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Aminotriazine 5-HT$_7$ antagonists

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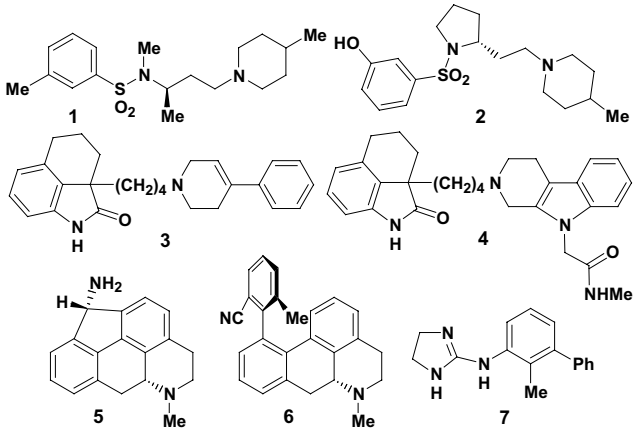
Abstract—The present studies have identified a series of aminotriazines as novel 5-HT$_7$ receptor antagonists. Compounds **10** and **17** have high affinity for the 5-HT$_7$ receptor and do not bind to either the 5-HT$_{2C}$ or 5-HT$_6$ receptors. These compounds produce no agonist effects by themselves, and shift the dose–response curve of 5-CT to the right in the manner of an antagonist.

The 5-HT$_7$ receptor has been implicated in a variety of therapeutic targets: cognition,[1] depression,[2] sleep disorders,[3] migraine,[4] and schizophrenia.[5] While three splice variants of the 5-HT$_7$ receptor have been identified,[6] and may mediate different functional actions, the development of selective agonists and antagonists remains of key importance in determining the actual therapeutic role of this receptor. While no selective 5-HT$_7$ agonists have been identified, pharmacophore models[7] for 5-HT$_7$ agonism and antagonism have been proposed. To date, only four general types of 5-HT$_7$ receptor antagonists have been reported to date: the sulfonamides, SB-258719[8] (**1**) and SB-269970[9] (**2**); the tetrahydrobenzindoles, **3**[10] and **4**;[11] the apomorphine derivatives, **5**[12] and **6**;[13] and most recently, the dihydroimidazolyl-biphenylamines, **7**.[14] We now report a new class of 5-HT$_7$ antagonists, the aminotriazines (**8**–**24**).



Compounds **8**–**11**, **14**, and **16**–**24** were prepared by the sequential displacement of the halogens on cyanuric chloride (Scheme 1).[15,16] Compounds **13** and **15** were similarly prepared from cyanuric fluoride and 2,4-dichloro-6-methyl-1,3,5-triazine,[17] respectively. Compound **12** was prepared by catalytic hydrogenation over Pd/C of the corresponding chlorotriazine intermediate.

From efforts that will be reported separately, the phenylpropyl amine, **8**, was distinguished by a moderate 5-HT$_7$ binding affinity[18] ($K_i = 60$ nM). An initial library designed to investigate the binding SAR of this lead compound produced the phenoxyethyl amine, **9**, with increased affinity ($K_i = 8$ nM), and determined that all the 5-HT$_7$ receptor affinity resided with the

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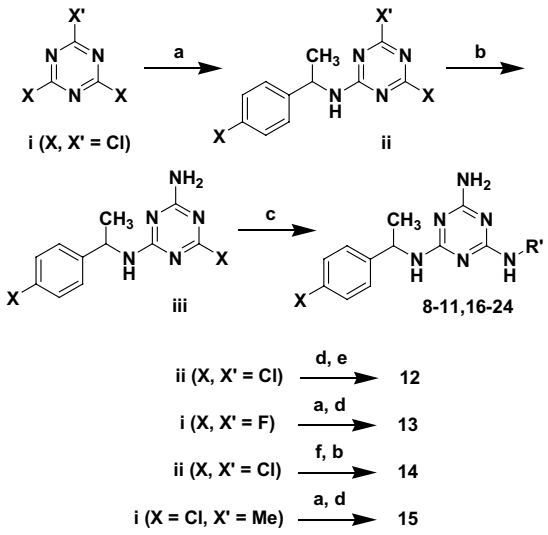
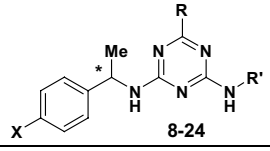


Scheme 1. Reagents and conditions: (a) RNH_2, $NEt(iPr)_2$, THF, 0 °C; (b) NH_4OH, THF, 25 °C; (c) $R'NH_2$, $NEt(iPr)_2$, THF, heat; (d) $R'NH_2$, $NEt(iPr)_2$, THF, 25 °C; (e) H_2, Pd/C, EtOH; (f) $NHMe_2$, THF, 25 °C.

(*S*)-1-phenethyl amine substituent (**10**, $K_i = 3$ nM) rather than in its enantiomer (**11**). Another library determined that the unsubstituted triazine (**12**) was as potent as the primary amine (**10**) with decreased activity with fluoro, methyl, and dimethyl amino substituent patterns (**13**–**15**). The fluoro compounds (**16**–**18**) demonstrated the same activity pattern as **9**–**11**, with the *S*-enantiomer (**17**) being the more active (Table 1).

Substituted side chains were investigated with compounds **19**–**24**. The 4-fluorophenethyl amine (**20**, $K_i = 2$ nM), 2-pyridylethyl amine (**21**, $K_i = 9$ nM), and the 2-thienylethyl amine (**24**, $K_i = 2$ nM) bound with

Table 1. 5-HT_7 binding of **8**–**24**.[20]



Compd	*	R	X	R′	5-HT_7 K_i (nM)
8	±	$-NH_2$	–H	$-(CH_2)_3$–Ph	60
9	±	$-NH_2$	–H	$-(CH_2)_2$–O–Ph	8
10	*S*	$-NH_2$	–H	$-(CH_2)_2$–O–Ph	3
11	*R*	$-NH_2$	–H	$-(CH_2)_2$–O–Ph	>1000
12	*S*	–H	–H	$-(CH_2)_2$–O–Ph	3
13	*S*	–F	–H	$-(CH_2)_2$–O–Ph	10
14	*S*	$-NMe_2$	–H	$-(CH_2)_2$–O–Ph	23
15	*S*	–Me	–H	$-(CH_2)_2$–O–Ph	54
16	±	$-NH_2$	–F	$-(CH_2)_2$–O-(4-F–Ph)	5
17	*S*	$-NH_2$	–F	$-(CH_2)_2$–O–(4-F–Ph)	2
18	*R*	$-NH_2$	–F	$-(CH_2)_2$–O–(4-F–Ph)	36
19	±	$-NH_2$	–F	$-(CH_2)_2$–Ph	8
20	*S*	$-NH_2$	–F	$-(CH_2)_2$–(4-F–Ph)	2
21	*S*	$-NH_2$	–F	$-(CH_2)_2$–(2-pyridyl)	9
22	±	$-NH_2$	–F	$-(CH_2)_2$–(3-pyridyl)	>41,000
23	±	$-NH_2$	–F	$-(CH_2)_2$–(4-pyridyl)	>1000
24	±	$-NH_2$	–F	$-(CH_2)_2$–(2-thienyl)	2

Table 2. 5-HT_6, 5-HT_{2C}, and α_1 binding[20] and oral bioavailability[19] (rat) of **10**, **17**, **20**, **21**, and **24**

Compd	% F_o rat	5-HT_7 binding K_i (nM)	5-HT_6 binding K_i (nM)	α_1 binding K_i (nM)	5-HT_{2C} binding K_i (nM)
10	12	3	>1000	300	>1000
17	57	2	>1000	100	>1000
20	44	2	172	200	20
21	15	9	>1000	>1000	160
24	—	2	380	>1000	10

high affinity, while the 3- and 4-pyridylethyl amines (**22** and **23**, respectively), possess little affinity for the 5-HT_7 receptor, relative to the unsubstituted phenethyl compound, **19**.

Since the oral bioavailability in rat of **10** was only 12% (Table 2), the in vitro stability of **10** with human liver microsomes[19] was studied. The results suggest the major route of metabolism of **10** is hydroxylation of the phenoxy ring with hydroxylation of the 2-phenylethyl moiety as a minor route. When 4-fluoro substituents were added to both phenyl groups in **17** to block metabolic hydroxylation, the bioavailability was raised to 57%. Likewise, the bioavailability of the the 4-fluorophenyl compound, **19**, was 44%, while the 2-pyridyl ethyl compound, **20**, was low at 15%. These results are consistent with the major metabolic route being *para*-hydroxylation on the two aromatic rings.

The selectivity of several compounds for the 5-HT_7 receptor versus several receptors was also assessed (Table 2). Compounds **20** and **24** possessed moderate affinity for the 5-HT_6 receptor while compounds **10**, **17**, and **20** demonstrated moderate affinity for the α_1 adrenergic receptor. Compounds **21**, **20**, and **24** demonstrated moderate affinity for the 5-HT_{2C} receptor. Compounds **10** and **17** were more selective with no affinity for either 5-HT_{2C} or 5-HT_6 receptors with only moderate affinity for the α_1 adrenergic receptor. None of the compounds in Table 2 exhibited significant affinity ($K_i > 1000$ nM) for the 5-HT_{1a} receptor, the D_{2L} receptor, or the serotonin transporter.

None of these compounds produced a functional agonist response up to a concentration of 10^{-5} M in the 5-HT_7 cyclase assay.[21] In addition, these compounds shifted the

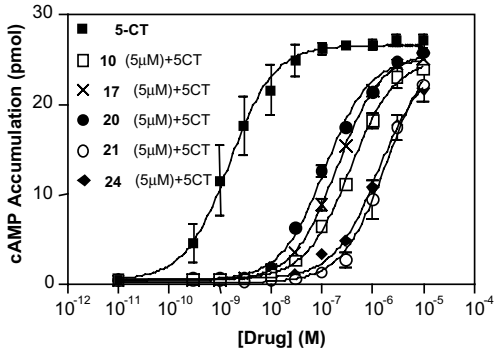


Figure 1. 5-HT_7 cyclase dose–response curves for 5-CT, alone, and in the presence of **10**, **17**, **20**, **21**, and **24** (5 μM).

dose–response curve for 5-CT (a potent 5-HT_7 receptor agonist) to the right in the manner of an antagonist (Fig. 1).

In conclusion, the present studies have identified 5-HT_7 receptor antagonists of a new structural type, the aminotriazines. Compounds **10** and **17** had high affinity for the 5-HT_7 receptor and did not bind to either the 5-HT_{2C} or 5-HT_6 receptors. These compounds produce no agonist effects by themselves and appear to function as potent 5-HT_7 receptor antagonists in vitro. In particular, **17** has demonstrated selectivity and good bioavailability in rat. This compound may prove to be a valuable tool in elucidating the role of the 5-HT_7 receptor as a therapeutic target.

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16. N-(2-Phenoxy-ethyl)-N′-[(1*S*)-(1-phenyl-ethyl)]-[1,3,5]triazine-2,4,6-triamine **10**. Cyanuric chloride (25.0 g, 0.135 mol) was dissolved in THF (500 mL) and diisopropylethylamine (17.5 g, 0.135 mol) in at 0 °C. A solution of (1*S*)-1-phenylethylamine (16.4 g, 0.135 mol) in THF (100 mL) was added dropwise while maintaining the reaction temperature at or near 0 °C. The mixture was allowed to warm to ambient temperature and was then concentrated in vacuo. The residue was dissolved in ethyl acetate (500 mL) and extracted with 1 N HCl (250 mL), H_2O (250 mL), and brine (250 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo to give (4,6-dichloro-[1,3,5]triazin-2-yl)-[(1*S*)-(1-phenyl-ethyl)]-amine as a white solid (35 g, 96%). MP 146–147 °C, ^{1}H NMR ($CDCl_3$): δ 7.39 (m, 5H), 6.40 (b d, 1H), 5.25 (m, 1H), 1.59 (d, 3H); MS (ESI^+) (M^+) 269.1 obsd.
A solution of the above intermediate in THF (500 mL) and NH_4OH (28%, 50 mL) was stirred for 48 h and then concentrated in vacuo. The residue was dissolved in methylene chloride (500 mL) and washed with water (2×250 mL). The organic layer was filtered, dried over sodium sulfate, and concentrated in vacuo to give 6-chloro-N-[(1*S*)-(1-phenyl-ethyl)]-[1,3,5]triazine-2,4-diamine as a white solid (29.7 g, 90%). MP 163–164 °C, ^{1}H ($CDCl_3$): δ 7.78 (m, 5H), 6.1–6.2 (m, 4H), 1.52 (d, 3H), MS (ESI^+) (M^+) 249.7 obsd.
A solution of the above intermediate (15.0 g, 0.056 mol), 2-phenoxyethylamine (8.6 g, 0.062 mol), diisopropylethylamine (25 mL, 0.178 mol), and THF (500 mL) was heated to reflux for 24 h, then cooled, and concentrated in vacuo. The amber residue was dissolved in CH_2Cl_2 and washed with 1 N HCl (300 mL). The product precipitated from the organic layer and was collected by filtration, washed with water and acetonitrile. The product was triturated in hot acetonitrile, collected by filtration, and dried to give N-(2-phenoxy-ethyl)-N′-[(1*S*)-(1-phenyl-ethyl)]-[1,3,5]triazine-2,4,6-triamine, **9**, as a white solid HCl salt (18.2 g, 88%). MP 207–208 °C, ^{1}H NMR ($CDCl_3$): δ 7.19–7.3 (m, 7H), 6.91–6.96 (m, 3H), 5.16–6.08 (b m, 3H), 4.90 (b s, 2H), 3.32–4.04 (m, 4H), 1.49 (d, 3H); MS (ESI^+) $(M+H)^+$ 351.2 obsd; C, H, N calcd for $C_{19}H_{22}N_6O$·HCl: C, 58.98; H, 5.99; N, 21.72; Found C, 59.02; H, 5.87; N, 21.67.
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18. 5-HT_7 binding assay. Membranes are prepared for binding using the human 5-HT_7 receptor expressed in CHO cells. Cells are collected and ruptured using a dounce homogenizer. The cells are spun at 18,000*g* for 10 min and the pellet is resuspended in assay buffer, frozen in liquid nitrogen, and kept at −80 °C until the day of the assay. The assay is carried out in 96-deep-well plates with a total of 30 μg protein used per well in an assay buffer of 50 mM HEPES. The membrane preparation is incubated at 25 °C for 60 min with 0.1–1000 nM test compound and 1 nM ^{3}H-5-carboxamidotryptamine. 10 μM serotonin is used as blocking agent to determine nonspecific binding. The reaction is terminated by the addition of 1 mL of ice cold 50 mM HEPES buffer and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters. The filter pads are counted in an LKB Trilux liquid scintillation counter. K_i values are determined using nonlinear regression by Exel-fit.

19. Compound **10** was incubated with human liver microsomes for 30 min at 10 μM concentration. The protein was removed by acetonitrile precipitation and the supernatant (100 μL) was injected on LC/MS. HPLC conditions utilized a Zorbax RX C8 column (4.6×250 mm) with an acetonitrile/10 mM ammonium acetate (pH 4.6) linear gradient from 40% to 100% acetonitrile in 25 min. LC/MS and LC/MS/MS analysis was performed with a Finnigan LCQ ion trap mass spectrometer using electrospray ionization in positive ion mode with an API source at 5.0 kV and capillary temp of 230 °C. The analysis of the 30 min incubation sample showed four detectable metabolites (M1–M4) eluting at the retention times 13.8, 18.2, 18.3, and 20.1 min, respectively, with residual **10** at 22.4 min. MS/MS analysis of **10** showed an $(M+H)^+$ ion at m/z 351 with a key product ions at m/z 247 (loss of the 2-phenylethyl moiety) and 153 (loss of both the 2-phenylethyl moiety and the phenoxy moiety). The minor metabolite, M1, gave an intense $(M+H)^+$ ion at m/z 247 with a fragment ion at m/z 153 consistent with the cleavage of the 2-phenylethyl moiety. The three other metabolites gave the same $(M+H)^+$ ion at m/z 367, 16 amu greater than parent, suggesting that they result from monohydroxylation. The MS/MS analysis of M2 (major) and M4 (minor) gave the same fragment ions at m/z 263 and 153 consistent with *ortho*- and *para*-hydroxylation on the phenoxy moiety. The fragment ions (m/z 247 and 153) of metabolite M3 (minor) is identical to the parent compound, suggesting the hydroxyl group may be on the 2-phenylethyl moiety.
20. IC_{50} values are the mean of two determinations run at five different concentrations with the radioligand at the K_d concentration. Each experiment was carried out in triplicate or more. Standard errors were typically ±20% of the mean value, for example: **10**, 5-HT_7 $K_i = 9 \pm 1.9$ nM ($n = 18$); **17**, 5-HT_7 $K_i = 4 \pm 0.3$ nM ($n = 25$).
21. 5-HT7 CAMP assay. C6/5HT_7 cells (C6 cells stably transfected with human 5-HT_7 receptors) were used in the cell-based functional assays. Cells were cultured in HAMS F10 medium with 15% heat-inactivated horse serum, 2.5% heat inactivated, dialyzed FBS, and 400 μg/mL G418 and incubated at 37 °C and 5% CO_2. All cell culture reagents were purchased from Gibco BRL (Rockville, MD) unless noted otherwise. Scintillation proximity assay (SPA) functional assays were performed using the Biotrak cAMP SPA direct screening assay system (Amersham Pharmacia Biotech, Piscataway, NJ). Novel reagents in this kit allow for the rapid extraction and direct measurement of intracellular cAMP. Cells were plated the day before an assay in 96-well microtiter plates (Isoplates™; Wallac OY, Turku, Finland) and incubated overnight at 37 °C and 5% CO_2. Basal test medium used in the SPA assays was RPMI-1640 (without phenol red) supplemented with 20 mM HEPES. IBMX (3-isobutyl-1-methylxanthine; Sigma–Aldrich, St. Louis, MO) a phosphodiesterase inhibitor was added to the test media at a final concentration of 100 μM. Cells were plated for assays at a density of 1×10^5 cells/mL (2×10^4 cells/well) in UltraCulture (BioWhittaker, Walkersville, MD) serum-free medium. After overnight incubation at 37 °C and 5% CO_2, media was aspirated and compounds added. The plate was incubated for 10 min at 37 °C and then compounds aspirated. The reaction was terminated by the addition of lysis reagent. After agitation on a plate shaker for 10 min (to enhance cell lysis), an immunoreagent solution including tracer (adenosine 3′,5′-cyclic phosphoric acid 2′-0-succinyl-3-[^{125}I]iodotyrosine methyl ester), antiserum (rabbit antisuccinyl cAMP serum), and SPA antirabbit reagent (donkey antirabbit IgG coupled to SPA fluomicrospheres) was added and the plate incubated overnight at ambient temperature. The amount of [^{125}I]cAMP bound to fluomicrospheres was determined the following day by counting (2 min per well) in a Trilux MicroBeta counter. Counts (CPM's) from the assay were converted to picomoles using an Excel macro and the data plotted using GraphPad Prism.