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SAR development of a selective 5-HT$_{1D}$ antagonist/serotonin reuptake inhibitor lead using rapid parallel synthesis

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Abstract—Incorporation of an SRI (serotonin reuptake inhibitor) pharmacophore into a selective 5-HT$_{1D}$ agonist has led to the discovery of a molecule having both 5-HT$_{1D}$ antagonist and SRI activity. RPS methodology was used to develop the SAR and identify potential approaches to reduce unwanted adrenergic α_1 and dopamine D_2 cross-reactivities.

The enhancement of serotonin (5-HT) function by selective serotonin reuptake inhibitors (SRIs) has revolutionized the treatment of major depression, producing therapeutic benefits and having better safety and patient compliance profiles than the tricyclic antidepressants. Although SRIs offer a significant advance in the treatment of major depression, there are limitations to their effectiveness. Some reports suggest that 30–50% of patients fail to show an adequate response.[1–3] In those that do respond, therapeutic improvement is not immediate but requires treatment for 2–4 weeks.[4,5] In addition, SRIs are associated with a number of undesirable side effects, such as sexual dysfunction, sleep disturbances, nausea, anxiety and reduced appetite. Thus an agent that overcomes the above limitations and, importantly, reduces the time necessary to achieve a clear therapeutic improvement, would represent a significant advance in the treatment of major depression.

One hypothesis proposed to explain the delayed onset of the antidepressant action of SRIs is that the increased synaptic concentrations of 5-HT lead to stimulation of somatodentritic 5-HT$_{1A}$ receptors and terminal 5-HT$_{1B/1D}$ autoreceptors.[5,6] This results in an inhibition of further 5-HT release, limiting the SRIs' ability to elevate synaptic concentrations of 5-HT. Only on desensitization of these receptors does the therapeutic action of SRIs set in. Blockade of the 5-HT$_{1B/1D}$ receptors by selective antagonists should therefore prevent this initial inhibition of 5-HT release.[7–9] A compound combining 5-HT$_{1B/1D}$ antagonist activity with 5-HT transport inhibition might be expected to both potentiate the increase in extracellular 5-HT and also reduce the time of onset of therapeutic action.[10,11]

For the first examination of this hypothesis, the 5-HT$_{1D}$ selective agonist PNU-109291[12] was identified from the

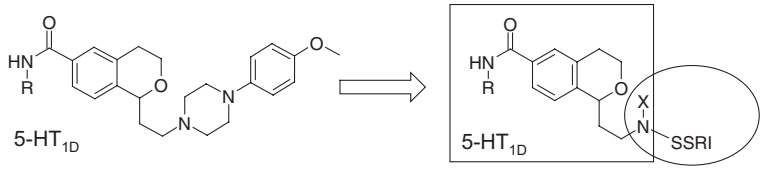

Figure 1. Incorporation of an SRI pharmacophore into the 5-HT$_{1D}$ agonist PNU-109291 (R = Me).

Keywords: 5-HT$_{1D}$ antagonist; Serotonin reuptake inhibitor; Rapid parallel synthesis.
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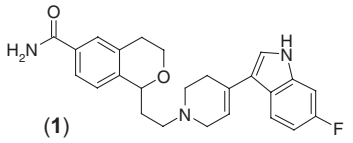


(1)

Figure 2. SRI/5-HT_{1D} antagonist lead compound (**1**).

literature as having the potential for incorporation of a SRI pharmacophore (Fig. 1).

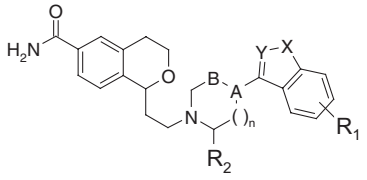
The initial compound synthesized (**1**) involved replacing the phenylpiperazine domain of PNU-109291 with the known SRI 3-(1,2,5,6-tetrahydropiperidin-4-yl)-6-fluoroindole[13] (Fig. 2). With this first derivative, in vitro radioligand binding indicated **1** possessed 5-HT_{1D} activity with potent SRI activity (Table 1). Importantly, it also showed oral bioavailability as measured in microdialysis, where increases in hypothalamic 5-HT levels exceeded those produced by an SSRI alone (Fig. 3). Furthermore, **1** showed little activity on the 5-HT_{1B} site, though unwanted α_1 and dopaminergic D_2 cross-reactivities were found.

The initial SAR development of **1** involved structural changes intended to influence the undesirable cross-reactivities. This was undertaken by modification of the indole; its replacement by other heterocycles; and by substitution of the piperidine by piperazine (Scheme 1). Rapid parallel synthesis (RPS) methodology was possible as many potential SRI pharmacophores were available to us from previous research efforts and were prepared by methods reported in the literature.[14]

Scheme 1 shows the synthesis of target molecules using RPS techniques by reaction of the mesylate intermediate (**2**)[15] with various potential SRI pharmacophores. Each of the secondary amines (0.03 mM) was heated at 80 °C in a sealed Reacti® vial with the mesylate (**2**) (50% excess) for 2–4 days in acetonitrile (2–4 mL) in the presence of potassium carbonate (0.06 mM) and a catalytic amount of potassium iodide (0.003 mM). Reaction mixtures were purified on an ion exchange cartridge (SCX, 0.5 g), eluting first with methanol to remove neutral components and then with 2 N ammonia/methanol, to yield the basic component in an average yield of 73%. Any unreacted secondary amine was removed by stirring the products overnight in DCM in the presence of a MeN-CO-P-resin. Solutions were blown dry under a stream of nitrogen and the residues analyzed by LC–MS.

Inhibition of the serotonin transporter in vitro was shown by the ability of compounds to displace [^{3}H]-

Table 1. Activities of heteroaryl 3,4-dihydro-1*H*-2-benzopyran derivatives



	X–Y	A–B	*n*	R_1	R_2	5-HT_{1D}	5-HT_{1B}	SRI	α_1	D_2	5-HT release (0.3 μM)
1	NH–CH	C=CH	1	6-F	H	56	281	0.11	16	4.7	36%
3	NH–CH	C=CH	1	6-Cl	H	145	>1000	0.38	—	—	—
4	NH–CH	C=CH	1	6-CF_3	H	122	>1000	0.99	190	8.6	—
5	NH–CH	C=CH	1	5-F	H	130	>1000	1.6	101	2.1	—
6	NH–CH	C=CH	1	5-MeO	H	31	316	3.5	75	26	42%
7	NH–CH	C=CH	1	7-F	H	26	>1000	0.76	26	3.8	—
8	NH–CH	C=CH	1	7-CN	H	130	>1000	2.1	134	15	—
9	NH–CH	C=CH	1	6,7-DiF	H	57	>1000	0.30	16	6.7	—
10	NH–CH	C=CH	1	6,7-DiCl	H	165	>1000	0.28	—	—	—
11	MeN–CH	C=CH	1	6-F	H	65	>1000	0.98	17	73	—
12	O–CH	C=CH	1	6-F	H	28	155	0.29	2.8	21	—
13	NH–N	C=CH	1	6-F	H	24	210	0.50	2.1	33	—
14	MeN–N	C=CH	1	6-F	H	51	>1000	0.54	16	183	—
15	O–N	C=CH	1	6-F	H	63	155	8.0	3.0	37	—
16	S–N	CH–CH_2	1	6-F	H	40	200	53	—	—	—
17	S–CH	C=CH	1	6-F	H	20	186	1.0	17	3.8	—
18	NH–CH	CH–CH_2	1	6-F	H	8.4	131	2.0	12	19	—
19	NH–CH	CH–CH_2	1	6-F	(*S*)-Me	28	>1000	0.78	20	86	—
20	NH–CH	CH–CH_2	0	6-F	H	40	96	2.1	1.2	28	—
21	NH–CH	N–CH_2	1	6-F	H	74	310	19	5.8	7.1	—
22	CH=CH–CH	C=CH	1	H	H	5.4	153	3.2	224	167	11%
23	CH=CH–CH	CH–CH_2	1	H	H	14	73	15	30	50	52%
24	CH=CH–CH	C=CH	1	7-F	H	26	210	13	202	87	—
27(*S*)	NH–CH	C=CH	1	7-F	H	15	387	0.3	2.8	7	—
28(*R*)	NH–CH	C=CH	1	7-F	H	25	199	0.2	1.7	48	—

Binding results are expressed as mean K_i (nM); assays were performed in triplicate.

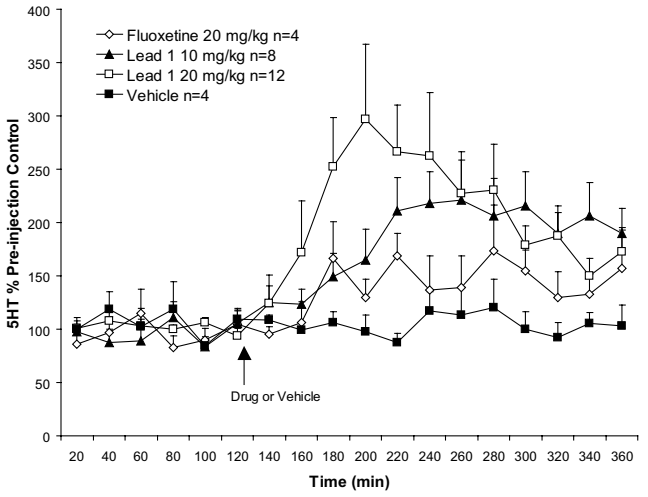
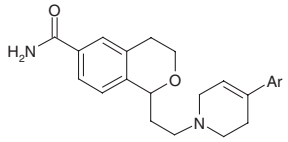


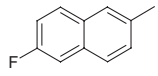
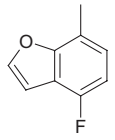
Figure 3. Effect of (**1**) (10 and 20 mg/kg p.o.) and fluoxetine (20 mg/kg p.o.) on the elevation of 5-HT in the hypothalamus of the freely moving guinea pig. Data expressed as a percentage of a pre-injection control period and represents the mean + SEM.

citalopram from its binding site on rat cerebral cortex membranes.[16] Human 5-$HT_{1B/D}$ binding affinity was determined using ^{3}[H]-GR125743 as radioligand in membrane homogenates prepared from L-M(tk-) cells expressing the cloned human 5-HT_{1B} or 5-HT_{1D} receptors.[16] Adrenergic α_1 binding affinity was determined in rat cortex membranes using [^{3}H]-prazosin,[17] and D_2 binding activity was determined in rat caudate membranes, using [^{3}H] raclopride.[18] In order to assess whether the lead compound was a functional antagonist at the 5-HT_{1D} receptor it was tested for its ability to potentiate potassium-stimulated [^{3}H]-5-HT release from preloaded guinea pig cortical slices. The assay[19] was carried out in the presence of a maximally effective concentration of the 5-HT transport inhibitor paroxetine so as to detect only the autoreceptor control on 5-HT release. For comparison, the 5-HT_{1D} antagonist GR127935 gave a 40–50% maximal potentiation of fractional release at 0.30 μM in this assay. Microdialysis studies[20] (Fig. 3) demonstrated that the dual pharmacology of **1** leads to an elevation of extracellular 5-HT levels in the guinea pig hypothalamus significantly above that obtained after a maximally effective acute dose of the SSRI fluoxetine.

Tables 1 and 2 summarize the biological results of targeted compounds **1**–**26**. Changes to the aromatic substitution (compounds **3**–**10**) in the lead indole (**1**) did not significantly affect the potency of the SRI activity. However, 6- or 7-fluoro (**1**, **7**), 6,7-difluoro (**9**) and

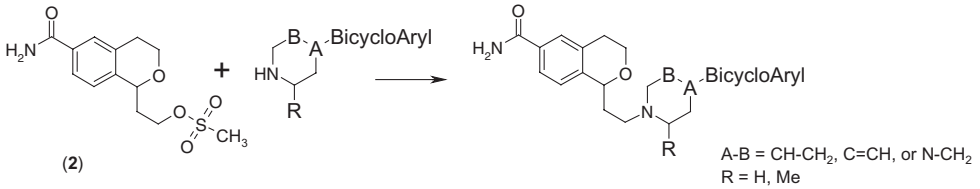
Table 2. Naphth-2-yl and 1-benzofuran-7-yl isochromans



Compds	Ar	5-HT_{1D}	5-HT_{1B}	SRI	α_1	D_2
25		71	>1000 nM	2.2	36	491
26		27	(19% @ 50 nM)	1.9	31	52

5-methoxy (**6**) substituents gave the best 5-HT_{1D} activity and this indicated scope for further derivatization. Furthermore, the substituent changes in compounds **4**, **5**, **6** and **8** indicated that α_1 cross-reactivity could be modified by simple changes to the lead (**1**). Compounds **1** and **6** also showed good activity on the 5-HT release assay, thus confirming potent functional 5-HT_{1D} antagonism. Saturation of the double bond in lead **1** to give **18** significantly potentiated the 5-HT_{1D} activity while 2-(*S*)-methylation of this analogue (**19**) also improved D_2 activity. Reduction in ring size of **1** to the pyrrolidine (**20**) retained the 5-HT_{1D} and SRI activity.

Replacement of piperidine with piperazine (**21**) retained 5-HT_{1D} activity with some reduction in SRI activity. The indole in (**1**) can be replaced with a number of heteroaromatics {6-*F*-indazole (**13**), benzofuran (**12**) and benzothiophene (**17**)} and still retain both 5-HT_{1D} and SRI activity, although the benzisoxazole (**15**) and particularly the benzisothiazole (**16**) gave a reduction in SRI activity. Interestingly, N-methylation of the indole (**11**) and indazole (**14**) reduced D_2 activity. Replacement of indole with naphthalene (**22**) significantly reduced α_1 and D_2 cross-reactivity while retaining the desired 5-HT_{1D} and SRI activities, though functional activity in the 5-HT release assay was poor. This latter activity was restored on reducing the double bond in the piperidine ring to give the naphthalene derivative (**23**). Thus the naphthalene analogues show good potential for further investigation. The 2-naphthyl compound (**25**) also showed the potential for removal of cross-reactivity. Separate preparation of the enantiomers of lead **1** from the optically pure mesylates of established stereochemistry[21] showed that the (*S*) isomer (**27**) (K_B = 33 nM) had



Scheme 1. K_2CO_3/KI/MeCN, reflux.

greater antagonist potency than the (*R*) isomer (**28**) ($K_B = 202$ nM) at the 5-HT_{1D} receptor, as shown by measurement of the inhibition of the 5-HT-induced increase in [^{35}S]-GTPγS binding in a membrane preparation derived from L-M(tk-) cells expressing the 5-HT_{1D} receptor.[16]

We have found that incorporating SRI pharmacophores into a structure exhibiting selective 5-HT_{1D} agonist activity produced a series of compounds that have both SSRI and 5-HT_{1D} antagonist activity. The α_1 and D_2 cross-reactivities originally found could be ameliorated by replacing the indole with an appropriate naphthalene derivative. By exploiting RPS techniques, leads with the potential for further investigation have been rapidly identified: their chemistry and biological evaluation will be described in fuller detail in future publications.

Acknowledgements

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