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Identification of non-furan containing A_{2A} antagonists using database mining and molecular similarity approaches

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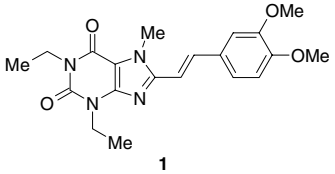
Abstract—Database searching led to the identification of potent A_{2A} antagonists which do not contain the privileged furan moiety and which show selectivity over A_1 receptors. Simple substructure searching on a proprietary database identified compounds with activities in the low nM range. A targeted approach to the identification of non-furan containing compounds resulted in the identification of two novel series, with potency, selectivity and directional SAR from screening 113 compounds.

Adenosine receptors comprise a family of G-protein coupled receptors consisting of four members: A_1, A_{2A}, A_{2B}, and A_3 receptors. This group of receptors belong to family one of the class A, rhodopsin-like subgroup of GPCRs, as indicated through sequence alignment.[1] A_{2A} is involved, via influence on cyclic AMP levels, in the regulation of the functional activity of D_2 dopamine receptors,[2] and antagonism of the A_{2A} receptor has been shown to be efficacious in relieving the symptoms in models of Parkinson's disease (PD). Several A_{2A} antagonists are now in clinical trials for the treatment of PD, for example, V2006[3] and KW-6002 (**1**),[4] the latter having a K_i of 35 nM against A_{2A} and 2.8 μM against A_1 when assayed in-house.



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Whilst a number of chemical classes are known to target the A_{2A} receptor, these can be broadly divided into xanthine and non-xanthine series. Further exploration of xanthine-related compounds is unattractive because of their poor solubility, issues with regard to drug-likeness and the wealth of prior art which already exists in this area. With the exception of the Roche series, for example, **2**,[5] and the recently reported 1,8-naphthyridines, for example, **3**,[6] the majority of non-xanthine A_{2A} antagonists discovered to date (e.g., triazolotriazine **4**[7]) bear a furan moiety off the core aromatic scaffold. The prevalence of this heterocycle suggested that it may be important in achieving compounds with good potency, selectivity and in vivo A_{2A} antagonist efficacy. Early in-house work also supported the notion that the furan moiety comprised a privileged fragment. Although Broughton et al.[8] noted that unsubstituted furans are present in some molecules which progress through clinical trials, it has been suggested that this group may sometimes carry a potential safety liability since, in some molecules, it is prone to oxidative metabolism which can lead to the formation of reactive species capable of forming covalent adducts. Therefore, additional studies are needed in order to progress a furan-containing compound to market. Whilst one possible strategy is to block the 2-position of the furan ring, with a view to reducing the ring-opening potential, it is also of interest to identify new series of A_{2A} antagonists which do not contain this moiety.

Keywords: A_{2A}; Database mining; Molecular similarity; Non-furan; Pharmacophore.
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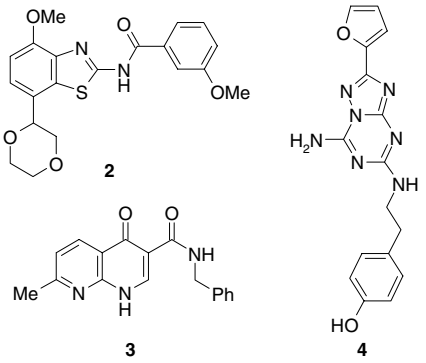


This work focuses on two strategies used to identify novel chemical series for investigation as potential A_{2A} antagonists. The first study comprised a simple substructure search which identified a series of highly potent molecules. The second study, on a larger database where simple substructure searching produced too many hits, aimed to select a small number of compounds for screening, using a targeted furan/non-furan approach. In this work, furan-containing molecules matching a substructure of interest were selected and screened, and then closely related non-furan containing near neighbours of the hits were assayed.

Analysis of public[7] and proprietary data[9–14] on A_{2A} antagonists showed that a number of chemical classes were known where the core scaffold was bicyclic, but contained a common pyrimidine or triazine substructure. In order to generalise from this, and to identify new scaffolds, the query shown in Figure 1 was developed and used to carry out a substructure search on a proprietary compound database in ISIS/Base format.[15–17]

Compound sets matching these substructures were combined and filtered according to drug-likeness criteria,[18] and 100 compounds were selected for screening against A_{2A}.[19] Nine compounds exhibited >50% inhibition at 1 μM and three, all of which were triazines, had K_i values ranging from 5 to 31 nM against the human A_{2A} receptor, as well as showing a degree of selectivity over A_1[19] (Table 1).

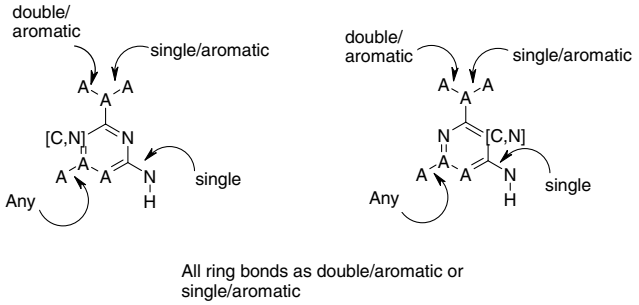
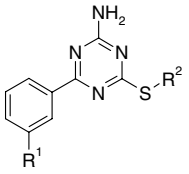
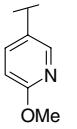
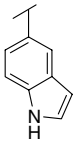


Figure 1. ISIS queries used to search a proprietary compound database.

Table 1. A_{2A} and A_1 binding affinities and selectivity data for triazines **5–7** identified from substructure search



Compound	R^1	R^2	A_{2A} K_i (nM)	A_1 K_i (nM)	A_1 K_i/A_{2A} K_i
5	H	Et	5	67	13
6		Me	23	840	36
7		Me	31	218	7

Radioligand binding assay using recombinant receptors.[19]

Compound **5** also demonstrated over 200-fold selectivity for A_{2A} over A_{2B} (unpublished results).

Subsequent IP searching identified a patent claiming triazines as A_{2A} antagonists.[20] Whilst this patent does cover closely related compounds, detailed analysis showed that compounds **5–7** lay outside of its scope.

Whilst it was of interest to apply a similar approach to identify a wider range of compounds for purchase, a search of rCat,[21] a proprietary database of commercially available screening samples, yielded too many hits for this approach to be tractable. Therefore, a second strategy was developed so as to strictly focus on the importance of the furan and the identification of tolerated replacements. This method involved a number of steps, outlined as follows.

1. Identification of compounds in the rCat ISIS database[15,21] matching the substructure queries (Fig. 2). These were then filtered to remove those with unwanted chemical functionality[18] and poor physicochemical properties with regard to drug-like space.[19]

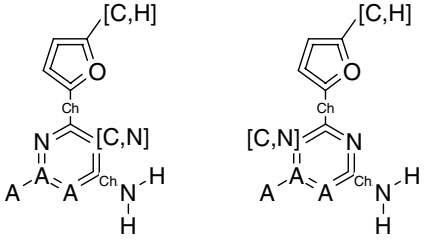
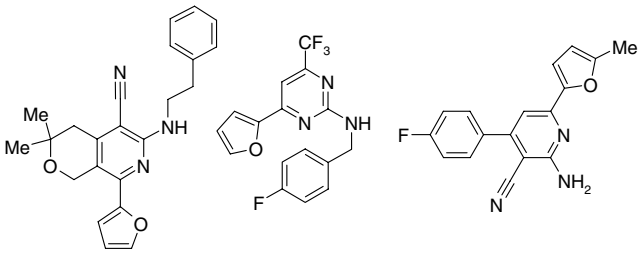


Figure 2. Substructure queries for identification of compounds in the rCat ISIS database.

2. Combination of hitsets and removal of the furan or methylfuran moiety using custom R-group clipping in SVL.[22]
3. Use of the resulting substructures to search a drug-like subset of rCat in smiles format. This was achieved using the Dayperl functionality,[23] shell scripts and smarts matching.[23]
4. Visual inspection of resulting compounds using the ISIS interface,[15] and manual filtering to remove any further unwanted functionality.

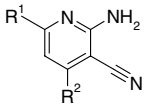
This strategy allowed the identification of a subset of compounds for which both furan and closely related non-furan analogues were commercially available. From this set, 34 furan-containing compounds were purchased and assayed. Of these, 14 showed >50% inhibition at 1 μM (a 41% hit rate) against the human A_{2A} receptor and five of these showed >70% inhibition. These structures broadly fell into three chemical series of pyrano[3,4-*c*]pyridines, 2-aminopyrimidines and pyridines, as illustrated by representative compounds **8**, **9**, and **10**.

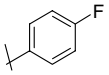
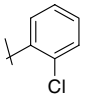
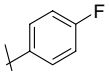
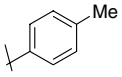
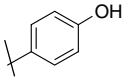
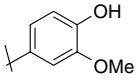
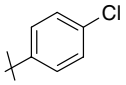
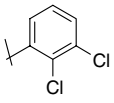
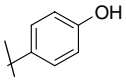
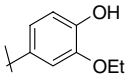
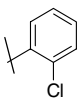
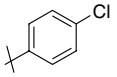


8 **9** **10**

The non-furan analogues of these compounds were then purchased and assayed. Of the 51 rCat-derived compounds that were screened, 17 had >50% inhibition at 1 μM, and these hits fell into two chemical families. In addition, screening 28 in-house analogues gave a further three hits, giving a total hit rate for this step of 25%. Fourteen of the 20 compounds had K_i values of <500 nM at the human A_{2A} receptor, as shown in Tables 2 and 3.

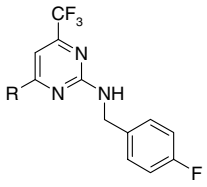
Table 2. A_{2A} and A_1 binding affinities and selectivity data for cyanoaminopyrimidines **11**–**19**

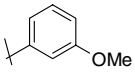
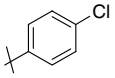


Compound	R^1	R^2	A_{2A} K_i (nM)	A_1 K_i (nM)	A_1 K_i/A_{2A} K_i
11			87	59	0.7
12			89	239	2.7
13			106	865	8.2
14			118	274	2.3
15			151	94	0.6
16			201	192	1
17			210	>10,000	
18			267	42	0.2
19			403	1744	4.3

Assayed using radioligand binding assay on recombinant receptors.[19]

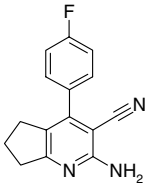
Table 3. A_{2A} and A_1 binding affinities for trifluoropyrimidines **21**–**24**



Compound	R	A_{2A} K_i (nM)	A_1 K_i (nM)
21		287	>10,000
22		287	>10,000
23		290	>10,000
24		454	>10,000

Assayed using radioligand binding assay on recombinant receptors.[19]

An additional member of this series is compound **20**, with a K_i against A_{2A} of 232 nM, and against A_1 of 1283 nM, being 5.5-fold selective.



20

Two compounds, both cyanopyridines (**11** and **12**), have sub-100 nM potency, although little selectivity. Some compounds, such as **15** and **18**, have reverse selectivity, whilst compounds such as **13** and **19** show moderate selectivity over A_1. Compound **17** is highly selective. The cyanopyrimidine data show clear evidence of directional SAR, and the compounds are drug-like in nature. The trifluoropyrimidine series (Table 3) are a little less potent but are highly selective over A_1.

As an additional aid to hit identification, a literature search was undertaken to identify compounds with good potency against A_{2A} and selectivity over A_1. Representative molecules (Fig. 3) were built in 3D and this dataset, augmented with additional in-house data (unpublished results), was then used to investigate whether common pharmacophore features could be identified.

After consideration of available SAR, it was decided to exclude xanthines, as they appeared to have distinct features and conformational preferences, which would

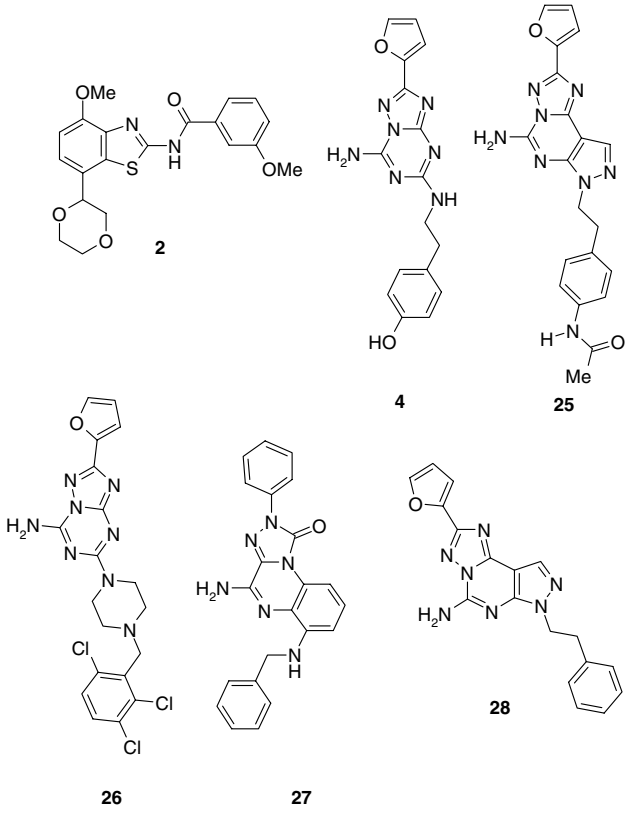


Figure 3. Literature compounds **2**,[5] **4**,[7] **25**,[24] **26**,[25] **27**[26] and **28**[27] used in the pharmacophore derivation.

suggest that they are likely to adopt a different binding mode with regard to the A_{2A} receptor.

An iterative flexible fitting approach was employed on the remaining compounds, using MOE.[22]

A consideration of the overlays (Fig. 4) showed that all molecules comprise a planar, aromatic core, with an exocyclic amine which may be monosubstituted. The absence of potent and selective compounds with a disubsti-

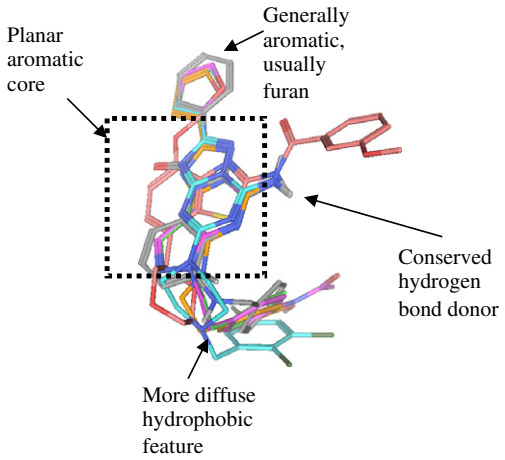


Figure 4. Overlay and common features illustrated with respect to the compounds shown in Figure 3. The conclusions drawn are general for the entire dataset considered.

tuted amine centre suggests that the polar hydrogen may be important for activity. The need for a hydrogen bond donor at this position was further supported by in-house SAR (unpublished results). The majority of compounds have a furan or other aromatic ring, which is able to sit almost co-planar to the central aromatic group, and around which little SAR is available. All molecules considered have at least two points of variation around the aromatic-amine core and, for most, this comprises a furan at the 3-position relative to the exocyclic amine, and a bulky substituent on the opposite side of the aromatic ring core from the furan. The latter, generally long and fairly flexible, side chains are disparate in character and occupy an area of space which can be best represented as a diffuse hydrophobic feature, since possible side-chain conformations sweep out a fairly large area of space and there are no conserved polar interactions with which to prioritise possible overlays in this region.

The new series identified from the rCat furan/non-furan work do appear to be compatible with the generic pharmacophore described above. The triazine series, in common with Roche compounds,[4] such as **2**, does not possess an obvious furan replacement, although given the scope for elaboration of the aryl group, it seems likely that the S-alkyl moiety is performing this function, whilst the exocyclic amine acts as the hydrogen bond donor.

In summary, molecular similarity methods, used in conjunction with databases of drug-like compounds, have been successfully employed in order to identify several novel series which bind to A_{2A} receptors, but do not contain a furan moiety. Potent and selective compounds were identified, which give rise to a variety of potential starting points for further optimization. The coupled furan/non-furan work provided a particularly effective way to identify new areas of interesting chemical space.

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19. Cell membranes expressing recombinant receptors were suspended in 50 mM Tris–HCl buffer containing 10 mM $MgCl_2$ (assay buffer). Assays were incubated in a total volume of 250 μl, containing radioligand ([^{3}H]-DPCPX (3 nM), [^{3}H]-CGS21680 (20 nM) [^{3}H]-ZM 241385 (20 nM) or [^{125}I]-AB-MECA (0.1 nM) for adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors, respectively), cell membranes, and additional drugs. Non-specific binding was determined in the presence of 100 nM DPCPX (A_1), 10 μM CGS21680 (A_{2A}), 1 μM ZM 241385 (A_{2B}), and 1 μM IB-MECA (A_3). Assays were terminated by rapid filtration.
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