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Synthesis and SAR evaluation of 1,2,4-triazoles as A_{2A} receptor antagonists

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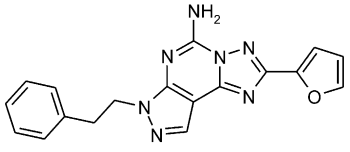
Abstract—The synthesis and in vitro structure–activity relationships (SAR) of a series of triazoles as A_{2A} receptor antagonists is reported. This resulted in the identification of potent, selective and permeable 1,2,4-triazoles such as **42** for further optimization and evaluation in vivo.

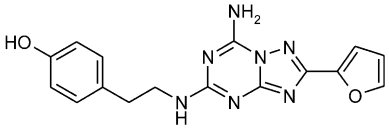
The neurotransmitter adenosine interacts with four G-protein coupled receptors the A_1, A_{2A}, A_{2B} and A_3 receptors.[1] The A_{2A} receptor, which is Gs coupled, is of particular interest in the area of psychiatry research with high receptor densities found to be located in the dopamine-rich areas of the striatum and nucleus accumbens.[2] More recently A_{2A} receptors have been shown to be co-localized and interact with both dopamine D_2 and metabotropic glutamate 5 ($mGlu_5$) receptors.[3] Thus, antagonism of the A_{2A} receptor may provide a subtle means of modulating the dopaminergic system, without the associated motor side effects of direct D_2 receptor interaction. Furthermore, animal behavioral studies have revealed the A_{2A} receptor as a potential new target for the development of antidepressants[4] and anti-Parkinsonian treatments.[5] Several drug canditates, which all contain a distinct purine motif, are currently being evaluated in the hope to exploit this receptor target.

These compounds suffer from a variety of drawbacks that call for the development of new classes of ligands for the A_{2A} receptor, due to the following: (a) the presence of the 2-mono-substituted furan moiety in **1** and **2** which carries metabolic liability, but is required for both high affinity and selectivity; (b) the styryl moiety in **3** is highly susceptible to photoisomerization, wherein the thermodynamically more stable *cis* photoisomer is almost 1000 times weaker in the rat A_{2a} [rA_{2a}] binding assay; finally (c) poly-annulated xanthinic and tricyclic structures invariably suffer from poor physiochemical characteristics (i.e., solubility, lipophilicity) that provide the opportunity to develop compounds with improved properties.



SCH 58261

1



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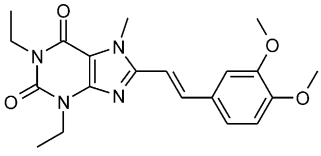

KW-6002

3

Keywords: 1,2,4-Triazoles; A2A receptor antagonists; SAR.
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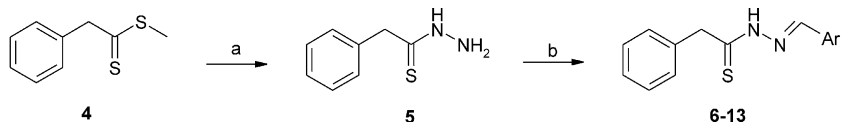


Scheme 1. (a) Hydrazine, EtOH, Δ, 73%; (b) ArCHO, EtOH, 55–89%.

In order to identify and develop novel, biologically active compounds in the A_2 receptor class, we chose to approach this task in two ways; (i) through an HTS screening campaign and (ii) de novo design based on a ligand derived pharmacophore model. The later approach will be described in more detail elsewhere.

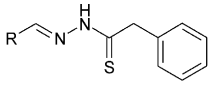
Screening the Roche compound depository using a scintilation proximity assay with [^{3}H]SCH-58261 resulted among other compounds in the identification of a series of thioacylhydrazones (**7**, **12**) as validated hits.[6] We were aware of the metabolic liabilities of such compounds but viewed this as a tractable entry point for further investigation of the binding and selectivity requirements (SAR), thus adding to our knowledge of the A_{2A} pharmacophore. In the initial synthetic approach to thioacylhydrazones **6**–**13**, we varied the appended aryl group, by construction of the thioacylhydrazide **5** from **4**, followed by condensation with a variety of aldehydes to afford the respective hydrazones (Scheme 1). By analysis of the first small array we uncovered some important findings: (a) geometry of the hydrazone double bond was labile, isomerizing at room temperature; and (b) there was a surprisingly large range of radioligand binding affinity (1000-fold) for these compounds despite rather conservative variations (i.e., **8** vs **12**), which could not be easily rationalized (Table 1). Steric bulk was not well tolerated as demonstrated by the loss in binding affinity with compounds **15** versus **8** and **9**; and **6** versus **13**.

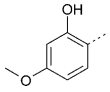
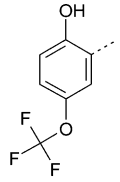
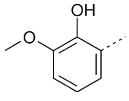
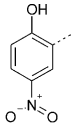
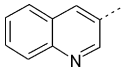
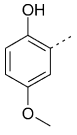
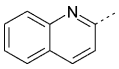
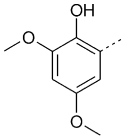
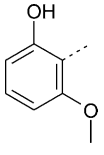
In order to address the problem with isomerization about the hydrazone double-bond, *N*-acyl dihydropyrazoles **19** was synthesized but was found to be completely inactive ($K_i > 5.2$ μM). Synthesis of the oxygen variant of thioacylhydrazone **20** eliminated the metabolically unstable thiocarbonyl and showed no isomerization, but unfortunately it was also inactive. One explanation for this finding might be the requirement for a certain minimal NH acidity, since there is considerable difference in the pK_a (6.6) **8** compared to the oxo analogue pK_a (2.9) **20**. In order to investigate this possibility, the sulfone derivative **18** (pK_a 6.5) was prepared but found to be inactive.

Due to these findings we decided to terminate this series of compounds. Since there exists a number of surrogates of the amide and hydrazide moiety, amongst them the triazole is described, our attention was drawn to a small series of modestly active biaryl 1,2,4-triazole hits **21**–**23** bearing a basic *meta* side-chain or an *ortho* phenolic moiety reminiscent of the thiohydrazides. Their profile was considered promising with regard to affinity, selectivity, stability and chemical tractibility. Interestingly, compound **21** had the best affinity despite the lack of an acidic NH. This suggested that the triazoles may offer an alternative binding mode or an additional exit vector that could be exploited.

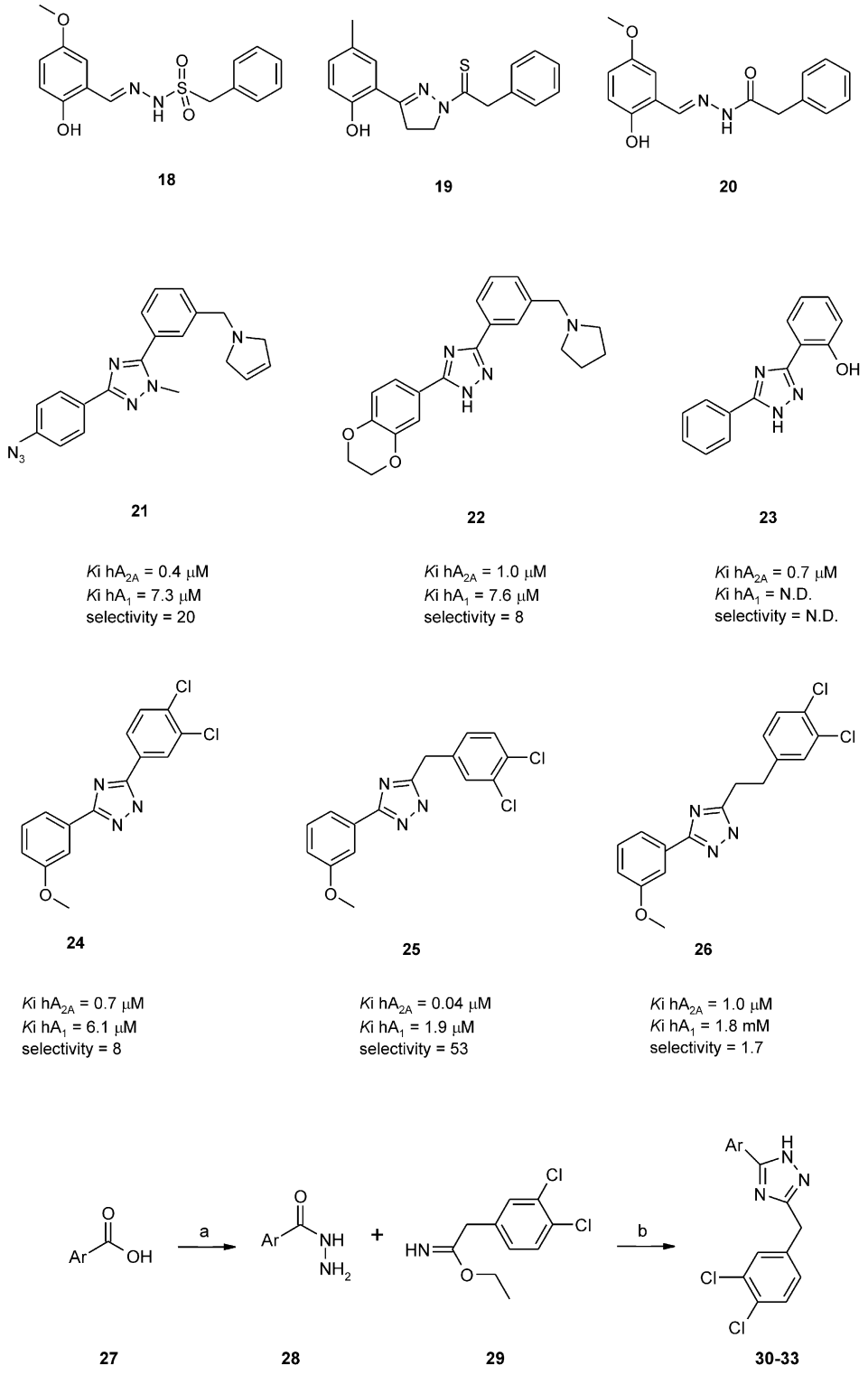
Despite the attraction of a terminal polar function for solubility purposes, we chose to remove this in order to

Table 1. Human recombinant A_{2A} [hA_{2A}] receptor affinities of compounds **6**–**13** [variation of R], determined by displacement of [^{3}H]SCH-58261 radioligand binding[7]



Compd	R	K_i [μM][a]	Compd	R	K_i [μM][a]	Compd	R	K_i [μM][a]	Compd	R	K_i [μM][a]
6		0.005	**9**		0.1	**12**		1.4	**15**		4.7
7		0.01	**10**		0.1	**13**		2.0	**16**		>5.2
8		0.02	**11**		1.2	**14**		4.2	**17**		>5.2

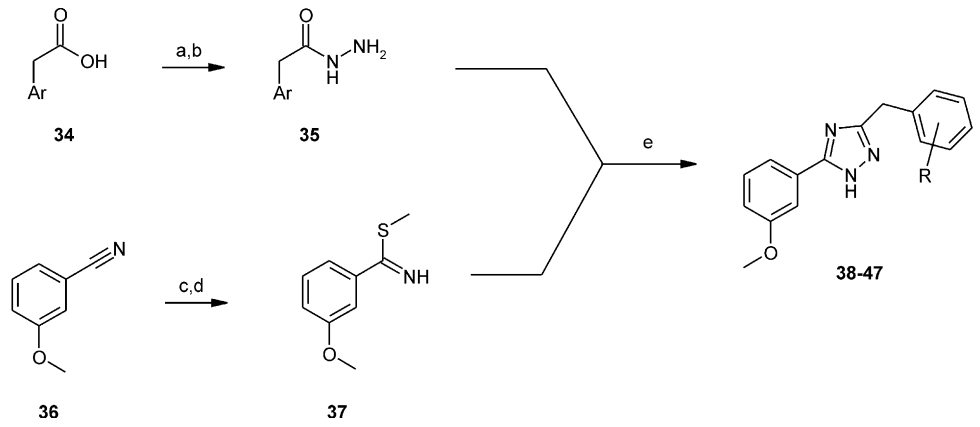
[a] Binding affinities are quoted as K_i values and are the mean of at least two experiments.



Scheme 2. Route to **30–33** via acyl hydrazide; Reagents and conditions: (a) CDI, THF, reflux, 82–93%; (b) EtOH, reflux, 17–97%.

avoid introducing an amphiphilic vector resulting in potential phospholipidosis. It was rapidly discovered that significant binding affinity could be gained by simply introducing a spacer atom between the triazole core and the aryl side chain in **24**, however this effect was sensitive to further elongation **26** or substitution and the optimum was found with a methylene linker **25**.

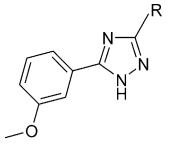
With these preliminary findings, a second focused array was built around the benzyl, aryl substituted triazoles **30–33** and was constructed via condensation of the acylhydrazide **28** with an ethyl benzylimidate **29** in refluxing ethanol, providing the triazole, Scheme 2. The starting acylhydrazide was obtained from the acid **27** with hydrazine using standard coupling conditions with CDI which consistently provided high yields.

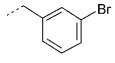
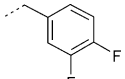
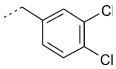
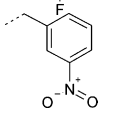
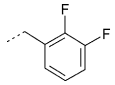
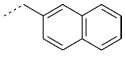
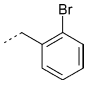
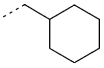
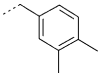
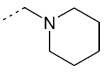


Scheme 3. Route to **38–47** via thioimidate; Reagents and conditions: (a) CDI, THF, reflux; (b) Hydrazine, 80–96% over two steps; (c) NaSH, NH_4Cl, MeOH, 98%; (d) MeI, acetone, 89%; (e) EtOH, reflux, 60–75%.

The results revealed that the *meta*-methoxy group on the aryl ring is essential for good A_{2A} receptor binding. This finding prompted us to concentrate on the benzyl portion of the triazoles and that required a change in the synthetic approach to allow expedient synthesis of the second-generation compounds for evaluation. The thioimidate **37** could be accessed by addition of sodium sulfide to 3-methoxy benzonitrile **36** followed by *S*-methylation of the resulting thioamide using methyl-iodide in acetone. Coupling of the thioimidate **37** with the respective acylhydrazide **35**, provided our second generation focused library of 3-*m*-methoxyphenyl-5-benzyl triazoles **38–47** (Scheme 3).

Table 2. hA_{2A} receptor affinities of compounds **38–47** [variation of R], determined by displacement of [^{3}H]SCH-58261 radioligand binding[7]



Compd	R_2	K_i [μM][a]	Compd	R_2	K_i [μM][a]
38		0.01	**43**		0.07
39		0.04	**44**		0.08
40		0.02	**45**		0.1
41		0.02	**46**		0.7
42		0.02	**47**		> 5.2

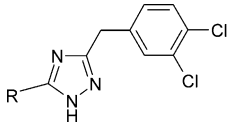
[a] Binding affinities are quoted as K_i values and are the mean of at least two experiments.

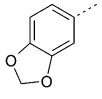
The intial SAR showed that the *m*-methoxyphenyl moiety was optimal with very little variation permitted. The benzyl moiety provided more scope for variation provided substituents are hydrophobic in nature but introduction of a polar group **47** dramatically abolishes binding activity (Tables 2 and 3).

A set of the active compounds **38**, **40**, **42**, **45** were further profiled for their selectivity against the human A_1 [hA_1] receptor as well as their activity in the rat A_{2A} receptor binding. We observed moderate selectivities as well as sufficient activity at the rat receptor to be considered for testing in the animal models. Additionally, these compounds showed good to medium permeability in the PAMPA assay and satisfactory solubility (2–20 μg/mL)[9] (Table 4).

In summary, we have identified a series of triazoles that show significant activity at the A_{2A} receptor and display attractive physiochemical properties compared to **1–3**. We are currently working towards refining these compounds and analyzing their in vivo activity and will report these results in due course.

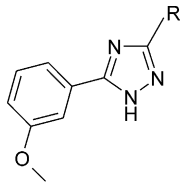
Table 3. hA_{2A} receptor affinities of compounds **30–33** [variation of R], determined by displacement of [^{3}H]SCH-58261 radioligand binding[7]

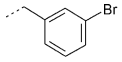
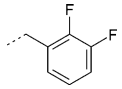
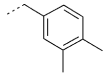
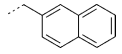


Compd	R	K_i [μM][a]	Compd	R	K_i [μM][a]
30		0.06	**32**		0.1
31		0.09	**33**		> 5.2

[a] Binding affinities are quoted as K_i values and are the mean of at least two experiments.

Table 4. Recombinant hA_{2A}, rA_{2A} and hA_1 receptor affinities of compounds **38**, **40**, **42**, **45** [variation of R], determined by displacement of [^{3}H]SCH-58261 [A_{2A}] or [^{3}H] DPCPX [A_1] radioligand binding[7]



Compd	R	hA_{2A} K_i [μM][a]	hA_1 K_i [μM][a]	sel.	rA_{2A} K_i [μM][a]	PAMPA[8] 10^{-6} s/cm
38		0.01	0.1	7	0.04	1.9 (high)
40		0.02	0.9	51	0.04	3.8 (high)
42		0.02	1.7	69	0.09	0.9 (high)
45		0.1	2.5	20	0.4	0.3 (med.)

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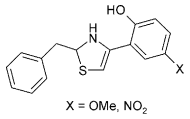
5. Richardson, P. J.; Kase, H.; Jenner, P. G. *Trends Pharmacol. Sci*. **1997**, *18*, 338.
6. The original hit was identified as the cyclic version and was later confirmed to be the open chain form.



7. $hA2_A$, hA_1 and rA_{2A} receptor affinities were determined in a 96 well plate assay, using [^{3}H]SCH-58261 (hA_{2A} and rA_{2A}; final concentration 1 and 0.4 nM, respectively) and [^{3}H] DPCPX (final concentration 0.6 nM) to radiolabel the receptors in the presence of 10 concentrations of competing compound or buffer. Non-specific binding was determined using 2 μM Xanthine amine congener (XAC). Assay buffer consisted of Tris–HCl (50 mM, pH 7.4), NaCl 120 mM, KCl 5 mM, $CaCl_2{\cdot}2H_2O$ 2 mM, $MgCl_2{\cdot}6H_2O$ 10 mM. Membrane preparations of receptors (approximately 2 μg/well in a 96-well plate) were mixed with adenosine deaminase (10 μg/mL) and scintillation proximity beads (SPA; 0.5 mg/well; Amersham) and added to the wells to initiate the incubation for 60 min (hA_{2A} and hA_1) or 90 min (rA_{2A}) at room temperature. The assay was terminated by centrifugation (3000 rpm, 3 min) and counted in a scintillation counter (Topcount, Packard). All assays were performed in duplicate in at least two separate experiments. Graphs were plotted with the % specific binding using the iterative curve fitting program XL-fit.
8. PAMPA = Parallel Artificial Membrane Permeation Assay: Kansy, M.; Senner, F.; Gubernator, K. *J. Med. Chem*. **1998**, *41*, 1007.
9. Solubility determined in 0.05 M phosphate buffer at pH 6.5.