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Spiro[pyrrolidine-3,3′-oxindoles] as 5-HT$_7$ receptor ligands

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ABSTRACT

Here we report the design and synthesis of spiro[pyrrolidine-3,3′-oxindole] derivatives representing a novel scaffold of 5-HT$_7$ receptor ligands. The synthesized analogues were validated as low nanomolar ligands showing selectivity in a panel of related serotonin receptor subtypes including 5-HT$_{1A}$R, 5-HT$_{2A}$R and 5-HT$_6$R.

The serotonin receptor subtype-7 (5-HT$_7$R) is expressed in both the central nervous system (CNS) and peripheral tissues, and coupled positively to $G_{\alpha s}$ (activation raises cAMP levels)[1] or $G_{\alpha 12}$ protein. The 5-HT$_7$R plays role in the regulation of body temperature, sleep-wake rhythm, circadian rhythm, and mood. Thus the receptor has become rapidly an important target for several important CNS-related indications with proved in vivo efficacy in the animal models of depression,[2,3] sleep disorders,[4] anxiety,[5] learning and memory deficits,[6] and autism spectrum disorders.[7]

To date the candidate drug with the pyrazolo[3,4-*d*]azepine core (JNJ-18308683 **1**) has reached the clinic (a phase 2 study is currently recruiting[8]), and a number of selective and non-selective antagonists[1] (e.g. SB-656104 **2**, DR-4004 **3**) and agonists have been proved as investigational compounds (see Fig. 1 for examples).

An early *3D* pharmacophore model was built on the basis of thirty known 5-HT$_7$R antagonists including DR-4004 and analogues, SB-258719 (structure not shown) and analogues, SB-269970 (structure not shown) analogue etc.[9] The 5-HT$_7$R binding pharmacophore has been updated[10] using broad structure-activity relationship (SAR) data and structure-based docking validated by site-directed mutagenesis experiments. Pharmacophore features of 5-HT$_7$R ligands include an aromatic ring (in stacking interactions with Phe$^{3.28}$ and Tyr$^{7.43}$), two further hydrophobic regions HYD$_1$ and HYD$_2$ (facing towards Phe$^{6.52}$), a hydrogen bond acceptor (binding to Ser$^{5.42}$ and/or Thr$^{5.43}$) next to HYD$_1$, and a positive ionizable moiety located at 5–6 Å distance from HYD$_1$ that contacts Asp$^{3.32}$ (Fig. 2).

Tetrahydrobenzindoles (e.g. DR-4004 (**3**))[11,12] and corresponding oxindoles[13,14] were validated as potent 5-HT$_7$R scaffolds. As an attempt to synthesize new, selective and less lipophilic compounds we designed spiro[pyrrolidine-3,3′-oxindoles] (**4**) as potential 5-HT$_7$R ligands. Arylpiperazine analogues attached to the common spiro[pyrrolidine-3,3′-oxindole] core could provide ligands with more favourable ADME characteristics by exchanging the carbocycle of the tetrahydrobenzindole to the more polar and less rigid pyrrolidine ring. In order to design selective compounds against other serotonin receptors (5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_6$R) we used the pharmacophore models by López *et al.*[9,10] and further modified by Medina *et al.*[15] These models suggest that decreasing the distance between PI (positive ionizable) and HBA (H-bond acceptor) features, facilitating interactions to Ser$^{6.55}$ (Ala$^{6.55}$ at 5-HT$_{1A}$R), introducing polar substituents at HYD$_1$-HYD$_2$ to contact Arg$^{7.36}$ (that is absent in 5-HT$_{1A}$R) would be beneficial. Following these suggestions we set our objective for exploring selectivity drivers around the spiro[pyrrolidine-3,3′-oxindoles] core.

The 5-HT$_7$R pharmacophore suggested that we need a four-atom linker between the spiro carbon *C-3* to the pyrrolidine-nitrogen and therefore connected this with two methylenes (Fig. 2) to the basic moiety of compounds with General Structure **4**. Thus in line with other studies[12,11,16–19] we focused on compounds with two atom spacer (Fig. 3, compound **5**, although compound **6** was also synthesized in

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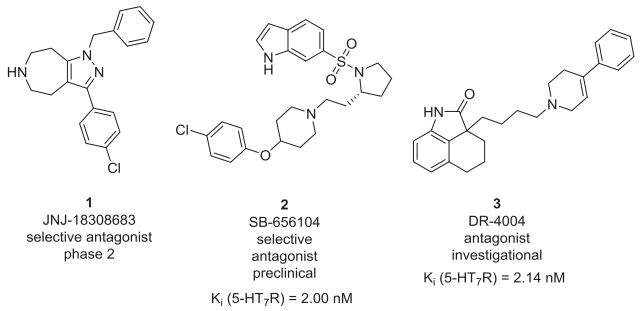


Fig. 1. Structures of the most prominent 5-HT$_7$R antagonists.

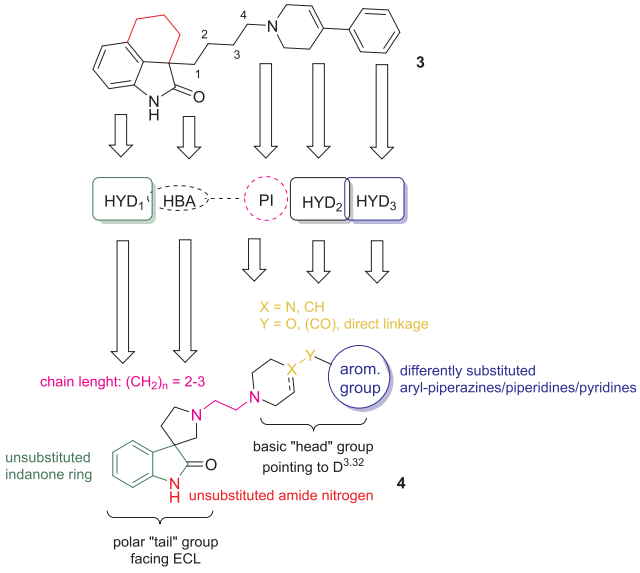
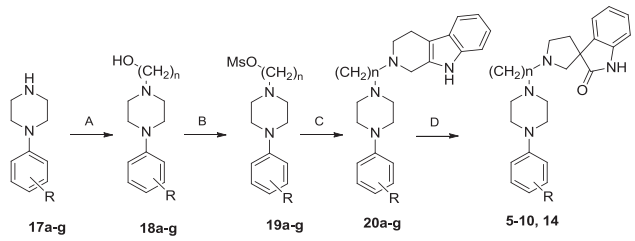


Fig. 2. Design concept for the SAR analysis of spiro[pyrrolidine-3,3′-oxindoles] (**4**).



Scheme 1. Synthesis of the aryl-piperazine derivatives (**5–10**, **14**). Reagents and conditions: (A) 2-bromoethanol (or 3-bromopropanol in case of **6**), K_2CO_3, MeCN, RT, overnight,; (B) MsCl, TEA, DCM, reflux, 2 h; (C) tryptoline/tetrahydro-β-carboline, K_2CO_3, MeCN, reflux, overnight; (D) NBS, cc. AcOH, THF, water, 1.5 h, 0 °C.

order to investigate its effect on flexibility and HBA-PI distances.

Halo-scan, however, was planned to explore the substituent effects around the phenyl ring of the aryl-piperazine moiety comparing the profile of the unsubstituted **7** and the meta-Cl **8**, ortho-Cl **9**, para-Cl **5**, and 3,4-dichloro-derivatives **10**. Halo-scan methodology explores the functionalization of the ligands thus filling subpockets, and probing the impact of introducing lipophilic, and possibly H-bonding halogen atoms.[20]

In order to explore the impact of the HYD$_2$/HYD$_3$ features on the 5-HT$_7$R affinity and selectivity we replaced the canonical aryl-piperazine by phenyl-, and phenoxy-piperidines **11**, **12**, **15**, 5,6-dihydropyridine **13**, and benzoyl-piperazine **16** moieties.

Aryl-piperazine derivatives **5–10**, **14** were synthesized from the corresponding secondary amines **17a–g**. First, cyclic secondary amines were alkylated by 2-bromoethanol (in case of 1-(4-chlorophenyl)piperazine **17a** either by 3-bromopropanol) followed by the mesylation of the appropriate alcohols using mesyl chloride. A protecting group on the basic nitrogen of the tryptoline is necessary to avoid decomposition during the spiro-rearrangement reaction. As depicted in Scheme 1, *N*-alkylation by the corresponding mesylates leading to intermediates **20a–g** provided the desired protection of the tetrahydro-β-carboline nitrogen for the final spirocyclization step to afford derivatives **5–10** and **14**.[21]

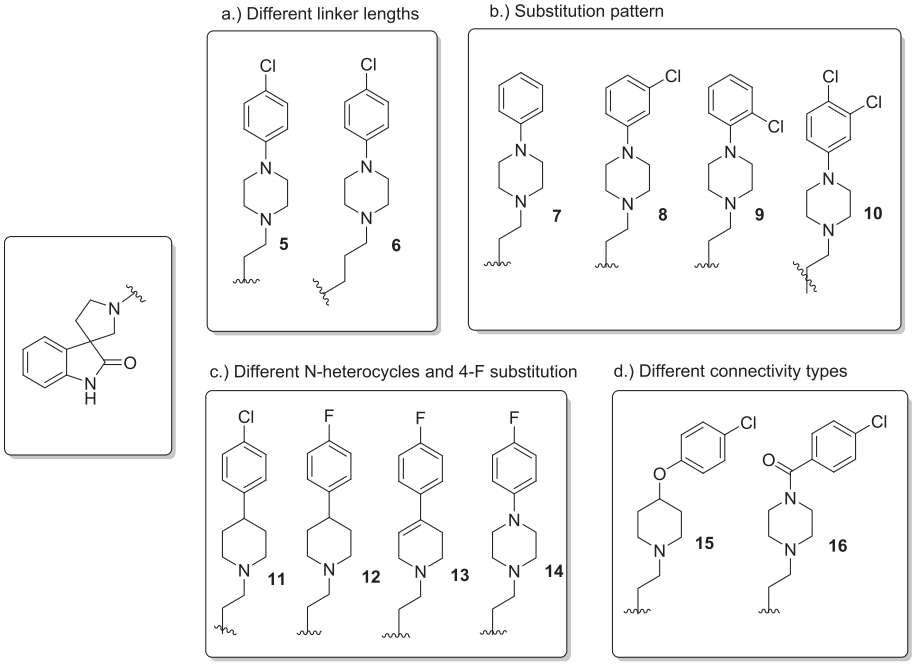
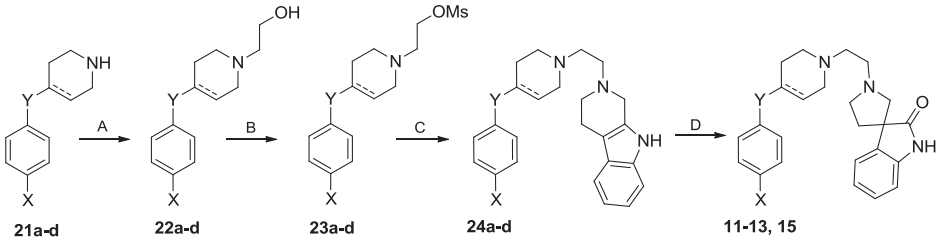
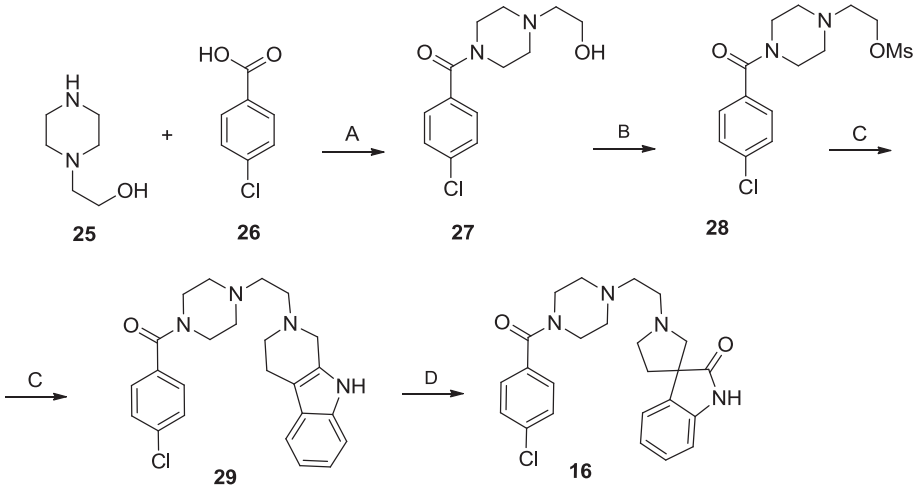


Fig. 3. Spiro[pyrrolidine-3,3′-oxindoles] designed for SAR studies.



Scheme 2. Synthesis of aryl-piperidine (**11–12**), and 5,6-dihydropyridine (**13**), and phenoxy (**15**) analogues. Reagents and conditions: (A) 2-bromoethanol, K_2CO_3, MeCN, RT, overnight; (B) MsCl, TEA, DCM, reflux, 2 h; (C) tryptoline/tetrahydro-β-carboline, K_2CO_3, MeCN, reflux, overnight; (D) NBS, cc. AcOH, THF, water, 1.5 h, 0 °C.



Scheme 3. Synthetic route leading to the amide-analogue (**16**). Reagents and conditions: (A) HATU, DIPEA, DMF, RT, overnight; (B) MsCl, TEA, DCM, reflux, 2 h; (C) tryptoline/tetrahydro-β-carboline, K_2CO_3, MeCN, reflux, overnight; (D) NBS, cc. AcOH, THF, water, 1.5 h, 0 °C.

The synthesis of the aryl-piperidine **11–12**, the *5,6*-dihydro-pyridine **13**, and the phenoxy **15** analogues (Scheme 2) was also started from the corresponding secondary amines **21a–d**, followed by the alkylation with 2-bromoethanol, to afford the alcohols **22a–d**. The crude mesylated derivatives **23a–d** were then used for alkylation of the tetrahydro-β-carboline to get **24a–d** that was spirocyclized to **11**, **12**, **13**, **15**, respectively.

1-(2-Hydroxyethyl)piperazine (**25**) was used as starting material for the synthesis of the benzoyl-piperazinyl analogue **16** (Scheme 3). The acylation[22] of **25** with the 4-chlorobenzoic acid **26** gave the corresponding amide **27**, which was treated with mesyl-chloride to yield the mesylate **28**. After the alkylation of tetrahydro-β-carboline with **28**, the spirocyclization of intermediate **29** afforded **16**.

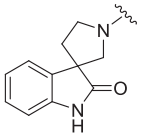
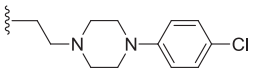
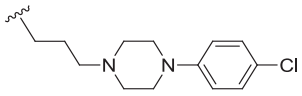
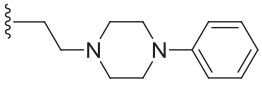
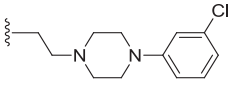
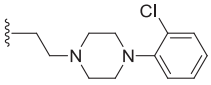
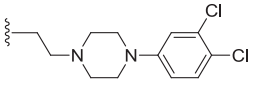
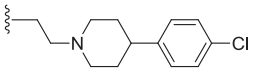
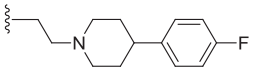
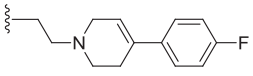
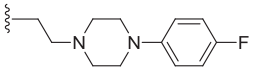
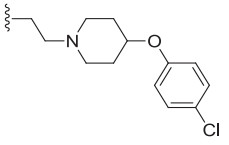
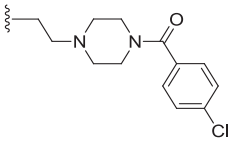
The synthesized compounds were investigated in competition binding assays against 5-HT_7R and other closely related serotonin receptor subtypes 5-HT_{1A}R, 5-HT_{2A}R and 5-HT_6R (Table 1).

The unsubstituted phenylpiperazine derivative **7** of the spiro[pyrrolidine-3,3′-oxindole] core showed reasonably high affinity towards the target, however its selectivity was moderate. Halo-scan around the phenyl ring revealed that the 5-HT_7R affinity and 5-HT_{1A} and 5-HT_6R selectivities are increasing from ortho **9** – meta **8** – para **5** direction. In fact, the para-Cl derivate (**5**) showed low nanomolar affinity for the target and more than hundred-fold selectivity against two of the three other serotonin receptors. Selectivity against these receptors was further improved for the para-F analogue **14**. As compared to compound **5**, the longer ($[CH_2]_3$) alkyl chain in **6** increased the distance between the PI and HBA features, accounting for an overall loss of affinity for the 5-HT_7R. Selectivity against 5-HT_{1A}R decreased, however the selectivity against the 5-HT_{2A}R subtype has improved. **6** showed lower selectivity (65.6-fold) towards 5-HT_6R than **7** (163.3-fold).

Starting from the most active 4-fluorine derivative (**14**) the 5,6-dihydropyridine derivative (**13**) showed decreased selectivity (91.8-fold) against 5-HT_{1A}R. Replacing the piperazine ring by piperidine (**12**) gave 10-times lower affinity that was further confirmed by the corresponding 4-chloro analogue (**11**). Selectivities against 5-HT_{1A}R and 5-HT_{2A}R did not change significantly, but the 5-HT_6R selectivity decreased ten-times. Similar to **11** and **12**, introduction of the phenoxy-piperidine moiety (**15**) improved the affinity towards 5-HT_6R. Finally, the benzoyl-piperazine derivative (**16**) showed reduced affinity to 5-HT_7R, however, it has the highest selectivity against 5-HT_{2A}R (32.8-fold).

In summary, preliminary SAR data demonstrates that spiro[pyrrolidine-3,3′-oxindoles] are potent and selective 5-HT_7R ligands. We confirmed that the 2-methylene linker ensures the ideal distance between HYD1 and HYD2 and therefore it is beneficial for the 5-HT_7R affinity. Although previous findings[13,23] suggested that the para substitution at the aryl-piperazine moiety may abolish 5-HT_7R affinity we showed that it provides spiro[pyrrolidine-3,3′-oxindoles] with reasonable affinity and selectivity against 5-HT_{1A}R and 5-HT_6R. Actually, the 4-fluoro analogue (**14**) showed the best affinity and most remarkable selectivity against 5-HT_{1A}R and 5-HT_6R. Selectivity against 5-HT_{2A}R might be improved by replacing the phenyl substituent of the piperazine by a benzoyl group. The present results show the potential of this novel chemotype and validate its further optimization for more detailed *in vivo* characterization in diseases models.

Table 1
Serotonergic activity of the spirooxindole derivatives as measured in binding assays of four serotonin receptors (K_i values are in μM). 5-HT_7R affinities and selectivities are shown in bold and italics, respectively. Assay reference compounds: serotonin in 5-HT_{1A}R and 5-HT_7R assays, chlorpromazine in 5-HT_{2A}R and methiothepine in 5-HT_6R assay. Results were expressed as means of at least three separate experiments (SD ≤ 24%).

ID	Structure	5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
					
5		3.755 *129.5*	0.068 *2.3*	4.737 *163.3*	**0.029**
6		2.891 *43.1*	0.715 *10.7*	5.559 *83.0*	**0.067**
7		6.005 *60.1*	0.496 *5.0*	6.556 *65.6*	**0.100**
8		2.687 *48.9*	0.351 *6.4*	2.005 *36.5*	**0.055**
9		1.096 *4.5*	0.452 *1.8*	2.843 *11.6*	**0.245**
10		4.054 *106.7*	0.270 *7.1*	1.131 *29.8*	**0.038**
11		14.900 *52.5*	0.404 *1.4*	4.577 *16.1*	**0.284**
12		52.010 *189.1*	0.259 *0.9*	5.285 *19.2*	**0.275**
13		3.949 *91.8*	0.075 *1.7*	6.267 *145.7*	**0.043**
14		14.160 *674.3*	0.059 *2.8*	4.744 *225.9*	**0.021**
15		13.790 *114.0*	0.343 *2.8*	1.693 *14.0*	**0.121**
16		48.240 *91.2*	17.330 *32.8*	7.746 *14.6*	**0.529**

Acknowledgments

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A. Supplementary data

Supplementary data (synthetic procedures, analytical data, description of the cell-based assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2018.06.019.

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