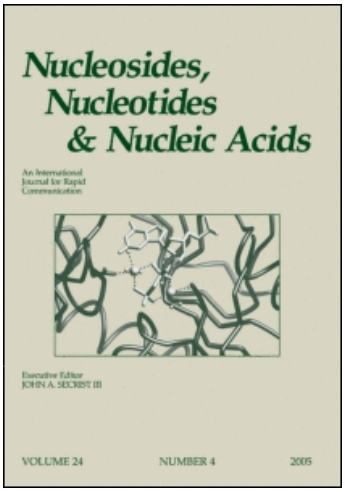

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MOLECULAR MODELLING OF THE ANTAGONIST BINDING SITE ON THE ADENOSINE A_1 RECEPTOR

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Abstract. With the aid of molecular modelling both adenosine and adenosine A_1 receptor antagonists belonging to various chemical classes were compared. A model for the antagonist binding site was developed. As a consequence 1*H*-imidazo[4,5-*c*]-quinolin-4-amines were synthesized, constituting a novel class of potent non-xanthine adenosine receptor antagonists.

INTRODUCTION

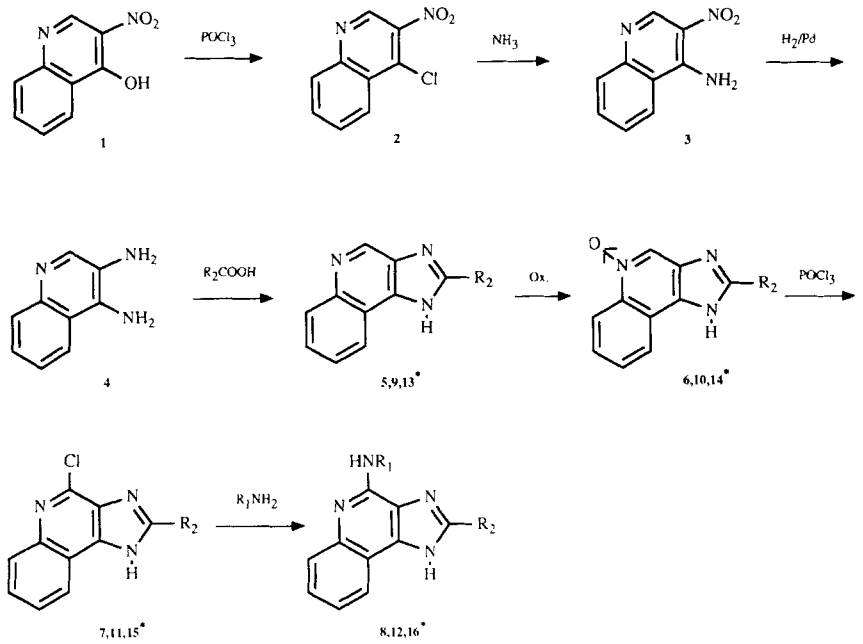
Computer-assisted molecular modelling (CAMM) is a relatively new and rapidly developing tool in drug design. Computer graphics techniques enable transformation of complex data sets obtained from theoretical chemical calculations into a picture on the computer screen. Calculated chemical structures and their properties may thus be visualized, manipulated and e.g., matched with other relevant molecules, significantly expanding the possibilities of conventional molecular models, such as Dreiding or CPK models. With regard to drug design it is often possible to delineate qualitative and quantitative features of ligands specificallly or selectively interacting with a receptor (subtype) or other biologically relevant proteins. It may help to further understand drug action and lead to a more rational approach towards drug design.

Recently, we have developed a model for the so-called N^6-region of the adenosine A_1 receptor, explaining the affinity of N^6-substituted adenosine agonists[1]. Here, we report on the molecular modelling of the antagonist binding site on the adenosine A_1 receptor. Adenosine and A_1 receptor antagonists belonging to various chemical classes were compared with respect to their minimum energy conformations and molecular electrostatic potentials (MEPs). On the basis of this modelling procedure a novel class of non-xanthine adenosine antagonists could be predicted, and was subsequently synthesized. Some of the compounds displayed nanomolar affinity for the adenosine A_1 receptor.

EXPERIMENTAL

Calculations. Studies were performed with a VAX 11/785 computer, located at the Dutch CAOS/CAMM Center in Nijmegen, and either a Visual 550 monochrome display or a Pericom MX7200 colour display. Manipulations of structures and construction of molecular electrostatic potentials were carried out with the molecular modelling software package Chem-X (July '88 update)[2]. Minimum energy conformations and charge distributions were calculated with the semi-empirical molecular orbital MOPAC program[3]. A detailed protocol of the methods followed was described by Van Galen et al.[4].

Synthesis. The synthetic route to substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amines is shown in SCHEME 1, and follows methods described by Gerster[5]. 3-Nitro-4-hydroxyquinoline (**1**) is treated with phosphorous oxychloride to afford 3-nitro-4-chloroquinoline (**2**). This is converted to 3-nitro-4-aminoquinoline (**3**) with NH_3, which is subsequently reduced by catalytic hydrogenation to 3,4-diaminoquinoline (**4**), with 5% palladium on charcoal as catalyst. The next step involves ring-closure with either formic acid, cyclopentyl carboxylic acid or benzoic acid to yield the imidazoquinolines **5**, **9** and **13**, respectively. Oxidation with 3-chloroperbenzoic acid affords the respective 5-oxides (**6**, **10** and **14**), which can be converted subsequently with phosphorous oxychloride into the 4-chlorides (**7**, **11** and **15**). Finally, reaction with the appropriate amines affords the N-substituted imidazoquinolin-4-amines (**8**, **12** and **16**). All syntheses were performed by the synthesis department of Duphar BV, Weesp, The Netherlands. Further details of the synthetic procedure are described by Van Galen et al.[6].



SCHEME 1. The synthesis of 1*H*-imidazo[4,5-*c*]quinolin-4-amines.

Receptor binding. The measurement of [^{3}H]DPCPX binding to A_1 receptors as present on rat brain membranes was performed as described by Lohse et al.[7]. [^{3}H]NECA binding to A_2 receptors of rat striatal membranes was determined as originally described by Bruns et al.[8]. IC_{50}-values were determined from pseudo-Hill plots of the displacement curves and transformed into K_i-values according to the equation described by Cheng and Prusoff [9].

RESULTS AND DISCUSSION

Since adenosine receptor antagonists such as the prototypic xanthine theophylline competitively displace radiolabelled adenosine receptor agonists, it is generally assumed that the antagonists bind to the same region of the receptor as agonists do. Both adenosine and theophyllline contain a purine ring, and therefore the most obvious way to superimpose them is with the atoms N1, N3, N7 and N9 coinciding (see CHART 1).

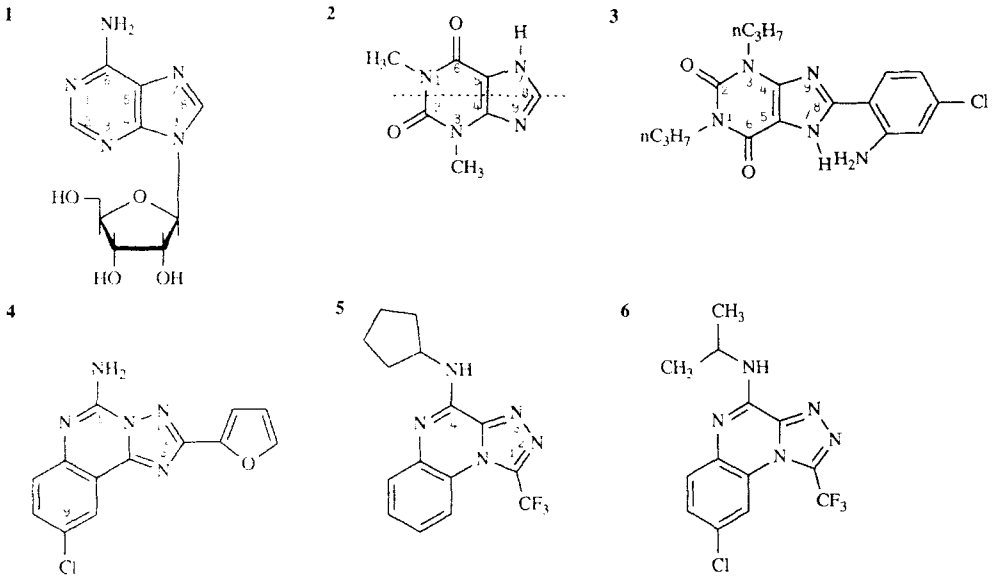


CHART 1. Structures and ring numbering of adenosine (**1**) and various antagonists: theophylline (**2**) (the dashed line indicates the long axis, as referred to in the text), PACPX (**3**), CGS 15943 (**4**), *N*-cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinolin-4-amine (**5**) and CP-66,278 (**6**). For further explanation see text.

Sterically equally acceptable is to first rotate theophylline 180° about the long axis and then superimposing the 6:5 fused ring systems. Of course, in this situation the nitrogen atoms do not all coincide anymore. Since drug-receptor interactions are not only governed by steric demands, but also by electrostatic and lipophilic requirements, we evaluated the two modes of superposition in electrostatic terms. It appeared that the second mode, i.e. with theophylline in its 'flipped' orientation yielded a much larger area of common molecular electrostatic potential. This better electrostatic fit is predominantly due to the common negative electrostatic potential of the lone pairs of N1 and N3 in adenosine and those of the carbonyl oxygens of theophylline. Thus, *each single part* of theophylline corresponds with a similarly charged region of adenosine. All these findings are in agreement with a similar suggestion by Olsson et al.[10], who reached the same conclusion by comparing the dipole moments of adenine and theophylline. Moreover, theophylline-7-riboside has moderate affinity for the adenosine receptor[11], whereas theophylline-9-riboside is virtually inactive[12].

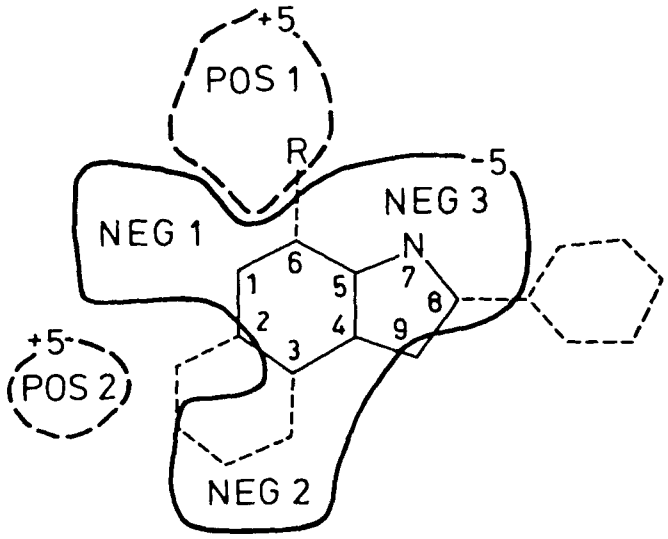


FIG. 1. Common steric and electrostatic properties of the adenosine antagonists used in this study. Additional areas where substitution may enhance potency are indicated with dashed lines. Ring numbering is followed as in adenosine.

Compounds **3**, **4**, **5** and **6** in CHART 1 all display nanomolar affinity towards the A_1 receptor, although they belong to different chemical classes. The graphical representation of the latter four compounds in CHART 1, different from the 'usual' way, leads upon superposition of the four compounds to the best steric and electrostatic fit, similar to the fit obtained with adenosine and theophylline.

In FIG. 1 a schematic model of this fit is shown in which a large Y-shaped area of negative electrostatic potential (EP) is apparent, resulting from the aromatic system of the 6:5 fused heterocycle. The Y-shaped area extends from the ring system at three points, designated NEG 1, NEG 2 and NEG 3.

In addition, there are two common areas of positive EP, i.e. POS 1 and POS 2. The negative EP of NEG 3 is in all compounds caused by the lone pair of a nitrogen atom (hydrogen bond acceptor?), whereas NEG 1 and NEG 2 are from various origin, as are POS 1 and POS 2. Three areas can be identified where hydrophobic substitution may increase adenosine receptor affinity. Two of these, adjacent to positions 6 and 8, are similar to the N^6-region in adenosine and the C8-region in xanthines, respectively. The third area where lipophilic substitution may lead to enhanced receptor affinity is the area occupied by the propyl substituent at N1 of compound **3** (PACPX) and the benzene rings of compounds **4** and **5** in CHART 1.

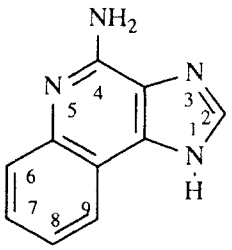


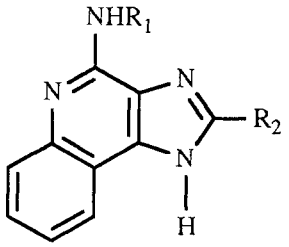
FIG. 2. Structure of 1*H*-imidazo-[4,5-*c*]quinolin-4-amine

Based on the model in FIG. 1 we aimed to design a novel class of non-xanthine adenosine antagonists, in order to test the model's predictive value. Of several likely candidates, 1*H*-imidazo-[4,5-*c*]quinolin-4-amine (FIG. 2) was chosen as a lead. The basic ring structure bears structural resemblance to [1,2,4]triazolo[1,5-*c*]quinazolin-4-amines[13] and [1,2,4]triazolo[4,3-*a*]-quinoxalin-4-amines[14], known to act as potent adenosine antagonists, but it has a different arrangement of the nitrogen atoms. It complies with the required MEP pattern (not shown) and it contains a nitrogen atom at position 3 (corresponding to position 7 in FIG. 1). Furthermore, positions 2 and 4 (positions 8 and 6 in FIG 1, respectively) can be substituted to enhance affinity. Thus, a series of compounds was synthesized according to these ideas.

In TABLE 1 the affinities of the novel compounds for rat A_1 receptors as well as rat A_2 receptors are presented. As predicted by our model the unsubstituted compound **8a** indeed has affinity for A_1 receptors, albeit moderate, from rat brain (K_i 1.6 μM), as well as for rat striatal A_2 receptors (K_i 1.4 μM) and thus it is nonselective. A hydrophobic cyclopentyl substituent at the exocyclic amino group (**8c**) greatly enhances receptor affinity, especially for the A_1 receptor. The R- and S-PIA 'analogs' **8d** and **8e** show stereoselectivity, **8d** is 5-fold more potent than **8e** on A_1 receptors. R_2-monosubstitution also enhances affinity (compounds **12a** and **16a**). Substitution with a phenyl group, however, is remarkably more effective than with a cyclopentyl moiety (K_i 34 nM vs 270 nM at rat A_1 receptors). Disubstitution leads to the most active compound at A_1 receptors, 2-phenyl-1*H*-imidazo[4,5-*c*]quinolin-4-cyclopentylamine (**16c**), displaying nanomolar affinity at A_1 receptors with 45-fold A_1 selectivity. In other cases, however, there appears to be lack of additivity of the affinity-enhancing effects of substitutions at position 2 and the exocyclic amino group: the dicyclopentyl substituted derivative (**12c**) is equal in potency to the N-cyclopentyl substituted compound (**8c**) at A_1 receptors of rat brain. Moreover, the diphenyl substituted **16b** is even less potent than the monosubstituted **16a**.

Finally, marked species differences were found in this class of imidazoquinolinamines (data not shown), as has been noticed for other chemical classes, whether agonists or antagonists [15,16].

TABLE 1. A_1 and A_2 adenosine receptor affinities of novel 1*H*-imidazo[4,5-*c*]-quinolin-4-amines.



Compound	R_1	R_2	A_1 K_i[a] rat	A_2 K_i[b] rat	A_2/A_1[c] rat
			nM	nM	
8a	H	H	1600	1400	0.9
8b	phenyl	H	2100	10500	5.0
8c	cyclopentyl	H	43	290	6.7
8d	(*R*)-1-methyl-2-phenylethyl	H	310	810	2.6
8e	(*S*)-1-methyl-2-phenylethyl	H	1500	2800	1.9
12a	H	cyclopentyl	270	740	3.0
12b	phenyl	cyclopentyl	230	6% (1μM)[d]	>4
12c	cyclopentyl	cyclopentyl	39	450	11
16a	H	phenyl	34	290	8.5
16b	phenyl	phenyl	460	9% (1μM)[d]	>2
16c	cyclopentyl	phenyl	10	450	45

[a] [^{3}H]DPCPX binding to rat brain cortical membranes. [b] [^{3}H]NECA binding to rat striatal membranes. [c] Ratio A_2- vs A_1-affinity in the rat. [d] Percentage of displacement at 1 μM.

CONCLUDING REMARKS

It has been suggested that the N^6-region of adenosine receptor agonists and the C8-region of xanthine antagonists might bind to the same part of the receptor[8]. This view is not compatible with the model in FIG 1, in particular because of huge discrepancies in MEP-patterns when both classes are superimposed with the N^6- and C8-regions coinciding. The results obtained with e.g., compound **16c** appear to bear this out: both substituents enhance A_1 receptor affinity and selectivity. These are obviously distinct sites and would correspond, according to our model, with the N^6- and C8-regions, respectively.

In conclusion, our model of the antagonist binding site of the adenosine A_1 receptor has successfully predicted the 1*H*-imidazo[4,5-*c*]quinolin-4-amines as a novel class of non-xanthine adenosine antagonists. The most potent compounds that have been synthesized so far in this series have nanomolar affinity for the A_1 receptor.

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