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Synthesis and evaluation of arylpiperazines derivatives of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine as 5-HT_{1A}R ligands

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Abstract

5-HT_{1A}R agonist or partial agonists are established drug candidates for psychiatric and neurological disorders. We have reported the synthesis and evaluation of a series of high affinity 5-HT_{1A}R partial agonist PET imaging agents with greater selectivity over α -1AR. The characteristic of these molecules are 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine skeleton tethered to an arylpiperazine unit through an alkyl side chain. The most potent 5-HT_{1A}R agonistic properties were found to be associated with the molecules bearing C-4 alkyl group as the linker. Therefore development of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine bearing arylpiperazine derivatives may provide high affinity selective 5-HT_{1A}R ligands. Herein we describe the synthesis and evaluation of the binding properties of a series of arylpiperazine analogues of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine.

Keywords

5-HT; 5-HT_{1A}R; agonist, α -1AR; arylpiperazine

The serotonin receptor, 5-HT_{1A}R, is a G protein-coupled receptor (GPCR).¹ It is widespread and heterogeneously distributed in CNS as somatodendritic autoreceptors in raphe nuclei and postsynaptically in cortical and subcortical brain regions.² In addition to 5-HT, 5-HT_{1A}R affects release and signaling by a variety of neurotransmitters, hormones and growth factors in brain.^{3–5} Alteration of 5-HT_{1A}R binding has been implicated in the pathophysiology of a variety of neuropsychiatric diseases and neurodegenerative disorders.^{6–9} 5-HT_{1A}R agonists (eg: buspirone and tandospirone) are currently approved drugs for anxiety and depression.^{10, 11} 5-HT_{1A}R agonists and partial agonists are being evaluated as antipsychotic drugs with fewer side effects for the treatment of schizophrenia.^{12–14} Furthermore, 5-HT_{1A}R desensitization and increased 5-HT_{1A}R

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postsynaptic activation has also been postulated as part of the antidepressant action of SSRIs and perhaps other antidepressants.^{2, 5}

Many 5-HT_{1A}R agonists and partial agonists belong to aminotetraline and arylpiperazine structural skeletons (Figure 1).¹⁵ Recent additions of compounds to these prominent structural skeletons include CUMI-101, S14506 (**1**), benzo[*d*]oxazole **2**, alkylthiobenzimidazole, benzothiazole, and benzofuro[7,6-*g*]quinoline **3**.^{16–18} Compounds belonging to several novel structural classes such as derivatives of 4-(aminomethyl)-4-fluoropiperidin-1-ones **4**, 5-spiroimidazolidine-2,4-dione **5**, indolylalkyl-amines, ergolines, dihydrofluoroaporphines, 1,3-oxathiolane, 1,3-dioxolanes and pyrroloimidazoles have also been identified recently as agonist ligands at 5-HT_{1A}R (Figure 1).^{16–23} Although several ligands with high affinity for 5-HT_{1A}R have been developed, a major drawback of these ligands is their significant affinity for alpha1-adrenergic receptor (α -1AR).

Positron Emission Tomography (PET) is a noninvasive method to measure 5-HT_{1A}R binding in living human brain.^{24, 25} Estimation of *in vivo* occupancy of agonist drugs at 5-HT_{1A}R is a valuable tool for drug development and monitoring the therapeutic intervention. Additionally, *in vivo* PET estimation of the sensitivity of binding of 5-HT_{1A}R ligands to competition by intrasynaptic serotonin would make it possible to compare serotonergic transmission in healthy and diseased populations.²⁴ A number of 5-HT_{1A}R antagonist radioligands such as [carbonyl-¹¹C] WAY-100635, [¹⁸F]MPPF and [¹⁸F]FCWAY have been routinely used for research PET studies in man.^{24,25} However, an agonist ligand would bind preferentially to the G-protein coupled high affinity (HA) state of the receptor and thereby offers advantages to an antagonist ligand.²⁴

Several studies attempted to develop a 5-HT_{1A}R agonist PET imaging agent with limited success *in vivo*.^{24, 25} We have recently identified several high affinity and selective 5-HT_{1A}R ligands as candidates for PET ligands.^{26–29} Of these [¹¹C]CUMI-101, is the only successful partial agonist PET tracer available so far for the *in vivo* measurement of high affinity 5-HT_{1A}R agonist binding in nonhuman primates and human subjects with PET.^{26, 30–33} The characteristic feature of the ligands we identified is the presence of a 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine skeleton tethered to an arylpiperazine unit through a C-4 alkyl side chain. Prior to our studies 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazines were reported as 5-HT_{1A}R ligands in a patent application with limited information.³⁴ Herein we describe the synthesis and evaluation of a series of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine derivatives as 5-HT_{1A}R ligands.

The candidate 5-HT_{1A}R ligands characterized by an 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine tethered to arylpiperazines through an alkyl side chain were prepared by refluxing a solution of 2-(4-chlorobutyl)-4-methyl-1,2,4-triazine-3,5-(2*H*,4*H*)-dione (**6**) preactivated with sodium iodide in acetonitrile with substituted arylpiperazine (**7**) in the presence of anhydrous K₂CO₃.²⁸ Thirty six analogues of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine (compounds **8–41**) were synthesized from the corresponding substituted arylpiperazines (Scheme 1).

Most of the arylpiperazines were commercially available and used as such without further purification. 2,4-Dimethoxyphenylpiperazine was prepared by the treatment of 2,4-

dimethoxyphenyl amine with bis(2-chloroethyl)amine hydrochloride in the presence of potassium carbonate in 2-butoxyethanol at 150 °C.³⁵ The naphthyl piperazines, for the synthesis of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine derivatives (compounds **39-43**) were prepared by the condensation of the corresponding naphthylamines with bis(2-chloroethylamine)-hydrochloride in polyethylene glycol under microwave irradiation.²⁸ Binding affinity values (K_i) of the compounds at 5-HT_{1A}R were determined by competition binding studies with [³H]8-OH-DPAT using racemic 8-OHDPAT as the reference compound.²⁶⁻²⁸ K_i of the compounds at α -1AR were determined by competition binding studies with [³H]-7-methoxyprazosin using phentolamine as the reference compound.²⁶⁻²⁸ The agonist properties of selected ligands were examined on 5-HT_{1A}R using [³⁵S]GTP γ S binding in membranes of Chinese hamster ovary cells stably expressing the human 5-HT_{1A}R (CHO-h5-HT_{1A} cells).²⁶⁻²⁸

The unsubstituted phenyl analogue **18** has a 2.5 nM K_i for 5-HT_{1A}R and 25 times selectivity over α -1AR (K_i = 64 nM). In the case of substituted phenyl derivatives (Table 1), a variety of substituents such as methoxy (**8**), ethoxy (**12**), thiomethyl (**17**), cyano (**20**), fluoro (**25**), fluoroethyl (**13**), fluoropropyl (**14**), hydroxyl (**24**), thiohydroxy (**31**) groups were well tolerated at *ortho*-position and exhibited nanomolar affinity toward 5-HT_{1A}R. A few of these ligands have meaningful selectivity over α -1AR such as 45, 210, 42, >10,000, and >10000 times for compounds **8,13,14,24** and **31** respectively. The methoxy (**9**) and hydroxyl (**25**) substitution at *meta*-position of the phenyl group also resulted in high affinity ligands with excellent selectivity for 5-HT_{1A}R compared to α -1AR. The substitution of dimethylamino (**15**) and methylamino (**16**) analogues did not provide any 5-HT_{1A}R specificity or selectivity. Substitution on the phenyl moiety resulted in a sharp decrease in affinity for the 5-HT_{1A}R receptor for 4-methoxy, 1,4-dimethoxy, 1-cyano-4-methoxy, 4-iodo analogs **10, 11, 23** and **19** respectively. The introduction of a pyridyl ring results in high affinity 5-HT_{1A}R binding with excellent selectivity over α -1AR (**27, 29, 32, 33**).

However, the pyridyl derivative with *meta*-substituted fluoro analog **28** had modest 5-HT_{1A}R binding affinity. Similarly replacement of pyrimidine ring instead of phenyl group results in a remarkable decrease in 5-HT_{1A}R binding affinity (**30**). Several substituted naphthyl derivatives also exhibited nanomolar affinity at 5-HT_{1A}R as well as possess reasonable selectivity over α -1AR (**39-41**, Table 2). The ideal length of alkyl linker was identified to be 4. When the chain length was decreased to 3 (**35-37, 43**), there was a dramatic drop in the binding affinity at 5-HT_{1A}R (Tables 1 and 2). Similarly we did not see considerable improvement in 5-HT_{1A}R binding affinity for linker with length 5 as shown for molecules **38** and **42**.

The promising compounds with higher ratios of 5-HT_{1A}R vs. α -1AR were tested for 5-HT_{1A}R functional measurements.³⁶ Agonist increased [³⁵]GTP γ S binding over basal level in a concentration dependent manner. Maximal ligand stimulated [³⁵]GTP γ S binding (E_{max}) for the selected compounds were in the range of 80–95% of that seen with 5-HT (Table 3).

In summary, a novel series of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine tethered arylpiperazines has been identified as candidate agonist ligands with high affinity for 5-HT_{1A}R. Several members of the series such as **32** have nanomolar affinity for 5-HT_{1A}R, higher selectivity to

5-HT_{1A}R over α -1AR in comparison to CUMI-101 and robust agonist profile as measured by GTP γ S binding.

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35. Typical Experimental procedure: **4-Methyl-2-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)-1,2,4-triazine-3,5(2H,4H)-dione (11)**. A mixture of 2-(4-chlorobutyl)-4-methyl-1,2,4-triazine-3,5(2H,4H)-dione, (217 mg, 1 mmol) and NaI (225 mg, 1.5 mmol) in acetonitrile (3 mL) was stirred under reflux for 30 min. Then, 2-pyridylpiperazine (325 mg, 2 mmol) and anhydrous K₂CO₃ (420 mg, 3 mmol) were added. The reaction mixture was stirred at 60 °C for 24 h. After cooling, the reaction mixture was diluted with EtOAc, filtered to remove excess K₂CO₃. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed under vacuum. The crude mixtures were purified by silica gel column chromatography using chloroform/methanol 98:2 (v/v) as the eluent. The combined product fractions were concentrated to yield the desired product as a colorless solid (320 mg, 93 %); Mp: 85 °C. ¹H NMR (400 MHz, CDCl₃) δ: 1.61(pentet, 2H, *J* = 7.6 Hz); 1.84 (pentet, 2H, *J* = 7.6 Hz); 2.45 (t, 2H, *J* = 7.6 Hz); 2.57 (t, 4H, *J* = 5.2 Hz); 3.37 (s, 3H); 3.57 (t, 4H, *J* = 5.2 Hz); 4.05 (t, 2H, *J* = 7.6 Hz); 6.63-6.67 (m, 2H); 7.42 (s, 1H); 7.50 (dt, 1H, *J* = 1.6, 7.2 Hz); 8.20-8.22 (m, 1H). HRMS Calcd for C₁₇H₂₅N₆O₂ (MH⁺): 345.2039; Found: 345.2018.
36. Agonist-stimulated [³⁵S]GTPγS binding of the ligands were measured as described previously.²⁸ Chinese hamster ovary (CHO) cells expressing 5-HT_{1A} receptors (CHO-h5-HT_{1A}) membranes (30 μg) were preincubated with specific concentrations of the test ligand for 5 min at room temperature in a buffer containing 20 mM HEPES pH 7.4, 3 mM MgCl₂, 100 mM NaCl, and 3 μM GDP in a final volume of 0.5 ml. [³⁵S]GTPγS (0.1 nM; 1250 Ci/mmol Perkin Elmer Life Science, Boston, MA) was added and the incubation was continued for 60 min at room temperature. Experiments were terminated by rapid filtration through Whatman GF/B filters followed by three washes with ice-cold 20 mM HEPES buffer, pH 7.4, using a cell harvester (Brandel, M-24R, Gaithersburg, MD). Bound radioactivity was determined by liquid scintillation spectrometry (Beckman, LS9000).

Figure 1.

Representative examples of recent 5-HT_{1A}R agonist ligands

Scheme 1.

Synthesis of azaurazil derivatives of aryl and heteroaryl peperazines

Substituted phenyl and heterocyclic derivatives of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine tethered piperazines

8-36		n= 1-4					
Compd	n	X	Y	R ¹	R	5-HT _{1A} R K _i (nM)	α 1 _{AR} K _i (nM)
8 (CUMI-101) ²⁶	3	C	C	OCH ₃	H	0.15	6.75
9 ²⁷	3	C	C	H	5, OCH ₃	1.1	413
10	3	C	C	H	4, OCH ₃	414	>10,000
11	3	C	C	OCH ₃	4, OCH ₃	190	>10,000
12	3	C	C	OCH ₂ CH ₃	H	0.66	1.8
13 ²⁹	3	C	C	OCH ₂ CH ₂ F	H	0.1	21.4
14	3	C	C	OCH ₂ CH ₂ CH ₂ F	H	0.8	280
15	3	C	C	N(CH ₃) ₂	H	180	350
16	3	C	C	NHCH ₃	H	220	450
17	3	C	C	SCH ₃	H	0.46	1.8
18	3	C	C	H	H	2.5	64
19	3	C	C	H	4, I	58.8	103
20	3	C	C	CN	H	0.835	7.3
21	3	C	C	CN	2, F	4.8	>10,000
22	3	C	C	CN	4, OMe	215	380
23	3	C	C	CN	2, Br	28	450
24	3	C	C	OH	H	1.7	>10,000
25	3	C	C	H	5, OH	2.8	>10,000
26	3	C	C	F	H	1.89	9.73
27	3	C	N	H	H	1.1	262
28	3	C	N	H	4, F	68	>10,000

Compd	n	X	Y	R ¹	R	5-HT _{1A} R K _i (nM)	α ₁ -AR K _i (nM)
29	3	C	N	H	2, F	1.2	>10,000
30	3	N	N	H	H	175	>10,000
31	3	C	C	SH	H	8	>10,000
32	3	N	C	H	3, F	0.2	>10,000
33	3	N	C	H	3, Br	1.4	>10,000
34	1	C	C	OCH ₃	H	240	>10,000
35	2	C	C	OCH ₃	H	180	>10,000
36	2	C	C	H	H	350	>10,000
37	2	C	C	F	H	250	>10,000
38	4	C	C	OCH ₃	H	150	>10,000

Table 2

Substituted naphthyl derivatives of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine tethered piperazines.

Compd	n	R	5-HT _{1A} R K _i (nM)	α1-AR K _i (nM)
8 ²⁶			0.15	6.75
39	3	OH	0.44	24.5
40 ^[28,34]	3	OCH ₃	1.6	32.0
41	3	OCH ₂ CH ₃	2.1	48
42	4	OCH ₃	240	>10,000
43	2	OCH ₃	175	>10,000

Table 3Agonist properties of selected 5-HT_{1A}R ligands

Compd	GTPγS EC ₅₀ , (nM)	GTPγS E _{max} (%)
8	0.1	80
9	0.7	95
13	0.65	77
18	0.3	93
27	0.08	85
29	0.8	80
32	0.2	95
40	0.05	95