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Triamino derivatives of triazolotriazine and triazolopyrimidine as adenosine A_{2a} receptor antagonists

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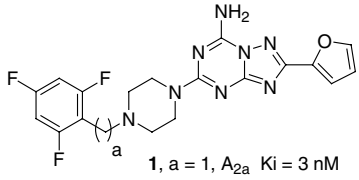
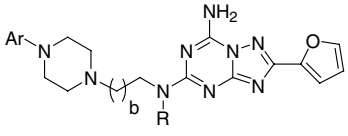
Abstract—Piperazine derivatives of 2-furanyl[1,2,4]triazolo[1,5-*a*][1,3,5]triazine have recently been shown to be potent and selective adenosine A_{2a} receptor antagonists. We now demonstrate that potent and selective A_{2a} receptor antagonists could still be obtained when the arylpiperazines are separated from the triazolotriazine core structure by an ethylenediamine spacer. Selected analogs bearing this triazolotriazine or the related triazolopyrimidine core structure have been found to be orally active in a mouse catalepsy model of Parkinson's disease.

The adenosine A_{2a} receptors belong to a family of seven *trans*-membrane G-protein-coupled receptors (GPCRs) consisting of four subtypes (A_1, A_{2a}, A_{2b}, and A_3).[1,2] The A_{2a} receptors are present abundantly in the basal ganglia; and within the striatum, they are selectively located on the GABA/enkephalin-containing neurons bearing the dopamine D_2 receptors.[3,4] The A_{2a} receptors, therefore, have the ability to modulate motor functions since they can indirectly regulate the striatal output activity.[5] In rats, intracerebroventricular injection with a selective adenosine A_{2a} agonist induces catalepsy, a motor disability that is similar to that exhibited by patients with Parkinson's disease.[6] More importantly, administration with selective adenosine A_{2a} receptor antagonists has been shown to reverse this cataleptic behavior.[6,7] A similar improvement in motor function has also been observed when selective adenosine A_{2a} receptor antagonists are administered to marmosets in a primate model of Parkinson's disease.[8]

Adenosine A_{2a} receptor antagonists can be classified into two categories: xanthine based and nonxanthine based. Research in the former category has already led to the discovery of KW-6002, a selective adenosine A_{2a} receptor antagonist that is currently being evaluated in clinical trials for Parkinson's disease.[7] The identification of nonxanthine A_{2a} receptor antagonists has been the subject of intense interest in recent years.[9] Much effort in this field has been focused on novel modifications of SCH-58261 and ZM-241385.[10–15] We have recently disclosed that compound **1** and other piperazine derivatives of [1,2,4]triazolo[1,5-*a*][1,3,5]triazine were potent and selective adenosine A_{2a} receptor antagonists.[12] As an extension of this series, we began to explore the possibility of varying the length of the spacer between the two ends of the piperazine moiety.



1, a = 1, A_{2a} Ki = 3 nM
2, a = 0, A_{2a} Ki = 29 nM



3 (where R = H or Me, b = 1 or 2)

In our first modification, the methylene group between the capping group and the piperazine moiety was removed. As shown in **2**, this type of aryl piperazine

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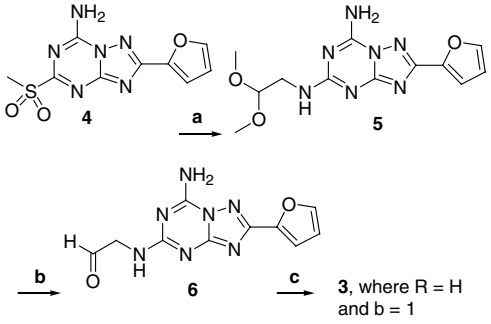
showed a fairly significant decrease in A_{2a} binding affinity. We then turned our attention to changing the length of the spacer between the aryl piperazine moiety and the [1,2,4]triazolo[1,5-*a*][1,3,5]triazine core. We were particularly interested in using a more flexible linker such as the one shown in structure **3**.

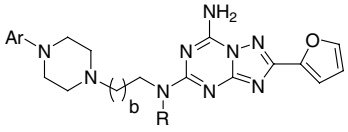
The chemistry shown in Scheme 1 was used to prepare compounds of the general structure **3** (where R = H and b = 1). Sulfone **4**[16] was reacted with aminoacetaldehyde dimethyl acetal to obtain the intermediate **5**. The dimethyl acetal group was then hydrolyzed to the corresponding aldehyde by treatment with TFA/H_2O/CH_2Cl_2. Aldehyde **6** was not stable and was not isolated. After the TFA had been neutralized with Et_3N, the reductive amination was carried out by adding the desired piperazine derivative along with $Na(OAc)_3BH$. Longer carbon spacers (as in general structure **3** where R = H and b = 2) could also be prepared using Scheme 1, employing a different protected amine derivative, namely aminoproprionaldehyde dimethyl acetal. Also, *N*-methylated derivatives (as in **3**, where R = CH_3 and b = 1) could be prepared by using the same Scheme 1, substituting the appropriate amine such as *N*-methylaminoacetaldehyde dimethyl acetal.

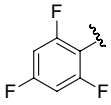
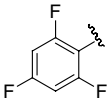
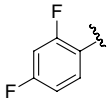
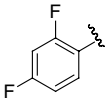
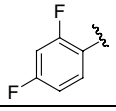


Scheme 1. Reagents and conditions: (a) aminoacetaldehyde dimethyl acetal, Et_3N, CH_3CN, reflux; (b) TFA/H_2O/CH_2Cl_2; (c) substituted arylpiperazine, CH_2Cl_2, $Na(OAc)_3BH$, rt.

Table 1 lists the various [1,2,4]triazolo[1,5-*a*][1,3,5]triazine analogs that have been prepared. Based upon the previous SAR involving the piperazine derivatives of [1,2,4]triazolo[1,5-*a*][1,3,5]triazine,[12] we knew that substituted aryl fluorides would afford some of the best A_{2a} activity.[12–14] Hence, for our preliminary evaluation, we employed only a small set substituted piperazines consisting of phenylpiperazine, 2,4-difluorophenylpiperazine and 2,4,6-trifluorophenylpiperazine. First of all, compounds **7** and **8** clearly showed that the two-carbon spacer was superior to the three-carbon spacer in terms of A_{2a} binding affinity. The same trend was also

Table 1.

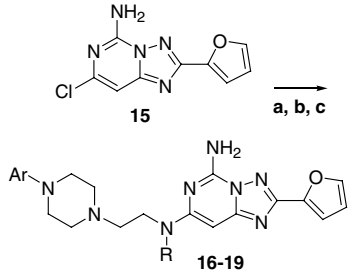


Compds	Ar	b	R	A_{2a} K_i (nM)	A_1 K_i (nM)
7	Phenyl	1	H	22	>500
8	Phenyl	2	H	190	>500
9		1	H	24	>500
10		2	H	100	>500
11		1	H	7.1	1100
12		2	H	93	>500
13	Phenyl	1	CH_3	11	1500
14		1	CH_3	4.0	820

For the A_{2a} receptor, membranes were prepared from rat brain tissues and the radioligand binding assay was performed using [^{3}H]ZM-241385. For the A_1 receptor, membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using [^{3}H]DPCPX. As a control for these radioligand binding assays, we routinely used SCH-58261, which had an A_{2a} K_i of 37 nM and an A_1 K_i of 390 nM. K_i values were calculated from binding curves generated from the mean of three determinations per concentration, with variations in individual values of <15%.

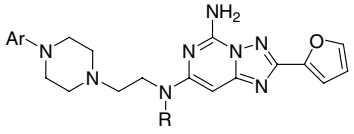
observed with the following two pairs of compounds (compounds **9/10** and compounds **11/12**). In terms of substitution on the phenyl ring, the 2,4-difluoro substitution pattern was clearly better than the 2,4,6-trifluoro pattern. Next, we were interested in methylating the NH position of compounds of general structure **3**. As shown in Table 1, methylation at this position did result in a slight twofold increase in A_{2a} binding affinity (compare the two pairs of compounds **7/13** and **11/14**).

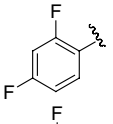
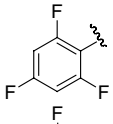
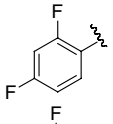
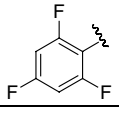
In a previous disclosure, we have demonstrated that the [1,2,4]triazolo[1,5-*c*]pyrimidyl core template could offer some unique in vivo potency over [1,2,4]triazolo[1,5-*a*]-[1,3,5]triazine.[14] Hence, selected analogs bearing this alternative triazolopyrimidine core were made. Scheme 2 illustrates how these analogs could be prepared from the chloro intermediate **15**.[11] The binding results are summarized in Table 2. As observed previously,[14] the triazolopyrimidine core gave compounds with slightly less A_{2a} activity than those having the triazolotriazine core (compare the two pairs of compounds **11/16** and **9/17**). Methylation of the NH group, as in **18** and **19**, also resulted in a gain of A_{2a} binding affinity. In terms of selectivity over the adenosine A_1 receptor, most of the analogs shown in Tables 1 and 2 were fairly selective (>100-fold in some cases).



Scheme 2. Reagents and conditions: (a) aminoacetaldehyde dimethyl acetal was used when R = H; CsF, DMSO, 110 °C *N*-methyl-aminoacetaldehyde dimethyl acetal was used when R = CH_3; (b) TFA/H_2O/CH_2Cl_2, rt; (c) substituted arylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt.

Table 2.

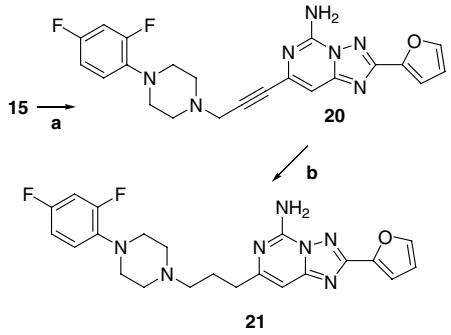


Compds	Ar	R	A_{2a} K_i (nM)	A_1 K_i (nM)
16		H	55	2600
17		H	92	>500
18		CH_3	6.5	750
19		CH_3	24	790

Refer to Table 1 for membrane preparation and details regarding the radioligand binding assay.

Thus far, all of the analogs discussed in Tables 1 and 2 contain an amino group that is directly attached to the core heterocyclic template. We were interested in finding out what the A_{2a} activity would be like if this nitrogen group had been replaced with just a methylene group as in **21**. The triazolopyrimidine template allowed us to quickly assemble the desired carbon framework using the procedure outlined in Scheme 3. Here, reaction between chloride **15** and a propargyl piperazine afforded **20**, which in turn, could be reduced to **21** by a simple hydrogenation. Compound **21**, with a A_{2a} K_i of 390 nM, was significantly less active than isoteres **11** and **16**.

After having examined the binding affinity of these compounds, we were interested in evaluating these compounds in our mouse catalepsy model. This is a widely used rodent model of Parkinson's disease where catalepsy is induced by subcutaneous injection of haloperidol (3 mg/kg). In this cataleptic state, the animals are unable to correct an externally imposed posture. For this study, the animals' forelimbs are placed on an aluminum bar that is suspended horizontally 4.5 cm above the surface of the bench. Catalepsy-free mice, that is those not treated with haloperidol, should be able to put one forelimb back on the bench almost immediately. On the other hand, cataleptic mice, when placed in this unnatural position, are unable to come down from the horizontal bar over a period of 120 s or more. For the efficacy study, a test compound is administered orally about 3 h after the haloperidol administration. Efficacious compounds are defined as those that allow the animals to come down from the bar within 60 s. Furthermore, the animals must remain in this catalepsy-free state for at least 60 min. Six compounds from Tables 1 and 2 were tested for oral activity in this rodent model for Parkinson's and the results are summarized in Table 3. The phenyl derivative **7** was essentially inactive in this mouse catalepsy model at 10 mg/kg. However, upon methylation of the NH group, as in **13**, oral



Scheme 3. Reagents and conditions: (a) 1-(2,4-difluorophenyl)-4-prop-2-ynyl-piperazine, $Pd(PPh_3)_4$, CuI, Et_3N, PPh_3, DMF, 110 °C, 12 h; (b) H_2, 1 atm, 10% Pd/C, MeOH, rt.

Table 3. Mouse catalepsy data

Compds	Active dose (p.o.), mg/kg
7	>10
13	3
11	10
14	3
16	>10
18	3

For the mouse catalepsy study, CD-1 mice (25–30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. Test compounds, formulated as the hydrochloride salt, were dissolved in saline and administered by oral gavage. More comprehensive details regarding the mouse catalepsy model can be found in Refs. 12,17.

activity was observed at 3 mg/kg. Likewise, the 2,4-difluoro derivative **11** was orally active at 10 mg/kg in the mouse catalepsy model. Methylating the NH group increased the in vivo activity and compound **14** showed oral activity at 3 mg/kg. This same trend of enhanced oral activity with the methylated derivative was also shown with the triazolopyrimidine derivatives **16** and **18**. Compound **18** showed significant oral activity at 3 mg/kg, whereas the unmethylated analog **16** was essentially inactive at 10 mg/kg.

In summary, we have demonstrated that a diamino ethylene spacer could be inserted between the piperazine group and the core heterocyclic template. The triazolotriazine or the related triazolopyrimidine core all afforded potent and selective adenosine A_{2a} receptor antagonists. Orally active leads such as **14** and **17** are being evaluated more thoroughly for their pharmacological properties and the results will be discussed in more detail separately.

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