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Synthesis and Biological Data of 4-Amino-1-(2-chloro-2-phenylethyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid Ethyl Esters, a New Series of A_1-Adenosine Receptor (A_1AR) Ligands

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Abstract—The synthesis of a new family of A_1-adenosine receptor (A_1AR) ligands **3a**–**n** has been performed in a straightforward way. Affinity data at A_1AR, A_{2A}AR and A_3AR in bovine membranes show that these new compounds bind the A_1AR in a selective way over A_{2A}AR and A_3AR and one of them (**3j**) presents a very high affinity, probably due to the phenethylamine substituent at C-4.

Adenosine, whose physiological function has been defined and reported in recent years,[1] is a neurotransmitter distributed through a wide variety of tissues in mammal.[2] This nucleoside modulates its effects through interaction with four subtype receptors designated as A_1, A_{2A}, A_{2B} and A_3.[3] They belong to the big family of the G-protein coupled receptors which consist of seven trans-membrane helices. Researching for selective agonists and/or antagonists for each type of receptor and also for different tissue is an expanding target in medicinal chemistry development.

Adenosine interacts in many aspects of the brain function by mediating central inhibitory effects.[4,5] It is believed that A_1-adenosine receptor (A_1AR) antagonists, which stimulate the activity of the central nervous system, will, in future, be used in the treatment of various forms of dementia, such as Alzheimer's disease.[6]

Xanthines (caffeine and theophilline) are prototypic adenosine receptor antagonists, but are non-selective and of moderate potency.[7] Many structurally different non-xanthine derivatives have been synthesized and studied as antagonists at A_1 and A_2ARs; they are generally flat molecules, aromatic, nitrogen-containing heterocycles, often 6,5-fused.[2,6] The pyrazolo[3,4-*b*]pyridines Tracazolate **1a** and Etazolate **1b** (Fig. 1) are among the first non-xanthine antagonists which have been found to inhibit A_1-adenosine brain receptor binding.[8,9]

A series of pyrazolo[3,4-*b*]pyridine-5-carboxylic acid derivatives **2** (Fig. 1), which show an interesting affinity to A_1 and A_2 receptors, have subsequently been reported,[10] while many other products with similar activity have recently been patented.[11]

We are going to report in this communication the synthesis and preliminary biological data of a new family of potent and selective A_1AR ligands **3** (Fig. 1) which are structurally related to **1** and **2**, but bear a 2-chloro-2-phenylethyl chain in order to improve the lipophilic character of the substituent in position 1, in comparison with the simple methyl or ethyl groups usually present in their analogues **1** and **2**.

The synthesis of compounds **3**[12] is outlined in Scheme 1.

Reaction of 2-hydrazino-1-phenylethanol **4**, prepared following a literature procedure,[13] with ethylethoxymethylenecyanoacetate **5** gave ethyl ester

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of 5-amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole-4-carboxylic acid **6** in a very good yield. Basic hydrolysis of **6**, followed by thermal decarboxylation,[10] quantitatively afforded 2-(5-amino-pyrazol-1-yl)-1-phenylethanol **7**.

Condensation of the latter with diethyl ethoxymethylenemalonate gave the intermediate **8**, which upon treatment with $POCl_3$ at reflux (36 h) underwent cyclization to the pyrazolo-pyridine nucleus with a concurrent chlorination of the hydroxylic side chain to **9** in yields of 50% after chromatographic purification.

Regioselective substitution of chlorine at C-4, with an excess of various amines, afforded the desired products **3** in good yield.

It is interesting to point out that, in all of the syntheses performed, the chlorine atom at the side chain has never been substituted by the amine in spite of its benzylic position, as shown by the ^1H NMR chemical shifts of the CH_2–CH side-chain protons, which give an ABX complex pattern owing to their non equivalence.[14]

Compounds were tested for their ability to displace [^{3}H]*N*6-cyclohexyladenosine ([^{3}H]CHA) to A_1 adenosine receptors, [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]-amino]-5-(*N*-ethyl-carbamoyl)adenosine ([^{3}H]CGS21680) to A_{2A} adenosine receptors, and, for the most active compounds, [^{125}I]-*N*-(3-iodo-4-aminobenzyl)-5′-*N*-methylcarboxamidoadenosine ([^{125}I]AB-MECA) to A_3 adenosine receptors in bovine membranes following a previously reported procedure.[15] The A_1, A_{2A}, A_3 receptor-binding affinities, expressed as K_i or % of inhibition binding, for compounds **3a**–**n** are reported in Table 1.

The data show that this family of compounds is very selective against the A_1 adenosine receptor. In fact, in the range of concentrations used for the tests, most compounds show moderate to high affinity for A_1 and no affinity for A_{2A} and A_3 adenosine receptors.

Compound **3j** showed the highest affinity value against A_1AR (50 nM), thus being the best of the series and comparable to the more active compounds reported in literature,[16] while compounds **3a**, **3b**, **3e**, **3f** and **3i**

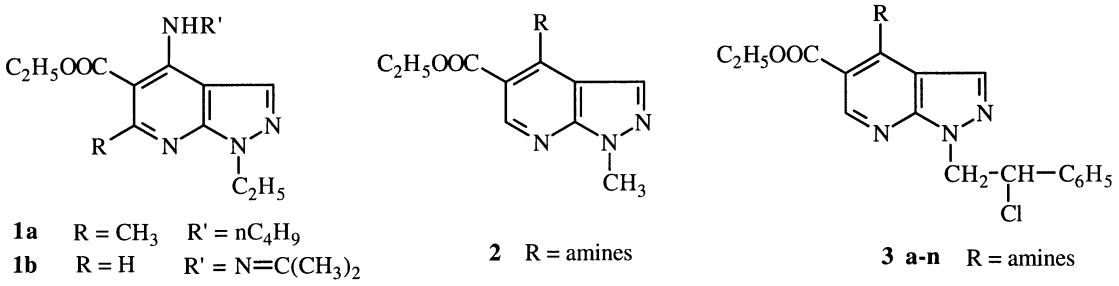
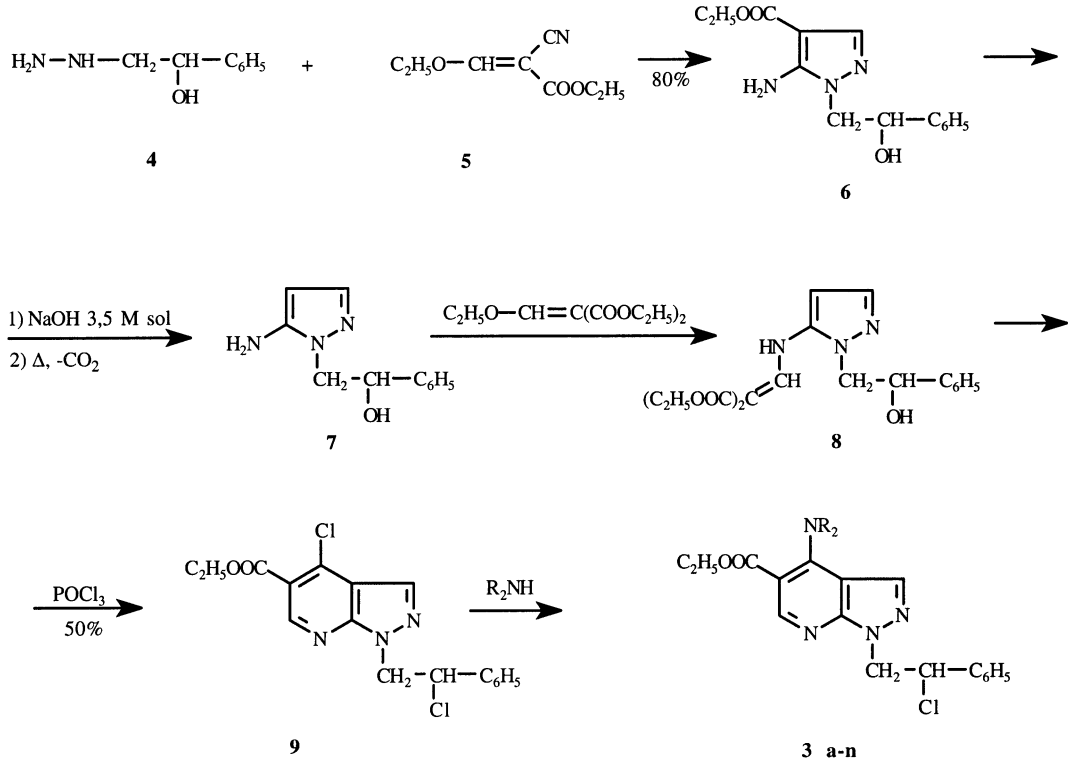


Figure 1.



Scheme 1.

Table 1. Affinity of **3a–n** derivatives at bovine brain A_1, A_{2A} and A_3ARs

Compounds	R	K_i (nM) or % inhibition[a]		
		bA_1[b]	bA_{2A}[c]	bA_3[d]
3a	$NH(CH_2)_2CH_3$	100±8.4	11%	23%
3b	NH cyclopropyl	112±9.6	10%	—
3c	$NH(CH_2)_3CH_3$	4100±23	3%	—
3d	$NHC(CH_3)_3$	4800±32	19%	—
3e	$NH(CH_2)_2OC_2H_5$	151±10	2%	—
3f	1-Pyrrolidino	98.2±7.3	17%	0%
3g	4-Morpholino	470±29	0%	—
3h	NHC_6H_5	348±21	11%	—
3i	$NHCH_2C_6H_5$	139±10	0%	—
3j	$NHCH_2CH_2C_6H_5$	50±3.7	4%	34%
3k	1-Piperidino	41%	23%	—
3l	1-Hexahydroazepino	35%	22%	—
3m	1-Piperazino	0%	6%	—
3n	1-(4-Methyl)piperazino	22%	2140±112	—

[a]The K_i values are means±SEM of three separate assays, each performed in triplicate.
[b]Displacement of specific [^{3}H]CHA binding in bovine cortical membranes or percentage of inhibition of specific binding at 10 μM concentration.
[c]Displacement of specific [^{3}H]CGS21680 binding in bovine striatal membranes or percentage of inhibition of specific binding at 10 μM concentration.
[d]Displacement of specific [^{125}I]AB-MECA binding in bovine cortical membranes or percentage of inhibition of specific binding at 10 μM concentration. Only compounds **3a**, **3f** and **3j** were tested.

showed good affinity data (in a range of 98.2–152 nM). Among the other compounds, **3g** and **3h** were also effective although with lower affinity (470 and 348 nM); compounds **3l**, **3m** and **3n** did not show any detectable affinity.

The structural requirements for high affinity in these pyrazolo[3,4-*b*]pyridine compounds appear to be an N-1 2-chloro-2-phenylethyl side chain together with a phenethylamine substituent at C-4 carbon atom. Many other differently N-1 substituted compounds have been synthesized and biologically tested, the data of which will be reported in full in due course. It is, however, worth pointing out that compounds with the N-1 2-chloro-2-phenylethyl side chain always showed higher affinity values.

The C-4 substitution plays a very important role in modulating the affinity of this class of compounds to the A_1 adenosine receptor. Variation of the nature of the amine function (from secondary to tertiary) at C-4 carbon atom dramatically drops the affinity (see Table 1), the only exception being **3f,** which still maintains a significant affinity value (98.2 nM). The length of the chain (see affinity of **3c** in comparison with **3a**) as well as its bulkiness are also very important (see **3d**). So as to better understand the SAR in this A_1AR antagonist family, a molecular modeling study is in progress and will be reported in a future paper.

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