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Synthesis of alkyne derivatives of a novel triazolopyrazine as A_{2A} adenosine receptor antagonists

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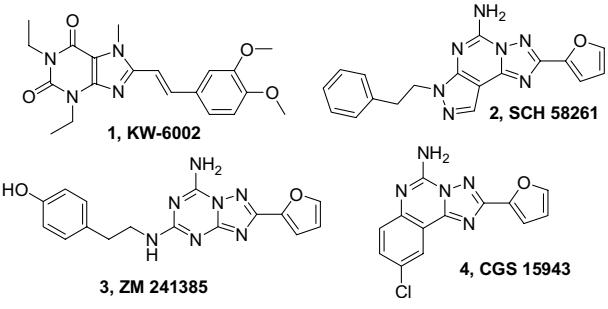
Abstract—A novel [1,2,4]triazolo[1,5-*a*]pyrazine core was synthesized and coupled with terminal acetylenes. The structure–activity relationship of the alkynes from this novel template was studied for their in vitro and in vivo adenosine A_{2A} receptor antagonism. Selected compounds from this series were shown to have potent in vitro and in vivo activities against adenosine A_{2A} receptor. Compound **12**, in particular, was found to be orally active at 3 mg/kg in both a mouse catalepsy model and a 6-hydroxydopamine-lesioned rat model.

Adenosine modulates physiological functions acting via specific cell surface receptors. Four subtype adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been identified, all of which belong to a family of seven *trans*-membrane G-protein coupled receptors (GPCRs).[1,2] The adenosine receptors are known to be associated with different second messenger systems: A_1 and A_3 mediate adenylate cyclase inhibition, whereas A_{2A} and A_{2B} stimulate the adenylate cyclase activity controlling intracellular cyclic-AMP level.[3] In recent years, the A_{2A} receptor has emerged as an attractive target for Parkinson's disease therapy, primarily because of its localized expression in striatum and motor enhancement function.[4] Recent genetic and pharmacological studies indicate that A_{2A} receptor antagonists also offer neuroprotective effects and may possibly modify chronic L-dopa-induced maladaptive responses in animal models of Parkinson's disease.[5]

Over the past few years, there has been extensive work to synthesize novel compounds, either xanthine or nonxanthine, interacting selectively with the A_{2A} adenosine receptor.[6] KW-6002 (**1**), a xanthine-based adenosine A_{2A} receptor antagonist, is currently in phase 2 clinical trial for the treatment of Parkinson's disease.[7] Potent and selective nonxanthine-based adenosine A_{2A} receptor antagonists such as SCH 58261 (**2**),[8] ZM 241385 (**3**)[9] and CGS-15943 (**4**)[10] have also been reported.

Efforts in our laboratory to develop nonxanthine-based A_{2A} adenosine receptor antagonists have led to the recent disclosure of several triazolo triazine and triazolo pyrimidine series as potent and selective A_{2A} adenosine receptor antagonists.[11] Harada et al. recently reported 2-alkynyl-8-aryladenine derivatives as adenosine antagonists.[12] In our effort to synthesize a novel class of adenosine A_{2A} receptor antagonist, we were interested in making the alkyne derivatives of the [1,2,4]triazolo[1,5-*a*]pyrazine core and evaluate them for the in vitro and in vivo adenosine A_{2A} receptor antagonism.



Keywords: A_{2A} antagonists; Parkinson's disease; Triazolopyrazine.
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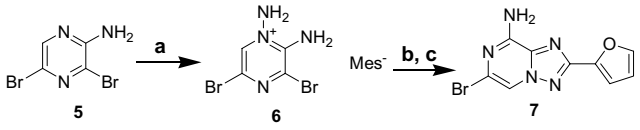
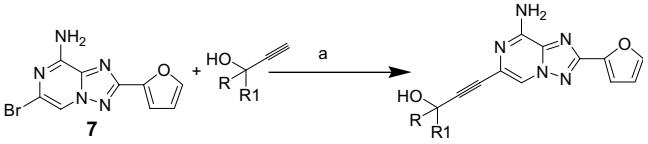


Scheme 1. Reagents and conditions: (a) *t*-butyl O-mesitylene carbamate, TFA, rt, 20 h, 73%; (b) MeOH, 70 °C, 5 h, furan-2-carbaldehyde, 90%; (c) NH_3, dioxane, rt, 45%.



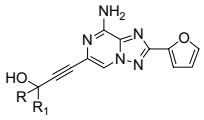
Scheme 2. General synthesis of hydroxy acetylene compounds. Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 100 °C.

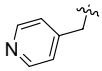
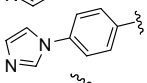
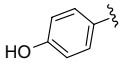
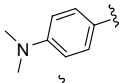
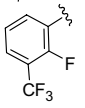
The key intermediate **7** (6-bromo-2-furan-2-yl-[1,2,4]-triazolo[1,5-*a*]pyrazin-8-ylamine) was prepared as shown in Scheme 1. Thus, treatment of 2-amino-3,5-dibromopyrazine[13] in methylene chloride with in situ generated aminating agent O-mesitylenesulfonylhydroxylamine[14] formed a pyrazinium salt **6**. This pyrazinium was condensed with 5 equiv of 2-furaldehyde in methanol to give an imine, which upon treatment with anhydrous ammonia in dioxane, gave the key intermediate bromo-triazolo[1,5-*a*]pyrazine **7** in an overall yield of 29%.

Substituted propargyl alcohols were coupled to **7** via the Sonogashira reaction (Scheme 2). The starting propargyl alcohols were either purchased from commercial sources or prepared from the corresponding aldehydes or ketones by the addition of ethynylmagnesium bromide in THF.[15]

The in vitro results against adenosine A_{2A} receptor are shown in Table 1. As a preliminary evaluation of selectivity, the affinities against adenosine A_1 receptor were also determined and are shown in Table 1. No effort was made at this stage to evaluate the selectivity of these compounds against the other two subtypes of adenosine receptor (A_{2B} and A_3), even though there is a possibility for this class of compounds to inhibit A_{2B} adenosine receptor as well.[12] Many of the tested compounds have affinity at A_{2A} receptor in the low nanomolar range. However, selectivity verses A_1 is still an issue with this class of compounds. Results from binding studies showed that: (a) the presence of alkyl or aryl groups on the terminus of the acetylene side chain is clearly beneficial for the adenosine A_{2A} receptor affinity, in view of the poor activity of **11**. Compounds **8**, **9**, **10**, **15**, and **24** all showed single-digit nanomolar potency, with compound **15** being most potent (A_{2A}, 1.1 nM) and selective (A_1/A_{2A} = 91). Propargyl alcohol gives only an inactive compound **11**; (b) The presence of a second substituent on the terminus of the acetylene (**12**, **16**, **17**, **18**, and **20**) can also be beneficial, especially when R_1 is a methyl group. However, the activity decreases dramatically when the methyl group is replaced with either hydrogen

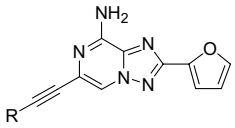
Table 1.

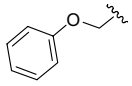
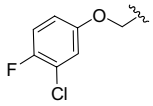
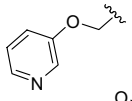
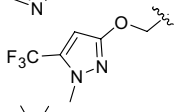
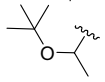
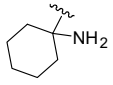
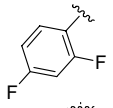


Compd	R	R_1	Binding assays K_i (nM)		Selectivity A_1/A_{2A}
			A_{2A}	A_1	
8		Me	3.7	24	6.5
9			7	8	1.1
10			9	33	3.7
11	H	H	>500	>500	N/A
12		Me	12	41	3.4
13		H	340	2400	7
14		CF_3	74	2200	30
15		Me	1.1	100	91
16		Me	25	170	7
17		Me	33	110	3.3
18		Me	33	340	10
19		H	260	73	0.28
20		Me	42	66	1.6
21		CF_3	1100	N/A	N/A
22		Me	1600	N/A	N/A
23			30	72	2.4
24			7.3	11	1.5
25			12	2.5	0.2

K_i values were calculated from binding curves generated from the mean of three determinations per concentration, with variation in individual values of <15%. For the A_{2A} receptor, membranes were prepared from rat brain tissues and the radioligand binding assay was performed using the radioligand [^{3}H]ZM-241385 according to standard radioligand binding procedures.[16a,b] For the A_1 receptor, membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using the radioligand [^{3}H]DPCPX.[16c] As a positive 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.[16d]

Table 2.



Compd	R	Binding assay K_i (nM)		Selectivity A_1/A_{2A}
		A_{2A}	A_1	
26		390	530	1.4
27		310	4300	14
28		92	110	1.2
29		89	1800	20
30		37	96	2.6
31		210	35	0.17
32		>1000	N/A	N/A
33		280	680	2.5

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

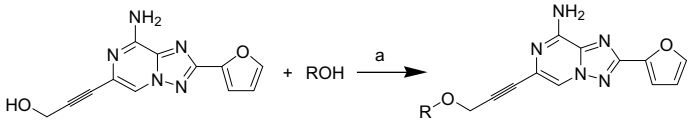
or CF_3 group (**13**, **14**, **19**, and **21**). Another observation is that when R equals *p*-(dimethylamino)phenyl, a basic group, or the bulkier 2-fluoro-3-(trifluoromethyl)phenyl, activity in the corresponding analogs (**21**, **22**) is totally lost. However, a phenol (**20**) is well tolerated. It is also noteworthy that compound **15**, which has a methylene spacer on the pyridyl group, is 10-fold more potent than **16**, which has a 4-pyridyl group directly attached to the terminus of the acetylene. Compounds **23**, **24**, and **25** showed that propargyl alcohols with cyclic alkyl substituents on the allylic position behave similarly to propargyl alcohols with noncyclic alkyl substituents in adenosine A_{2A} receptor binding affinity and selectivity.

In addition to the propargyl alcohols, compounds without the hydroxy group were also evaluated, as shown in Table 2. Ethers were derived from the propargyl alcohol via Mitsunobu reaction (Scheme 3). The ethers showed moderate A_{2A} activity. The *tert*-butyl ether **30** containing a methyl group on the terminus of the acetylene side chain was the most potent with 37 nM A_{2A} binding affinity and a relative A_1/A_{2A} selectivity of 2.6-fold. It is noteworthy that ether **29** not only had good A_{2A} activity, but it also showed 20-fold A_1/A_{2A} selectivity. Propargyl amine **31** exhibited much lower binding affinity than its alcohol counterpart **24** to adenosine A_{2A} receptor and showed a reversed selectivity toward A_1 receptor.

In order to determine the importance of the hydroxy group, we also made examples that contain either an alkyl group or an aryl group capping the alkyne (**32**, **33**). In general, they are much less potent that the propargyl alcohols. In general it was found that the presence of both a hydroxy and an alkyl group on the terminus of the acetylene side chain is essential for obtaining good in vitro adenosine A_{2A} receptor affinity.

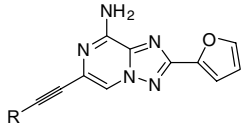
To evaluate the in vivo activity of these alkynyl derivates of [1,2,4]triazolo[1,5-*a*]pyrazine, the mouse catalepsy model was used. This is a widely used rodent model for Parkinson's disease where catalepsy (i.e., immobility) is induced by subcutaneous injection with haloperidol (3 mg/kg).[17a,b] In addition, we also made use of a rat catalepsy model. This type of rat study also allowed us to correlate the in vivo activity with the in vitro binding data, which were obtained using rat membranes. As a third measurement of efficacy, we employed the 6-hydroxydopamine-lesioned rat model.[18] This is a frequently used rodent model for Parkinson's disease in which efficacy is defined as the ability to potentiate L-dopa-induced turning behavior. In this model the MED (minimum dose that caused a significant increase in rotational behavior) for the clinical candidate KW-6002 was determined to be 3 mg/kg po. Seven compounds from Table 1 were tested for oral activity in this rodent model of Parkinson's disease and the results are summarized in Table 3. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Most of the alkyl and aryl substituted triazolo[1,5-*a*]pyrazin propargyl alcohols showed good oral activity in the mouse catalepsy model at 3 mg/kg, with the exception of **14**, which has a trifluoromethyl group on the terminus position of the acetylene side chain. Compounds **10**, **12**, **23**, and **24** also exhibited oral activity in the rat catalepsy model at 3 mg/kg, while **10** is not active in the rat catalepsy model at 3 mg/kg. Of these active compounds,



Scheme 3. Reagents and conditions: (a) DIAD, PPh_3, THF, rt.

Table 3. Summary of efficacy data in rodent models of Parkinson's disease[a]



Compd	Mouse catalepsy PO (ED_{50}) (mg/kg)	Rat catalepsy PO (ED_{50}) (mg/kg)	Rat 6-hydroxydopamine PO (MED) (mg/kg)
8	3	>3	NT
9	3	NT	NT
10	3	3	NT
12	3	3	3
14	>3	NT	NT
23	3	3	>10
24	10	NT	NT
KW-6002	1	1	3

[a] For the mouse catalepsy study, male CD-1 mice (25–30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. For the rat catalepsy study, male Sprague–Dawley rats (225–275 g) were treated with haloperidol (1 mg/kg sc) in order to induce catalepsy. ED_{50} refers to the minimum dose that causes at least a 50% reduction in catalepsy. For the 6-hydroxydopamine studies, male Sprague–Dawley rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway were given test compound 30 min before a subthreshold dose of L-dopa (3.7 mg/kg ip), and rotational behavior was measured for 2 h. MED refers to the minimum dose that caused a significant increase in rotational behavior. In all of these studies, test compounds, formulated as the hydrochloride salts, were dissolved in saline and administered by oral gavage. Details regarding the mouse catalepsy model can be found in Refs. 11e,17a,b.

12 and **23** were further tested in the 6-hydroxydopamine rat model. Among them, compound **12** is particularly promising, showing oral activity at 3 mg/kg. In contrast, compound **23**, which has a cyclo alkyl substituent, did not exhibit activity at 3 mg/kg.

In summary, we have demonstrated for the first time that 2-furan-2-yl-[1,2,4]triazolo[1,5-*a*]pyrazin-8-ylamine core, when coupled with branched propargyl alcohols, can afford potent adenosine A_{2A} receptor antagonists with both in vitro and in vivo activities. Selected compounds from this series have been shown to be orally active in three different rodent models of Parkinson's disease (e.g., compound **12**).

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15. Typical synthetic procedure for **16** and analogs thereof: A solution of 4-acetylpyridine (1 equiv) in dry THF under argon was cooled to 0 °C with an ice bath. Ethynylmagnesium chloride (2.5 equiv) was added dropwise, and the solution was stirred at room temperature for 1–3 h. The reaction was quenched by the addition of a saturated ammonium chloride solution in water, and the THF was evaporated in vacuo. The residue was then extracted with ethyl acetate and the combined organic extracts were dried over magnesium sulfate, and concentrated in vacuo to yield the desired propargyl alcohol, which was used in the coupling reaction without further purification.
To a solution of 6-bromo-2-furan-2-yl-1,2,4triazolo[1,5-*a*]pyrazin-8-ylamine (1 equiv) and the above prepared alkyne (2 equiv) in a 1:1 mixture of DMF and TEA were added copper(I) iodide (20 mol %) and palladium (0) catalyst (15 mol %). The reaction vessel was degassed and heated at a 100 °C for 6 h. After cooling, water was added and the residue was extracted with ethyl acetate. The

combined organic extracts were dried over magnesium sulfate, and concentrated in vacuo to yield a crude product, which was purified using preparative HPLC to afford the desired product **8**. ^{1}H NMR (MeOH-d_4) δ 8.84 (br s, 2H), 8.31 (d, J = 6.0 Hz, 2H), 8.29 (s, 1H), 7.74 (d, J = 1.5 Hz, 1H), 7.19 (d, J = 3.3 Hz, 1H), 6.65 (dd, J = 3.3 Hz, 1.5 Hz, 1H), 1.92 (s, 3H) ppm. MS m/z = 347 amu (M^+ + H).

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