, 4H), 8.10−8.23 (m, 2H), 8.22−8.39 (m, 4H), 8.50−8.80 (m, 3H). Monoisotopic mass 443.14, $[M + H]^+$ 444.2. HRMS calcd for $C_{24}H_{22}N_5O_2S$, 444.1494; found, 444.1491.

*(R,S)-1-[(5-Methylbenzo[b]thiophen-2-yl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline dihydrochloride (**27**).* White solid, 65% yield, t_R = 5.23, Mp 221−223 °C, Anal. ($C_{24}H_{24}Cl_2N_4O_2S_2·H_2O$) C, H, N. MW 553.52. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.20 (d, J = 4.10 Hz, 1H), 2.36 (s, 4H), 3.25−3.32 (m, 2H), 3.50−3.56 (m, 2H), 5.25 (bs, 1H), 7.39 (dd, J = 8.65, 1.32 Hz, 1H), 7.50 (bs, 1H), 7.63 (t, J = 7.18 Hz, 1H), 7.79 (s, 1H), 7.92 (d, J = 8.50 Hz, 1H), 8.12−8.18 (m, 1H), 8.31 (d, J = 17.59 Hz, 1H), 8.57 (s, 1H), 8.86 (d, J = 8.50 Hz, 1H), 9.50 (bs, 2H). Monoisotopic mass 462.12, $[M + H]^+$ 463.0. HRMS calcd for $C_{24}H_{23}N_4O_2S_2$, 463.1262; found, 463.1258.

In Vitro Pharmacology. *Cell Culture and Preparation of Cell Membranes for Radioligand Binding Assays.* HEK293 cells with stable expression of human 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_{7b}, and D_{2L} receptors (prepared with the use of Lipofectamine 2000) or CHO-K1 cells with plasmid containing the sequence coding for the human serotonin 5-HT_{2A} receptor (PerkinElmer) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and grown in Dulbecco's modified Eagle's medium containing 10% dialyzed fetal bovine serum and 500 mg/mL G418 sulfate. For membrane preparation, cells were subcultured in 150 cm^2 diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS) and pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at −80 °C.

Radioligand Binding Assays. Cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer, and centrifuged twice at 35 000*g* for 15 min at 4 °C, with incubation for 15 min at 37 °C in between. Composition of the assay buffers was as follows. for 5-HT_{1A}R: 50 mM Tris HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate. 5-HT_{2A}R: 50 mM Tris HCl, 0.1 mM EDTA, 4 mM $MgCl_2$ and 0.1% ascorbate. 5-HT_6R: 50 mM Tris HCl, 0.5 mM EDTA and 4 mM $MgCl_2$. 5- HT_{7b}R: 50 mM Tris HCl, 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate. Dopamine D_{2L}R: 50 mM Tris HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate. All assays were incubated in a total volume of 200 μL in 96-well microtiter plates for 1 h at 37 °C, except for 5-HT_{1A}R and 5-HT_{2A}R, which were incubated at room temperature and 27 °C, respectively. The process of equilibration was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester, and radioactivity retained on the filters was quantified on a Microbeta plate reader (PerkinElmer). For displacement studies, the assay samples contained as radioligands (PerkinElmer): 2.5 nM [^{3}H]-8-OH-DPAT (135.2 Ci/mmol) for 5-HT_{1A}R; 1 nM [^{3}H]-ketanserin (53.4 Ci/mmol) for 5-HT_{2A}R; 2 nM [^{3}H]-LSD (83.6 Ci/mmol) for 5-HT_6R; 0.8 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R or 2.5 nM [^{3}H]-raclopride (76.0 Ci/mmol) for D_{2L}R. Nonspecific binding was defined with 10 μM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas 20 μM of mianserin, 10 μM of methiothepine or 10 μM of haloperidol were used in 5-HT_{2A}R, 5-HT_6R and D_{2L}R assays, respectively. Each compound was tested in triplicate at 7 concentrations (10^{-10}−10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation.[42] Results were expressed as means of at least two separate experiments.

Preliminary Agonist/Antagonist Screening. The functional activity of compounds on intracellular cAMP levels, studied in CHO cells stably expressing the human 5-HT_6 receptor, was determined at CEREP. cAMP concentration was measured by the HTRF method. Adenylate cyclase activity was expressed as the percentage of the maximal effect

obtained with 300 nM serotonin. Compounds were tested at a 10^{-6} M concentration. Serotonin was used as a reference compound for determination of the agonist effect and methiothepin for the antagonistic effect. Experimental conditions are described online at www.cerep.fr.

Functional cAMP Assay Protocol. The antagonistic properties of compounds at the 5-HT_6R were evaluated, as their ability to inhibit cAMP production induced by the agonist 5-CT (100 nM) in HEK293 cells overexpressing 5 HT_6R. Each compound was tested in triplicate at 8 concentrations (10^{-11}– 10^{-4} M). Cells (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's modified Eagle's medium containing 10% dialyzed fetal bovine serum and 500 mg/mL G418 sulfate. For functional experiments, cells were subcultured in 25 cm^2 diameter dishes, grown to 90% confluence, washed twice with prewarmed (37 °C) phosphate buffered saline (PBS) and were centrifuged for 5 min (160*g*). The supernatant was aspirated, and the cell pellet was resuspended in stimulation buffer (1 × HBSS, 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA). Total cAMP was measured using the LANCE cAMP detection kit (PerkinElmer), according to the manufacturer instructions. For cAMP levels quantification, cells (5 μL) were incubated with compounds (5 μL) for 30 min at room temperature, in a 384-well white opaque microtiter plate. After incubation, the reaction was stopped and cells were lysed by addition of 10 μL working solution (5 μL Eu-cAMP and 5 μL ULight-anti-cAMP). The assay plate was incubated for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TR-FRET) was detected by an Infinite M1000 Pro (Tecan) using instrument settings from LANCE cAMP detection kit manual. K_b values were calculated from Cheng–Prusoff equation[42] specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1 + A/EC_{50})$ where A is the agonist concentration, IC_{50} is the antagonist concentration producing a 50% reduction in the response to agonist, and EC_{50} is the agonist concentration, which causes a half of the maximal response.

Determination of cAMP Production as 5-HT_6R Constitutive Activity. cAMP measurement was performed in NG108-15 cells transiently expressing 5-HT_6 receptor using the bioluminescence resonance energy transfer (BRET) sensor for cAMP, CAMYEL (cAMP sensor using YFP-Epac-RLuc).[43] NG108-15 cells were cotransfected in suspension with 5-HT_6 receptor and CAMYEL constructs, using Lipofectamine 2000, according to the manufacturer protocol, and plated in white 96-well plates (Greiner), at a density of 80 000 cells per well. At 24 h after transfection, cells were washed with PBS containing calcium and magnesium. Coelanterazine H (Molecular Probes) was added at a final concentration of 5 μM, and left at room temperature for 5 min. BRET was measured using a Mithras LB 940 plate reader (Berthold Technologies).

In Vivo Pharmacology. *Novel Object Recognition Protocol.* Procedures are based on earlier studies of Popik et al.[44] The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology. Male Sprague–Dawley rats (Charles River, Germany) weighing ∼250 g at the arrival were housed in the standard laboratory cages, under standard colony A/C controlled conditions: room temperature 21 ± 2 °C, humidity (40–50%), 12 h light/dark cycle (lights on at 06:00) with ad libitum access to food and water. Rats were allowed to acclimatize for at least 7 days before the start of the experimental procedure. During this week animals were handled for at least 3 times. Behavioral testing was carried out during the light phase of the light/dark cycle. At least 1 h before the start of the experiment, rats were transferred to the experimental room for acclimation. Rats were tested in a dimly lit (25 lx) "open field" apparatus made of a dull gray plastic (66 × 56 × 30 cm^3). After each measurement, the floor was cleaned and dried. Procedure consisted of habituation to the arena (without any objects) for 5 min, 24 h before the test and test session comprised of two trials separated by an intertrial interval (ITI). For PCP-induced memory impairment paradigm, 1 h ITI was chosen. For memory improvement paradigm, 24 h ITI was chosen. During the first trial (familiarization, T1) two identical objects (A1 and A2) were presented in opposite corners, approximately 10 cm from the walls of the open field. In the second trial (recognition, T2) one of the objects was replaced by a novel one (A = familiar and B = novel). Both trials lasted 3 min, and animals were returned to their home cage after T1. The objects used were the glass beakers filled with the gravel and the plastic bottles filled with the sand. The heights of the objects were comparable (∼12 cm) and the objects were heavy enough not to be displaced by the animals. The sequence of presentations and the location of the objects was randomly assigned to each rat. The animals explored the objects by looking, licking, sniffing or touching the object while sniffing, but not when leaning against, standing or sitting on the object. Any rat spending less than 5 s exploring the two objects within 3 min of T1 or T2 was eliminated from the study. Exploration time of the objects and the distance traveled were measured using the Any-maze video tracking system. Based on exploration time (E) of two objects during T2, discrimination index (DI) was calculated according to the formula: DI = (EB − EA)/(EA + AB). Phencyclidine (PCP), used to attenuate learning, was administered at the dose of 5 mg/kg (ip) 45 min before familiarization phase (T1). The compounds **14** or **15** were administrated ip 30 min before PCP injection. In nondisturbed paradigm, memory improvement, compounds were administrated ip 30 min before familiarization phase (T1).

Vogel Conflict Drinking Test. The testing procedure was based on a method of Vogel et al.[37] as a conditional model where a noxious stimulus is applied. Anxiety Monitoring System Vogel test produced by TSE Systems (Germany) was used. It was consisted of polycarbonate cages (dimensions 26.5 × 15 × 42 cm^3), equipped with a grid floor made from stainless steel bars and drinking bottles containing tap water. Experimental chambers were connected to PC software by control chassis and electric shocks' generator. On the first day of the experiment, the rats were adapted to the test chambers and drink water from the bottle spout for 10 min. Afterward, the rats were returned to their home cages and were given 30 min free access to water followed by a 24 h water deprivation period. The adaptation session and water deprivation protocols were repeated on the second day of the experiment. On the third day, the rats were placed again in the test chambers 60 min after the compounds administration and were given free access to the drinking tube. Recording data started immediately after the first lick and rats were punished with an electric shock (0.5 mA, lasting 1 s) delivered to the metal drinking tube every 20 licks. The number of licks and the number of shocks received during a 5 min experimental session were recorded automatically.

The experiments were performed on male Wistar rats (230–260 g). The animals were housed in polycarbonate Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm^3) in an environmentally controlled room (ambient temperature 21 ± 2 °C; relative humidity 50–60%; 12:12 light/dark cycle, lights on at 08:00), in groups of four rats. Standard laboratory food (LSM-B) and filtered water were freely available. Animals were assigned randomly to treatment groups. Each experimental group consisted of 6–8 animals/dose, and the animals were used only once in each test. All the experiments were performed by three observers unaware of the treatment applied between 9:00 and 14:00 on separate groups of animals. All the experimental procedures were approved by the First Local Ethical Committee on Animal Testing at the Jagiellonian University in Kraków. Compounds **14**, **15**, and SB-722457 were dissolved in distilled water and injected intraperitoneally (ip) at a volume of 2 mL/kg 60 min before the test procedures. Control animals received a vehicle injection according to the same schedule.

Modified Forced Swim Test in Rats. The experiment was carried out according to the method of Porsolt et al. modified by Detke et al.[38,39] On the first day of an experiment, the animals were gently individually placed in Plexiglas cylinders (50 cm high, 18 cm in diameter) containing 30 cm of water maintained at 23–25 °C for 15 min. On removal from water, the rats were placed for 30 min in a Plexiglas box under a 60-W bulb to dry. On the following day (24 h later), the rats were replaced in the cylinder and the total duration of immobility, swimming, and climbing was recorded during the whole 5 min test period. The swimming behavior entailed active swimming motions, for example, moving horizontally around in the cylinder. Climbing activity consisted of upward directed movements of the forepaws along the side of the swim chamber, and immobility was assigned when no additional activity was observed other than that necessary to keep the rat's head above the

water.[41] Fresh water was used for each animal. Compounds **14**, **15**, and SB-742457 were dissolved in distilled water and injected intraperitoneally (ip) 60 min before the test.

Open field test in rats was performed using Motor Monitor System (Campden Instruments, Ltd., UK) consisting of two Smart Frame Open Field (OF) stations (40 × 40 × 38 cm^3) with 16 × 16 beams, located in sound attenuating chambers and connected to PC software by control chassis. Individual vehicle- or drug-injected animals were gently placed in the center of the station. An automated Motor Monitor System the total distance covered by a rat for 5 min.

The experiments were performed on male Wistar rats (230−260 g). The animals were housed in polycarbonate Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm^3) in an environmentally controlled room (ambient temperature 21 ± 2 °C; relative humidity 50−60%; 12:12 light/dark cycle, lights on at 08:00), in groups of four rats. Standard laboratory food (LSM-B) and filtered water were freely available. Animals were assigned randomly to treatment groups. Each experimental group consisted of 6−8 animals/dose, and the animals were used only once in each test. All the experiments were performed by three observers unaware of the treatment applied between 9:00 and 14:00 on separate groups of animals. All the experimental procedures were approved by the First Local Ethical Committee on Animal Testing at the Jagiellonian University in Kraków.

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Funding

The study was financially supported by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 671540, the project "Prokog", UDA-POIG.01.03.01-12-063/09-00, cofinanced by the European Union from the European Fund of Regional Development (EFRD), and by the University Montpellier, CNRS. F.L. and P.M. were supported by a grant from Institut Carnot Chimie Balard and Pôle BioSanté Rabelais. K.G. thanks the French Embassy in Warsaw for the cotutelle fellowship.

Notes

The authors declare no competing financial interest.

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