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Diaminopyrimidine and diaminopyridine 5-HT$_7$ ligands

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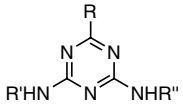
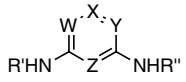
Abstract—The present studies have identified a series of diaminopyrimidines and diaminopyridines as novel 5-HT$_7$ receptor ligands. Three regiosiomeric classes of pyrimidines and four regioisomeric classes of pyridines were synthesized and analyzed for binding to the 5-HT$_7$ receptor. The 5-HT$_7$ binding affinities of different regioisomers show clearly the structure–activity relationship with position of ring nitrogens.

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] In a previous paper[6] we outlined the discovery of a novel class of 5-HT$_7$ ligands, the aminotriazines **1**. To explore the limits of the pharmacophore, ring nitrogens were replaced by CH groups to arrive at the diaminopyrimidines **2**–**4** and the diaminopyridines **5**–**8**. Several analogs from each series were synthesized and tested for binding to the 5-HT$_7$ receptor, and the results demonstrate the importance of the ring nitrogens in the positions denoted by W and Y in **2**–**8**.

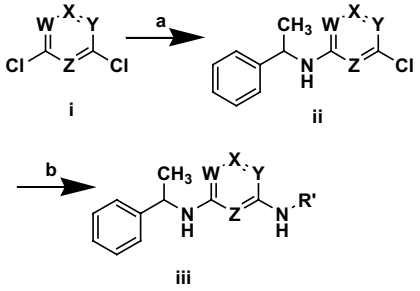


1



2 (W,Y = N; X,Z = CH) **5** (W = N; X,Y,Z = CH)
3 (W,Z = N; X,Y = CH) **6** (X = N; W,Y,Z = CH)
4 (Y,Z = N; W,X = CH) **7** (Y = N; W,X,Z = CH)
8 (Z = N; W,X,Y = CH)

The diaminopyrimidines, **iii**, were prepared by the sequential displacement of the halogens on the appropriate dichloropyrimidine **i** (Scheme 1). The choice of amines was guided by previous experience from the triazine class, which indicated that one of the amines was preferably a branched amine ((*S*)-α-methylbenzylamine preferred). The branched amine sidechain was introduced by refluxing the dichloropyrimidine with α-methylbenzylamine and diisopropylethylamine in acetonitrile to give **ii**. In the case of 2,4-dichloropyrimidine, this reaction gave a mixture of 2- and 4-amino products, which were separated by flash chromatography and carried on to the desired products separately. A neat mixture of the appropriate primary amine and the intermediate **ii** was heated at 140 °C to give the diaminopyrimidine products, **iii**.[7]



Scheme 1. Reagents and conditions: (a) RNH$_2$, NEt(*i*Pr)$_2$, THF; (b) R′NH$_2$, heat.

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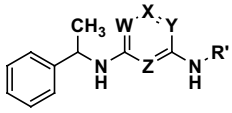
Table 1. 5-HT_7 binding[9] of **9**–**17**



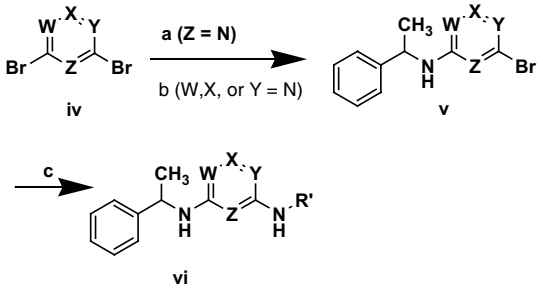
9-17

#	*	W	X	Y	Z	R′	5-HT_7 K_i (nM)
9	S	N	CH	N	CH	–$(CH_2)_2$–Ph	33
10	S	N	CH	N	CH	–$(CH_2)_2$–(4-F–Ph)	5
11	S	N	CH	N	CH	–$(CH_2)_2$–O–(4-F–Ph)	5
12	±	N	CH	CH	N	–$(CH_2)_2$–Ph	6
13	±	N	CH	CH	N	–$(CH_2)_2$–O–(4-F–Ph)	1
14	S	CH	CH	N	N	–$(CH_2)_2$–Ph	0.6
15	±	CH	CH	N	N	–$(CH_2)_2$–(4-F–Ph)	6
16	S	CH	CH	N	N	–$(CH_2)_2$–O–Ph	2
17	S	CH	CH	N	N	–$(CH_2)_2$–O–(4-F–Ph)	0.3

The influence of amines on the 5-HT_7[8] binding SAR of pyrimidines followed trends similar to those observed for the triazines.[6] One amine was fixed as α-methylbenzylamine (either the *S* enantiomer or the racemate), and the other amine was preferentially phenethylamine, phenoxyethylamine, or their 4-fluoro derivatives. The results of this SAR for the pyrimidines are shown in Table 1.

The 4,6-disubstituted pyrimidines, **9**–**11** (nitrogen in positions W and Y), showed moderate binding affinity to the 5-HT_7 receptor. The 2,4-disubstituted pyrimidines, **12**–**13** (nitrogen in positions W and Z) demonstrated slightly improved affinity, while the third class of 2,4-disubstituted pyrimidines, **14**–**17** (nitrogen in positions Y and Z), appear to possess the highest affinity 5-HT_7 binding. The most potent pyrimidine from this series was **17**, with a binding affinity of 0.3 nM.

Replacement of another ring nitrogen by a CH group led to diaminopyridines, which were synthesized as shown in Scheme 2. The diaminopyridines, **vi**, were prepared by the sequential displacement of the halogens on the appropriate dibromopyridine **iv**. The branched amine sidechain was introduced by reacting the dibromopyridine with α-methylbenzylamine (using heat for 2,6-dibromopyridine and palladium catalysis[10] for other dibromopyridines). In the case of 2,4-dibromopyridine, this reaction gave a mixture of 2- and 4-amino products, which were separated by flash chromatography and carried on to the desired products separately. The appropriate primary amine was reacted with the intermediate **v**, using palladium catalysis to give the diaminopyridine products, **vi**. The second amine addition was routinely done in parallel reactions using premixed stock solutions of catalyst and ligand.[11]



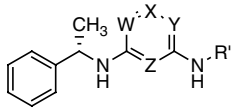
Scheme 2. Reagents and conditions: (a) RNH_2, K_2CO_3, DMF, heat; (b) RNH_2, NaOBu*t*, Pd_2dba_3, BINAP, toluene, 70 °C; (c) $R'NH_2$, NaOBu*t*, Pd_2dba_3, BINAP, toluene, 90 °C.

As can be seen from Table 2, changes in the location of ring nitrogens in the pyridine series caused significant effects on 5-HT_7 binding. 2,6-Disubstituted pyridines **30**–**31** (Z = nitrogen) showed a loss of activity, whereas the 3,5-disubstituted pyridines **22**–**25** (X = nitrogen) provided moderate activity. The 2,4-disubstituted pyridines **18**–**21** (W = nitrogen) and **26**–**29** (Y = nitrogen) both demonstrated high affinity for the 5-HT_7 receptor, with **26**–**29** being slightly more potent. Compound **29** demonstrated the highest affinity binding of the pyridine class with an K_i of 0.2 nM. Compounds **32** and **33** are included to show that at least one nitrogen does appear to be necessary for activity.

Several compounds were also assayed against other receptors to determine selectivity for 5-HT_7 (Table 3). Good to moderate affinity for the α_1 adrenergic receptor was shown by pyrimidines **13** and **17** and by pyridines **21** and **29**, while pyridine **25** showed poor binding. Good to moderate affinity binding to the 5-HT_{2c} receptor was shown by pyrimidine **13** and pyridines **21** and **29**, and again pyridine **25** showed poor binding (no value obtained for pyrimidine **17**). Only pyridine **29** showed some binding to the 5-HT_6 receptor. None of the compounds in Tables 1 and 2 exhibited significant affinity ($K_i > 1000$ nM) for the 5-HT_{1a} receptor, the D_{2L} receptor (excepting the potent pyridine **29**), or the serotonin transporter. One can note that the selectivity ratios for binding to 5-HT_7 versus binding to α_1 and 5-HT_{2c} are similar for the compounds in Table 3, even though each has different nitrogen positions. Thus, for the compounds represented in Table 3, the SAR for binding to α_1 and D_{2L} roughly parallels the SAR for binding to 5-HT_7.

In conclusion, the present studies have identified 5-HT_7 receptor ligands of two new structural types, the diamino-pyrimidines and -pyridines, and a clear SAR

Table 2. 5-HT_7 binding[9] of **18**–**33**



18-33

#	W	X	Y	Z	R′	5-HT_7 K_i (nM)
18	N	CH	CH	CH	–$(CH_2)_2$–Ph	3
19	N	CH	CH	CH	–$(CH_2)_2$–(4-F–Ph)	4
20	N	CH	CH	CH	–$(CH_2)_2$–O–Ph	0.4
21	N	CH	CH	CH	–$(CH_2)_2$–O–(4-F–Ph)	0.4
22	CH	N	CH	CH	–$(CH_2)_2$–Ph	13
23	CH	N	CH	CH	–$(CH_2)_2$–(4-F–Ph)	16
24	CH	N	CH	CH	–$(CH_2)_2$–O–Ph	4
25	CH	N	CH	CH	–$(CH_2)_2$–O–(4-F–Ph)	4
26	CH	CH	N	CH	–$(CH_2)_2$–Ph	0.3
27	CH	CH	N	CH	–$(CH_2)_2$–(4-F–Ph)	0.4
28	CH	CH	N	CH	–$(CH_2)_2$–O–Ph	0.4
29	CH	CH	N	CH	–$(CH_2)_2$–O–(4-F–Ph)	0.2
30	CH	CH	CH	N	–$(CH_2)_2$–Ph	>1000
31	CH	CH	CH	N	–$(CH_2)_2$–O–Ph	>1000
32	CH	CH	CH	CH	–$(CH_2)_2$–Ph	>1000
33	CH	CH	CH	CH	–$(CH_2)_2$–O–Ph	>1000

Table 3. 5-HT_6, 5-HT_{2C}, and α_1 binding[9] (rat) of **13**, **17**, **21**, **25**, **29**

Compd	5-HT_7 Binding K_i (nM)	D_{2L} Binding K_i (nM)	α_1 Binding K_i (nM)	5-HT_{2C} Binding K_i (nM)
13	1	>1000	57	40
17	0.3	>1000	14	—
21	0.4	>1000	27	13
25	4	>1000	379	476
29	0.2	370	8	15

emerged from the regiochemistry of the ring heteroatoms. The most active compounds were pyridines with one nitrogen in positions denoted W or Y in the general structure. The addition of a second nitrogen produced pyrimidines with similar or slightly diminished activity compared to the pyridines. The most active pyrimidines were those with a nitrogen in either position W or Y and another nitrogen in the Z position. These studies have helped to further define the SAR of the amino-triazine 5-HT_7 antagonists and have produced ligands which may be useful in elucidating the role of the 5-HT_7 receptor as a therapeutic target.

References and notes

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7. Procedure for synthesis of **11**: A mixture of 4,6-dichloropyrimidine (14.9 g, 0.10 mol), (*S*)-(−)-α-methylbenzylamine (13.3 g, 0.11 mol), and diisopropylethylamine (15.5 g, 0.12 mol) in acetonitrile (100 mL) were heated to reflux for 4 h. The reaction was cooled, then partitioned between ether (500 mL) and water (250 mL). The organic layer was separated, dried over sodium sulfate, and concentrated in vacuo. Purification by flash chromatography on silica gel with hexane/ethyl acetate (3:1) yielded pure (*S*)-6-chloro-*N*-(1-phenylethyl)pyrimidin-4-amine (18.6 g, 80%).
   The above intermediate (1.40 g, 6.0 mmol) and 4-fluorophenoxyethylamine (2.14 g, 13.8 mmol) were heated neat in a sealed tube at 150 °C for 3 h. After cooling, the solidified mass was dissolved in dichloromethane (150 mL) and extracted with saturated aqueous sodium carbonate and water. The organic layer was dried over sodium sulfate, partially concentrated, and precipitated with hexane. The solid brown product was filtered and washed with dichloromethane/hexane (2:1) to give compound **11** (1.30 g). Conversion to the HCl salt was accomplished by dissolving this material in dichloromethane (40 mL) and treating with a solution of 1 N HCl in ether (20 mL). Concentration yielded an oil, which was co-evaporated from acetonitrile to yield the HCl salt as a foam (1.33 g, 63%).
8. 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 ug 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.

9. IC_{50} values are the mean of 1–4 determinations run at five different concentrations with the radioligand at the K_d concentration. Each experiment was carried out in duplicate. Standard errors were typically ±20% of the mean value.
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11. Procedure for synthesis of **28**: To a solution of 2,4-dibromopyridine (0.45 g; 1.9 mmol) and (*S*)-α-methylbenzylamine (0.23 g; 1.9 mmol) in toluene (10 mL) was added sodium *t*-butoxide (0.22 g; 2.3 mmol), tris(dibenzylideneacetone)palladium(0) (35 mg; 0.038 mmol), and (+) BINAP (47 mg; 0.076 mmol; either enantiomer would work). The reaction was heated at 70 °C for 2 h and then diluted with H_2O (20 mL), extracted with ethyl acetate (2×10 mL), dried with $MgSO_4$, and evaporated to dryness. The products were easily separated by flash chromatography on a silica gel column, eluting with 15:85 ethyl acetate/hexanes to obtain **v**(W = N) (128 mg, 24%) and with 25:75 ethyl acetate/hexanes to obtain **v**(Y = N) (102 mg; 19%). The regioisomers were identified by NOE studies of their ^{1}H NMR spectra.

Compound **v**(W = N) (20 mg; 0.072 mmol), phenoxyethylamine (16 mg; 0.11 mmol), and sodium *t*-butoxide (8 mg; 0.08 mmol) were added to toluene (1 mL) in a 1 dram glass vial. A catalyst stock solution was prepared by adding Pd_2dba_3 (12 mg; 0.013 mmol) and (+) BINAP (18 mg; 0.029 mmol) to toluene (6 mL) and heating gently until most solids dissolved. A portion of this stock solution (1 mL) was added to the vial of amine and bromide prepared earlier. The vial was sealed with a teflon-backed lid and heated at 90 °C for 2 h. The solvent was removed in vacuo and the residue was dissolved in methanol and filtered before being purified by preparative reverse phase HPLC with conditions: column = YMC ODS-A 20×100 mm S5; solvent A = 10:90:0.1 methanol/H_2O/TFA; solvent B = 90:10:0.1 methanol/H_2O/TFA; elution gradient from 30% B to 100% B; gradient time = 10 min; flow rate = 20 mL/min. Obtained product **28** as a TFA salt in a yield of 11 mg (25%). ^{1}H NMR ($CDCl_3$) δ 8.80 (m, 1H), 7.46 (m, 1H), 7.17–7.38 (m, 6H), 6.98 (m, 2H), 6.79 (d, J = 7.8 Hz, 2H), 6.63 (d, J = 7.6 Hz, 1H), 5.98 (d, J = 5.8 Hz, 1H), 5.50 (d, J = 5.5 Hz, 2H), 4.51 (m, 1H), 3.96 (m, 2H), 3.44 (m, 2H), 1.59 (d, J = 6.6 Hz, 3H); MS (ESI^{+}) $(M+H)^{+}$ 334.0 obs.