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Naphthyl piperazines with dual activity as 5-HT$_{1D}$ antagonists and 5-HT reuptake inhibitors

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Abstract—SAR around a known molecule with dual 5-HT$_{1D}$ antagonist and 5-HT$_{transporter}$ inhibitory activity has led to the discovery of molecules with improved dual activity and reduced cross-reactivity toward other aminergic receptors (5-HT$_{1B}$, α_1, and D_2).

Major depression is a common illness affecting 5–10% of the western European population.[1] It is well established that a significant component of the symptomatology of depression can be attributed to a reduction in serotonergic function. Serotonin (5-HT) reuptake inhibitors (SRIs) have been widely used as antidepressant drugs, with their effect being believed to be due to an enhancement of postsynaptic 5-HT levels.[2] Although SRIs are effective drugs, their onset of clinical action may be delayed by 2–4 weeks.[3,4] One hypothesis for this delay in symptomatic improvement is that time is required for desensitization of the 5-HT$_{1B}$/$_{1D}$ and 5-HT$_{1A}$ autoreceptors.[5] The increased release of 5-HT mediated by the SRI stimulates these receptors, which in turn results in an inhibition of further 5-HT release, thus limiting the effect of the SRI. Only on desensitization of these autoreceptors does the SRI produce its therapeutic effect. According to this mechanism of action, blockade of the autoreceptors by an antagonist would prevent the initial lack of efficacy of the SRI.

Combined administration of a 5-HT reuptake inhibitor and a 5-HT$_{1B/1D}$ antagonist to guinea pigs demonstrated a significant increase in the extracellular levels of 5-HT above those evoked by a SRI alone, and to a level only observed after chronic SRI treatment.[6] It has also been demonstrated that pindolol, a 5-HT$_{1A}$ antagonist, increases the clinical effectiveness of a SRI, fluoxetine.[7] These results indicated that the effect of a 5-HT reuptake inhibitor is more pronounced and long-lasting with the co-administration of a 5-HT$_{1A}$ or 5-HT$_{1B/1D}$ antagonist. Therefore, the identification of a drug with dual activity as a 5-HT reuptake inhibitor and a 5-HT$_{1A}$, 5-HT$_{1B}$ or a 5-HT$_{1D}$ antagonist should produce a similar effect, potentially resulting in an antidepressant drug with a superior profile to an SRI alone, both in terms of efficacy and onset of clinically meaningful antidepressant effects. While the dual activity 5-HT$_{1B}$/SRI and 5-HT$_{1A}$/SRI has been studied elsewhere,[8–10] we were interested in the identification of a drug with dual activity as a 5-HT reuptake inhibitor and a 5-HT$_{1D}$ antagonist in order to determine the role of the 5-HT$_{1D}$ autoreceptor[11] in the augmentation of extracellular serotonin. With this aim, selectivity toward 5-HT$_{1A}$ and 5-HT$_{1B}$ was also required.

Initial studies in our laboratories demonstrated that the incorporation of a SRI pharmacophore into a known 5-HT$_{1D}$ agonist ligand, PNU-109291 **1**,[12] (Fig. 1) led to compounds with dual 5-HT$_{1D}$ antagonist/SRI activity.[13] Microdialysis studies in guinea pigs demonstrated that **2** gave an elevation of the extracellular 5-HT levels significantly above that obtained after a maximally effective acute dose of the SRI fluoxetine. However, this indole derivative was also a potent α_1 and D_2 ligand. Although

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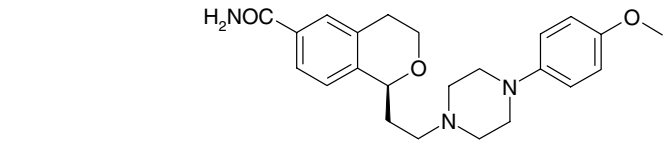
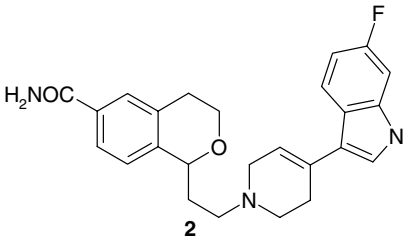
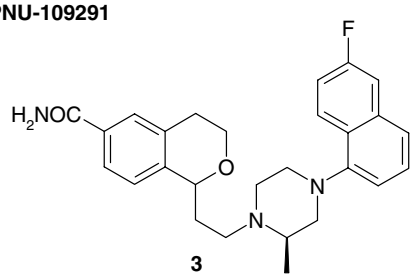


1 PNU-109291



2

Binding affinities	Ki (nM)
h5-HT$_{1D}$	8.3
h5-HT$_{1B}$	203
r5-HT$_{transporter}$	0.11
α_1	0.68
D_2	42



3

Binding affinities	Ki (nM)
h5-HT$_{1D}$	56
h5-HT$_{1B}$	281
r5-HT$_{transporter}$	0.78
α_1	16
D_2	4.7

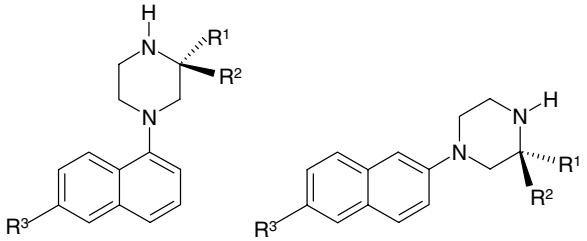
Figure 1. 5-HT$_{1D}$ agonist PNU-109291 (**1**) and 5-HT$_{1D}$/SRI ligand lead compounds (**2** and **3**).

the cross-reactivity at the α_1 receptor could be somewhat diminished by replacing the indole with a naphthylpiperazine, as in **3**, further improvement of the selectivity against both receptors would be desirable.

We report herein the effect on this dual activity of modifying the basic structure of compound **3** in order to reduce the cross-reactivity on α_1 and D_2 receptors.

A series of naphthyl piperazines **4**–**8** were synthesized for this study as potential SRI pharmacophores.[14] (Fig. 2) They were combined with the isochroman present in **3** or with a dihydroisobenzofuran.

The racemic isochroman (*RS*)-**16** was synthesized as described.[12] The isobenzofuran (*RS*)-**15** was synthesized following the reaction conditions shown in Scheme 1. Commercially available 5-chlorophthalide **9**[15] was reduced to its lactol, which in turn was transformed into the ester **10** by Horner–Emmons reaction. This ester was reduced with DIBAH, followed by $NaBH_4$, to the corresponding alcohol **11**.[16] The alcohol was protected as the TBDMS derivative **12**. Palladium(0)-catalyzed coupling of the aromatic chloride



4 R^1=Me, R^2=H, R^3=F
5 R^1=Me, R^2=H, R^3=CN
6 R^1=H, R^2=H, R^3=CN

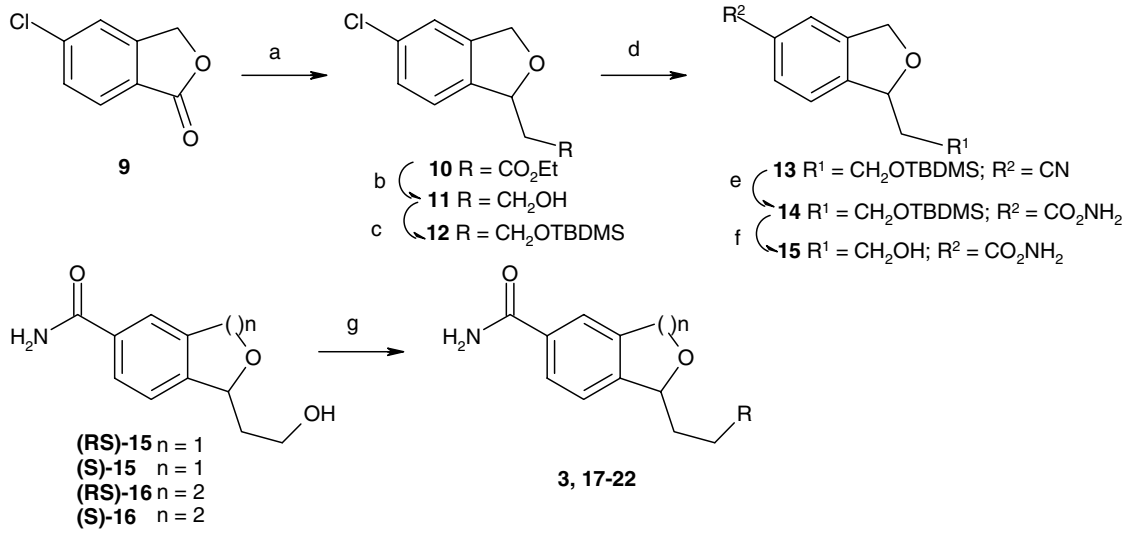
7 R^1=Me, R^2=H, R^3=F
8 R^1=H, R^2=H, R^3=F

Figure 2. Naphthyl piperazines **4**–**8**.

with $Zn(CN)_2$ and hydrolysis of the resultant nitrile **13** with basic H_2O_2 provided the primary amide **14**. Deprotection of the primary alcohol provided the racemic alcohol **15**. Activation of the primary alcohol of (*RS*)-**15** or (*RS*)-**16** as the mesylate and reaction with **4** provided compounds **3** and **21**, respectively, as a mixture of diastereoisomers.

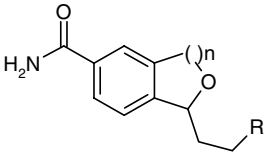
The two diastereomers of **3** and **21** were separated by chiral HPLC (**3a** and **3b** from **3**; and **21a** and **21b** from **21**, Table 1).[17] The affinity for the 5-HT$_{1D}$ receptor and the 5-HT$_{transporter}$ was greater for one isomer in both cases, necessitating an enantioselective synthesis of intermediates **15** and **16**. Compound (*S*)-**16** was synthesized as described.[12] We also applied these conditions to resolve the two enantiomers of **10** by lipase-catalyzed hydrolysis of the ethyl ester (Scheme 2). Thus, hydrolysis of (±)-**10** by Amano lipase at pH 7 to 50% conversion provided the ester (+)-**10** in a 45% yield and the acid (−)-**23** in a 45% yield, both compounds being of 95% ee.[18] A single recrystallization of (−)-**23** in ether/hexane provided this compound essentially enantiomerically pure (>98%). The absolute configuration of (−)-**23** was determined by derivatization to the corresponding (*R*)- and (*S*)-phenylglycine amides according to the method described in the literature.[19] Reduction of the free acid with borane, followed by TBDMS protection, provided enantiomerically pure (−)-**12**. Standard conditions used for the synthesis of racemic **15** (Scheme 1) provided (*S*)-**15** enantiomerically pure. Synthesis of **3a** and **21a** from (*S*)-**16** and (*S*)-**15**, respectively, demonstrated that the diastereomer that possesses the dual activity in both scaffolds is (*S*). Enantiopure (*S*)-**15** and (*S*)-**16** were used for the synthesis of **17**–**20** and **22**.

The undesired enantiomer (+)-**10** was racemized with NaOEt to provide (±)-**10** quantitatively, which could be resubmitted to the enzymatic hydrolysis conditions (Scheme 2).



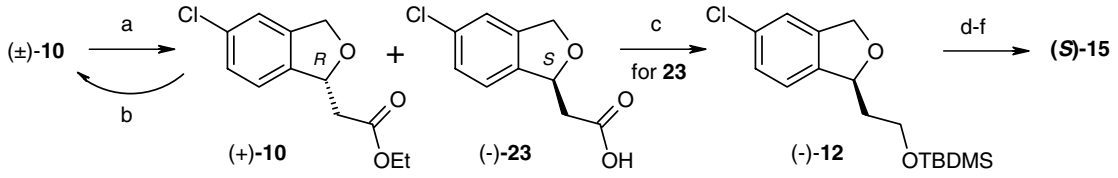
Scheme 1. Reagents and conditions: (a) i—DIBAH, CH_2Cl_2, −78 °C, 84%; ii—$(EtO)_2P(O)CH_2CO_2Et$, Cs_2CO_3, THF, 0 °C–rt, 73%; (b) DIBAH, THF, −78 °C then $NaBH_4$, MeOH, 0 °C, 73%; (c) TBDMS-Cl, imidazole, CH_2Cl_2, 90%; (d) $Zn(CN)_2$, $Pd_2(dba)_3$, *t*-Bu_3P, dioxane, 120 °C, 70%; (e) H_2O_2, NaOH (2N), Bu_4NHSO_4; CH_2Cl_2, 0 °C–rt, 90%; (f) TBAF, THF, rt, 83%; (g) i—MsCl, Et_3N, DMF, 70%; ii—**4**–**8**, K_2CO_3, CH_3CN, 70 °C.

Table 1. Receptor binding affinities at h5-HT_{1D}, h5-$HT_{transporter}$, h5-HT_{1B}, h5-HT_{1A}, rat α_1 and D_2 receptors expressed as K_i (nM) (mean of runs in triplicate); functional affinity at h5-HT_{1D} expressed as K_B or % displacement at 30 nM



Compound	*n*	Configuration	R[a]	5-HT_{1D}	5-HT_{1D} function	5-$HT_{transporter}$	5-HT_{1B}	5-HT_{1A}	α_1	D_2
2	2	*RS*		8	4.3	0.1	203	N.D.[b]	0.7	42
3	2	*RS*	**4**	56	0.01	0.8	281	N.D.[b]	16	4.7
3a	2	*S*	**4**	7	0.01	3.0	103	115	80	71
3b	2	*R*	**4**	88	11.1	7.4	91	105	74	78
17	2	*S*	**7**	14	4.05	2.1	>1000	307	91	1170
18	2	*S*	**8**	10	0.86	8.1	>1000	N.D.[b]	96	704
19	2	*S*	**5**	0.9	(124%)	2.2	21	26	353	287
20	2	*S*	**6**	1.0	(101%)	5.7	15	16	178	88
21a	1	*S*	**4**	14	0.05	1.3	114	13	64	26
21b	1	*R*	**4**	67	1.9	8.8	>1000	13	43	107
22	1	*S*	**5**	1.8	0.18	1.3	88	N.D.[b]	338	346

[a] R corresponds to the naphthyl piperazines **4**–**8** without the H attached to the piperazine.
[b] N.D., not determined.



Scheme 2. Reagents and conditions: (a) Amano lipase, buffer, pH 7, 24 h; (b) NaOEt, EtOH, quant.; (c) i—$BH_3.SMe_2$; ii—TBDMS-Cl, imidazole, CH_2Cl_2, 92%; (d) $Zn(CN)_2$, $Pd_2(dba)_3$, *t*-Bu_3P, dioxane, 120 °C, 70%; (e) H_2O_2, NaOH (2N), Bu_4NHSO_4; CH_2Cl_2, 0 °C–rt, 90%; (f) TBAF, THF, rt, 83%.

In vitro inhibition of the serotonin transporter was measured by the ability of compounds to displace [^{3}H]-citalopram from human 5-$HT_{transporter}$ expressed in HEK293 cells.[20] Human 5-HT_{1B} and 5-HT_{1D} binding affinities were 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.[20] Human 5-HT_{1A} binding affinity was determined using [^{3}H]-8-OH-DPAT as radioligand in membrane homogenates prepared from L-M(tk-) cells expressing the cloned human 5-HT_{1A} receptor.[21] Adrenergic α_1 binding affinity was determined in rat cortex

membranes using [^{3}H]-prazosin,[20] and D_2 binding activity was determined in rat caudate membranes using [^{3}H]-raclopride.[20]

Functional 5-HT_{1D} antagonism was measured by the dextral shift of the 5-HT dose–response curves for the binding of GTP-γ [^{35}S] to human 5-HT_{1D} receptor stably expressed into LM (tk-) cells, and the results were expressed as K_B values or % displacement at 30 nM.[22]

The biological results of compounds **3** and **17**–**22** are summarized in Table 1.

The two diastereomers of **3** (**3a** and **3b**) show that one diastereomer (**3a**, *S* configuration at the isochroman center) has higher affinity for the 5-HT_{1D} receptor and the 5-$HT_{transporter}$, while both isomers display similar cross-reactivity on the other receptors.

Comparison of the 2-naphthylpiperazine **17** with the 1-naphthylpiperazine **3a** shows that the 2-naphthylpiperazine maintains the binding affinity for the 5-HT_{1D} receptor, although there is a significant reduction in the functional 5-HT_{1D} antagonism.

The substitution of the naphthyl fluorine in **3a** by nitrile (compound **19**) retains the 5-HT_{1D} and SRI activity, and reduces the binding affinity of the compound for the α_1 and D_2 receptors.[23]

Elimination of the chiral center on the piperazine of **17** or **19** by removal of the methyl substituent provided compounds **18** and **20**, respectively. The absence of the methyl group reduced the SRI activity while increasing the affinity for the D_2 receptor.

Interesting results were obtained when the isochroman was replaced by an isobenzofuran. As observed on the isochroman series, a higher affinity for the 5-HT_{1D} and the 5-$HT_{transporter}$ on the isobenzofuran series resides in the (*S*) isomer.

A single change on **21a**, from fluorine to nitrile in the 6-position of the aromatic ring, generated **22**, a compound with a high affinity for 5-HT_{1D} and the 5-$HT_{transporter}$, and greater than 50-fold selectivity over the undesired receptors.

Compounds **19** and **22** have the best profile in terms of potency on 5-HT_{1D} and the 5-$HT_{transporter}$ (K_i < 3 nM) and selectivity (>100-fold over α_1 and D_2, and >20-fold of 5-HT_{1D} affinity over 5-HT_{1B}).

Microdialysis studies[24] (Fig. 3) demonstrated that the dual pharmacology of **3a** and **19** leads to an elevation of extracellular 5-HT levels in the guinea pig[25] hypothalamus (at 10 mg/kg po). For **19**, the increase obtained (for a 3 h period post-drug administration) was significantly greater than that obtained after a maximally effective acute dose of the SSRI fluoxetine. Interestingly, the effect is higher with **19** than with **3a**. These two compounds display similar affinity for the transporter but

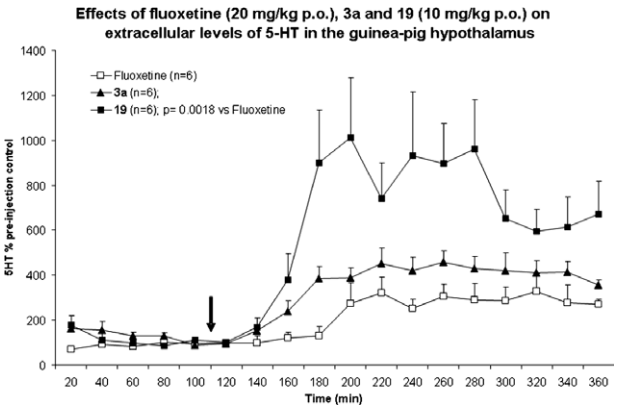


Figure 3. Effect of **3a** or **19** at 10 mg/kg po and fluoxetine at 20 mg/kg po on the elevation of 5-HT in the hypothalamus of the freely moving guinea pig. Drug administered at arrow. Data expressed as a percentage of a pre-injection control period and represent means + SEM. The effect of **3a** was not statistically different to fluoxetine.

the potency of **19** is sevenfold higher toward the 5-HT_{1D} receptor than that of **3a**.[26] Dose–response studies performed with **19** show that this compound displays a considerable elevation of 5-HT at all doses (1–20 mg/kg) (Fig. 4).

In summary, we have identified a series of isochromans and isobenzofurans with dual 5-HT_{1D} antagonism and 5-$HT_{transporter}$ inhibition. Isomer (*S*) shows a better profile in both series. 1-Napththyl- and 2-naphthyl-piperazines have been shown to be good 5-HT_{1D}/SRI pharmacophores in this study, with the presence of a 3-methyl group in the piperazines improving the affinity for the transporter. We have identified two compounds **19** and **22** with K_i < 3 nM at 5-HT_{1D} and 5-$HT_{transporter}$, and >50-fold selectivity over α_1 and D_2.

These two compounds are viable candidates for further development: details will be reported in due course.

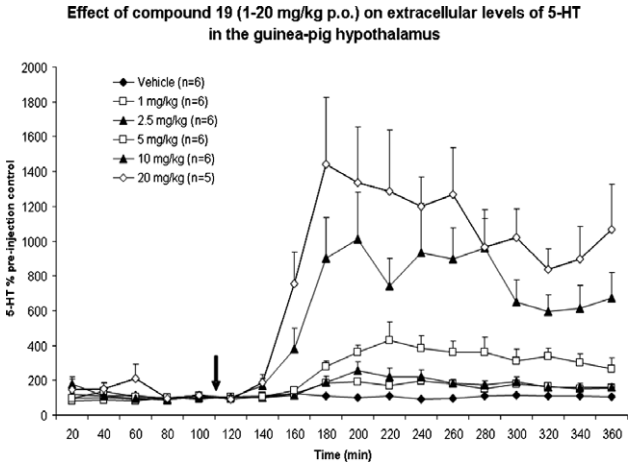


Figure 4. Dose–response effect of **19** (1–20 mg/kg po) on the elevation of 5-HT in the hypothalamus of the freely moving guinea pig. Drug administered at arrow. Data expressed as a percentage of a pre-injection control period and represent means + SEM (p < 0.05).

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16. DIBAH reduction stopped at the aldehyde. Further reduction with $NaBH_4$ after removal of the aluminum salts was necessary to obtain the alcohol.
17. Chiral HPLC conditions: Chiracel OJ column, eluting with 1:1 hexane (0.2% dimethylethylamine)/ethanol at a 1 ml/min flow rate.
18. The enantiomeric excess was analyzed by chiral HPLC using a Chiralpak AD column, eluting with hexane/ethanol in isocratic mode (70% of B) at a 1 ml/min flow rate. Derivative (+)-**10** was hydrolyzed to the corresponding acid to determine its ee.
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