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N-Tetrahydrothiochromenoisoxazole-1-carboxamides as selective antagonists of cloned human 5-HT$_{2B}$

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ABSTRACT

The serendipitous discovery of *N*-cyclohexyl-8-fluoro-3,3a,4,9b-tetrahydro-1*H*-thiochromeno[4,3-*c*]isoxazole-1-carboxamide as a selective human serotonin 5-HT$_{2B}$ antagonist with K_i of 42 ± 5 nM is reported herein. A subsequent functional assay indicated little agonist activity compared to 5-HT itself.

The importance of selective serotonin 5-HT$_{2B}$ antagonists is evident from the on going research probing the significance of this receptor in fields such as migraine, irritable bowel syndrome, pulmonary hypertension, and hypertrophy.[1–4] During the collection of data in another project, we noted one tetrahydrothiochromenoisoxazole-1-carboxamide that exhibited selective inhibition of serotonin receptor 5-HT$_{2B}$ over 5-HT$_{2A}$ and 5-HT$_{2C}$. While not the focus of our project, we nevertheless recognized the importance of such a selective inhibitor as a research probe and report our findings here. By way of comparison with the tetrahydrothiochromenoisoxazole-1-carboxamides disclosed here, Figure 1 lists the structures of some commercially available, selective antagonists of 5-HT$_{2B}$ used as assay standards.

Recent growing interest in designing molecules that interact with multiple receptors (polypharmacology) has identified a need for software tools suitable for this aim.[5–8] This prompted us to expand our existing software tools from an on-target/off-target system to a more robust polypharmacology design system.[9] In order to further develop these software tools, we required assay results against multiple targets for a common set of molecules.

A series of 8-fluorotetrahydrothiochromenoisoxazole-1-carboxamides **5**–**9** were prepared for this purpose in an analogous manner to that previously reported for tetrahydrothiochromenoisoxazoles as indicated in Scheme 1.[10]

2,5-Difluorobenzaldehyde **1** was treated with allyl mercaptan to form the thioether **2**. Treatment of **2** with 5-hydroxypentanal oxime under Abiko conditions[11] forms a nitrone that undergoes an intramolecular 1,3-dipolar cycloaddition reaction with the allyl moiety to form the desired tetrahydrothiochromenoisoxazole ring system **3** with concomitant formation of an *N*-tetrahydropyranyl protecting

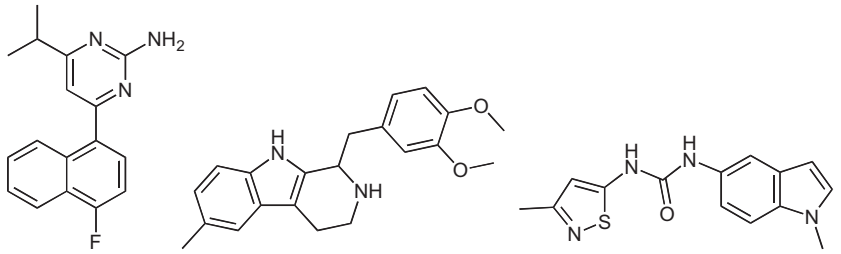

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Figure 1. Structures of some commonly available selective 5-HT$_{2B}$ antagonists. Source: Tocris Biosciences, UK.

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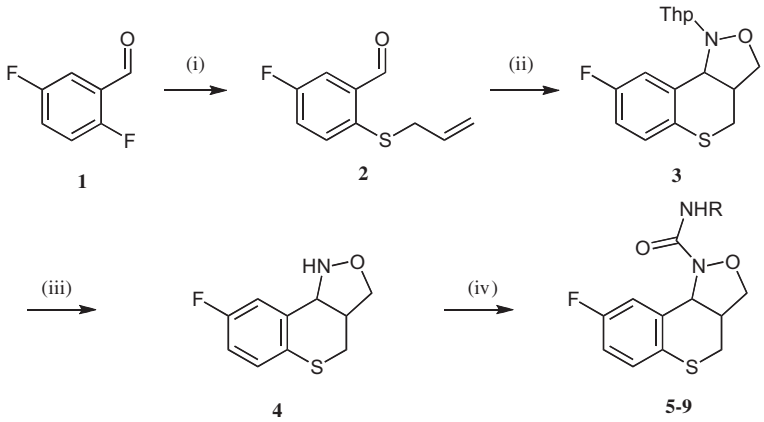


Scheme 1. Reagents and conditions: (i) allyl mercaptan, K_2CO_3, DMF, 80 °C, 51%; (ii) 5-hydroxypentanal oxime, Bu_2SnO, $PhCH_3$, Dean–Stark; (iii) aq HCl, MeOH, rt, 81% (two steps); (iv) RNCO, THF, rt, 77%.

Table 1
Selectivity of human 5-HT_2 assay results for compounds **5**–**17**

Compd	R	PDSP[b]	% Inhibition at 10 μM[a]			K_i[c] (nM)		
			$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$
5	Ph	10,630	33.6	95.3	61.2	>10,000	223	5171
6	*i*Pr	10,631	2.2	89.3	55.4	>10,000	569	>10,000
7	*n*Pr	10,632	−23.2	93.0	37.4	>10,000	384	>10,000
8	C_6H_{11}	10,633	6.2	102.5	30.0	>10,000	42	>10,000
9	*n*Bu	10,634	−15.6	92.0	34.9	>10,000	489	>10,000
11	Ph	9397	11.0	71.6	56.7	>10,000	1606	>10,000
12	*i*Pr	9398	−0.2	14.8	28.9	>10,000	>10,000	>10,000
13	*n*Pr	9399	2.4	53.5	14.7	>10,000	6596	>10,000
14	C_6H_{11}	9400	5.5	75.5	28.1	>10,000	1773	>10,000
15	*t*Bu	9401	12.0	43.0	25.9	>10,000	>10,000	>10,000
16	*n*Bu	9402	0.8	35.4	23.4	>10,000	>10,000	>10,000
17	2-MePh	9403	12.8	52.2	40.2	>10,000	3999	>10,000

[a] Average for $n = 4$. Assay details available at PDSP.
[b] PDSP access code.
[c] Binding values determined only when inhibition at 10 μM was over 50%.

group. The tetrahydropyranyl protecting group was removed with aqueous acid to form 8-fluorotetrahydrothiochromenoisoxazole **4**. Treatment of **4** with an appropriate isocyanate in THF formed the N-substituted isoxazolidine-1-carboxamides **5**–**9**.[12]

Testing of the carboxamides **5**–**9** in a number of assays indicated that the *N*-cyclohexyl isoxazolidine-1-carboxamide **8** completely inhibited 5-HT_{2B} (102.5%) at the test concentration of 10 μM, but not 5-HT_{2A} (6.2%) or 5-HT_{2C} (30.0%). A K_i value of 42 nM for carboxamide **8** was subsequently measured in a binding experiment with 5-HT_{2B} using [^{3}H]LSD (1 nM) as the competitive ligand. 5-HT itself exhibits $K_i = 11$ nM in this assay.

This selectivity prompted us to further explore the structure–activity relationship of this group of molecules.

As can be seen from the results in Table 1, dramatic loss of activity against 5-HT_{2B} occurred when the *N*-cyclohexyl group in **8** was replaced by short chain alkyl groups in compounds **6**, **7** and **9**. Replacement of the *N*-cyclohexyl group with a phenyl group in **5** resulted in a fivefold reduction in binding affinity to 5-HT_{2B}, and loss of selectivity with mild activity in the 5-HT_{2C} assay.

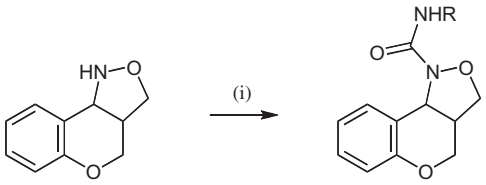
Compound **8** assay results for other receptors are shown in Table 2, indicating a strong selectivity for 5-HT_{2B}. Of note, the cyclohexyl carboxamide **8** exhibited poor activity against other serotonin receptors, even at the higher 10 μM concentration. Dopamine, histamine, muscarinic, and opiate receptors show little activity towards **8**. Carboxamide **8** does not show activity in the serotonin transporter assay.

Table 2
Assay results for compound **8** showing selectivity for 5-HT_{2B}[a]

Receptor	% Inhibition[b]	K_i (nM)	Receptor	% Inhibition	K_i (nM)
5ht1a	36.9		D5	−14.2	
5ht1b	1.3		DAT	66.3	>10,000
5ht1d	4.5		DOR	−3.2	
5ht1e	−7.8		H1	−4.3	
5ht2a	6.2		H2	30.8	
5ht2b	102.5	42 ± 5	H3	−10.0	
5ht2c	30.0		H4	−0.5	
5ht3	−8.7		KOR	34.6	
5ht5a	46.6		M1	23.4	
5ht6	−18.0		M2	23.7	
5ht7	27.1		M3	20.6	
Alpha1A	37.8		M4	44.9	
Beta1	2.4		M5	31.8	
Beta2	3.7		MOR	8.9	
Beta3	7.1		NET	34.1	>10,000
D1	14.0		SERT	20.4	>10,000
D2	8.1		Sigma 1	16.5	
D3	3.2		Sigma 2	1.1	
D4	12.0				

[a] Assay details available at PDSP. Data available as at July 2010.
[b] % Inhibition at 10 μM. Average for $n = 4$.

Compound **8** was then subjected to further testing in a functional assay to determine the nature of its activity against 5-HT_{2B}. Compound **8** displays negligible activity in the 5-HT_{2B} agonist assay at 10 μM (0.5% efficacy compared to 5-HT), but high



Scheme 2. Reagents and conditions: (i) RNCO, THF, rt.

activity in the antagonist assay ($pIC_{50} = 7.37 \pm 0.07$) (see Supplementary data).

We had also prepared *N*-carboxamide derivatives of tetrahydrochromenoisoxazole **10** as part of our polypharmacology project, and report their activities here by way of comparison. The parent ring system **10** was prepared according to Abiko,[11] then treated with an appropriate isocyanate in THF to form the *N*-carboxamides **11**–**17** (Scheme 2).

Although a similar trend can be seen for the activity of this series of compounds **11**–**17** against the 5-HT_2 receptors (Table 1), the activities are significantly lower, with compound **14**, the series analogue of **8**, recording more than a 40-fold reduction in binding activity against 5-HT_{2B}.

In summary, compound **8** was found to be an antagonist inhibitor of 5-HT_{2B} with high selectivity over 5-HT_{2A} and 5-HT_{2C}.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.074.

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12. *General procedure for carboxamide formation. N-cyclohexyl-8-fluoro-3,3a,4,9b-tetrahydro-1H-thiochromeno[4,3-c]isoxazole-1-carboxamide* **8**: To a solution of **4** (53 mg, 0.25 mmol) in THF (2 ml) was added cyclohexyl isocyanate (34 μl, 0.26 mmol, 1.05 equiv) and the solution stirred overnight. Methanol (one drop) was added to the reaction and stirring continued for 15 min. The reaction solution was then filtered through a small plug of silica to remove polar impurities using ethyl acetate as eluent. Concentration of the filtrate afforded **8** as a yellow oil (65 mg, 77%). MS (ESI$^+$) *m*/*z* 337.2 (100%, M+H), 359.2 (70%, M+Na), 673.3 (2M+H), 695.3 (2M+Na); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46 (ddd, *J* 9.9, 2.8, 0.8 Hz, 1H), 7.21 (dd, *J* 8.6, 5.5 Hz, 1H), 6.87 (dt, *J* 8.3, 2.9 Hz, 1H), 5.92 (d, *J* 8.3 Hz, 1H, N*H*), 5.31 (d, *J* 8.71 Hz, 1H), 4.00 (m, 2H); 3.70 (m, 1H), 3.30 (m, 1H), 3.03 (dd, *J* 13.0, 5.1 Hz, 1H), 2.63 (dd, *J* 13.0, 10.5 Hz, 1H), 2.10-1.00 (m, 10H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 161.22 (d, J_{CF} 245.9 Hz), 160.54, 136.89 (d, J_{CF} 7.3 Hz), 129.40 (d, J_{CF} 7.7), 128.96 (d, J_{CF} 3.0), 117.47 (d, J_{CF} 23.3), 114.60 (d, J_{CF} 22.3), 74.07, 58.46, 49.17, 45.01, 33.67, 33.24, 30.26, 25.45, 24.88, 24.82; ^{19}F NMR {1H} (188 MHz, $CDCl_3$) δ −116.13.