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New 2-(2′-phenyl-9′-benzyl-8′-azapurin-6′-ylamino)-carboxylic acid methylesters as ligands for A_1 adenosine receptors

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Abstract

Synthesis of a series of new 2-phenyl-9-benzyl-8-azaadenines bearing on N^6 an alkyl or aralkyl chain having a carbonyloxymethyl group on the carbon bound to N^6 were reported. The ester group could assure to the molecule a better water-solubility than the 8-azaadenines 2, 6 and 9 substituted with lipophilic groups synthesised in the past. Compounds synthesised demonstrated only little capability of binding A_1 adenosine receptors. © 2001 Elsevier Science S.A. All rights reserved.

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1. Introduction

In recent years we have synthesised a great number of inhibitors of A_1 adenosine receptors varying substituents in the positions 3, 6 and 9 of the nucleus of 8-azaadenine or adenine and some of these were very effective having a K_i in the range of nanomolar [1–6]. All these compounds were characterised by a high lipophilicity because the substituents on the bicyclic nucleus were hydrophobic as alkyl, cycloalkyl or alkyl-aromatic groups. This can make these compounds nearly water-insoluble causing the determination of activity in in vitro binding assays to be uncertain in some cases. The scarce water-solubility also leads to a low bioavailability of the compounds so that they are unsuitable for in vivo studies.

Therefore we projected to obtain some A_1 antagonists more water-soluble than the active antagonists discovered in the past, introducing on the N^6 a substituent having a polar function as a methoxycarbonyl group.

2. Chemistry

The synthetic route (Scheme 1) employed known 1,3 dipolar addition reactions starting from benzylazide, cyanoacetamide, ethyl benzoate to obtain the 2-phenyl-9-benzyl-8-azahypoxanthine (**1**) [7]. The reaction of **1** with phosphorus oxychloride afforded 6-chloroazapurine (**2**) which, by nucleophilic displacement of chlorine atom operated by suitable aminoesters [4], led to N^6-substituted-8-azaadenines.

3. Experimental

3.1. Biological evaluation

The 8-azaadenines **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10** and **11** were tested in radioligand binding assays for affinity at A_1 adenosine receptors in bovine brain cortical membranes. [^{3}H]N^6-cyclohexyladenosine (CHA) was used as the radio-ligand. The experimental details were reported in a previous paper [1].

3.2. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra

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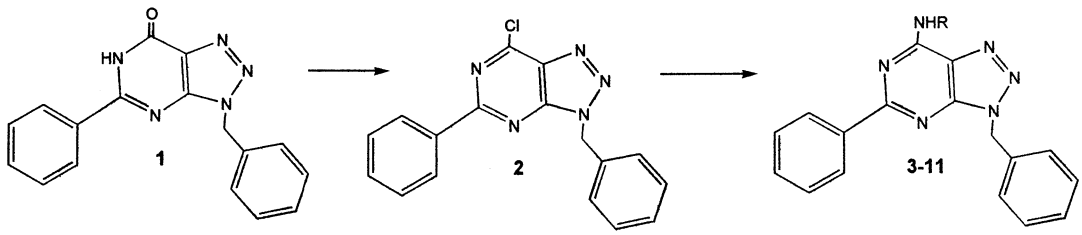

3 R = $CH(CH_3)COOCH_3$
5 R = $CH(CH_2C_6H_5)COOCH_3$
7 R = $CH(COOCH_3)CH_2CH_2COOCH_3$
9 R = $CH(CH_2CH_2CH_3)COOCH_3$
11 R= $CH(COOCH_3)CH(CH_3)CH_3$

4 R =$(CH_2)_2COOCH_3$
6 R =$CH(C_6H_5)COOCH_3$
8 R = $CH(CH_2CH_2SCH_3)COOCH_3$
10 R= $CH(CH_2CH_2CH_2CH_3)COOCH_3$

Scheme 1.

were recorded on a Bruker AC 200 spectrometer in δ units from TMS as an internal standard; the compounds were dissolved in chloroform-D1. Mass spectra were performed on a Hewlett–Packard GC/MS System 5988A. TLC was performed on precoated silica gel F_{254} plates (Merck). Flash-column chromatographies were performed using Merck Kieselgel 60 (230–400 mesh). Microanalyses (C, H, N) were carried out on a Carlo Erba elemental analyser (Model 1106) and were within $\pm 0.4\%$ of theoretical values.

3.2.1. General procedure to prepare aminoesters hydrochloride

Aminoesters hydrochloride of (D,L)-alanine, β-alanine, (D,L)-phenylalanine, (D,L)-phenylglicine, (D,L)-glutamic acid, (D,L)-methionine, (D,L)-norleucine, (D,L)-norvaline, (D,L)-valine and (D,L)-histidine were prepared starting from corresponding aminoacids. To a solution of aminoacid in methanol, cooled at -18 °C, an equimolar amount of $SOCl_2$ was dropped slowly. The mixture was refluxed for 12 h, then evaporated and the residue was crystallised from methanol–ethyl ether.

*3.2.2. General procedure to prepare 6-aminosubstituted-2-phenyl-9-benzyl-8-azaadenines (**3–11**)*

In a well-stoppered flask a mixture of **2** (200 mg, 0.623 mmol), toluene (2 ml), the suitable amino ester hydrochloride (1 mmol) (see Table 1) and *N*,*N*-diethylaniline (0.1 ml, 0.67 mmol) were stirred at room temperature for 24 h. Then the solution was evaporated at reduced pressure, diluted with chloroform and washed with 10% HCl and water. After evaporation of the organic layer the residue was flash-chromatographed using as eluent hexane–ethyl acetate 3:1. (see Tables 1 and 2).

4. Biological results and conclusions

None of the compounds showed a significant activity as A_1 adenosine receptors ligands. Compounds **4**, **7**, **8**, **9** and **11** showed a K_i included between 300 and 500 nM, while compounds **3**, **5**, **6** and **10** showed an inhibition $< 65\%$ at 10 μM. Results demonstrated that for 2-phenyl-9-benzyl-8-azaadenines a carboxymethyl

Table 1
Reaction data for compounds **3–11**

Comp.	Reagent	Yield (%)	m.p. (°C)	Formula (anal.)
3	$NH_2CH(COOCH_3)CH_3$	71	137–138	$C_{21}H_{20}N_6O_2$ (CHN)
4	$NH_2(CH_2)_2COOCH_3$	55	157–159	$C_{21}H_{20}N_6O_2$ (CHN)
5	$NH_2CH(CH_2C_6H_5)COOCH_3$	86	134–135	$C_{27}H_{24}N_6O_2$ (CHN)
6	$NH_2CH(C_6H_5)COOCH_3$	50	129–130	$C_{26}H_{22}N_6O_2$ (CHN)
7	$NH_2CH(CH_2CH_2COOCH_3)COOCH_3$	33	140–141	$C_{24}H_{24}N_6O_2$ (CHN)
8	$NH_2CH(CH_2CH_2SCH_3)COOCH_3$	38	164–165	$C_{23}H_{24}N_6O_2S$ (CHN)
9	$NH_2CH(CH_2CH_2CH_3)COOCH_3$	52	143–144	$C_{23}H_{24}N_6O_2$ (CHN)
10	$NH_2CH(CH_2CH_2CH_2CH_3)COOCH_3$	65	157–158	$C_{24}H_{26}N_6O_2$ (CHN)
11	$NH_2CH(COOCH_3)CH(CH_3)CH_3$	62	119–120	$C_{23}H_{24}N_6O_2$ (CHN)

Table 2
Spectroscopic data of compounds **3**–**11**

Comp.	^{1}H NMR				MS
	Aromatic H	Benzylic H	Aliphatic H	Exchang. H	*m/z* (%)
3	8.50 (m, 2H); 7.53–7.48 (m, 5H), 7.36–7.32 (m, 3H)	5.82 (s, 2H)	5.18–5.05 (m, 1H, CH); 3.80 (s, 3H, OCH_3); 1.69 (d, 3H, CH_3)	6.65 (1H)	388 (M^+, 2); 329 (8); 287 (1); 91 (100)
4	8.50 (s, 2H); 7.49 (m, 2H); 7.31 (m, 2H)	5.80 (s, 2H)	4.10 (t, 2H, CH_2); 3.73 (s, 3H OCH_3); 2.83 (t, 2H, CH_2)	6.64 (1H)	388 (M^+, 4); 329 (2); 91 (100)
5	8.49 (m, 2H); 7.49 (m, 5H); 7.39–7.22 (m, 8H)	5.80 (s, 2H); 3.37 (m, 2H)	5.40 (m, 1H, CH); 3.75 (s, 3H, CH_3)	6.62 (1H)	464 (M^+,0.5); 373 (13); 273 (18); 91 (100)
6	8.50–8.45 (m, 3H); 7.61–7.27 (m, 12H)	6.05 (d, 1H); 5.81 (s, 2H)	3.80 (s, 3H, OCH_3)	7.11 (1H)	450 (M^+, 2); 391 (7); 91 (100)
7	8.49 (m, 2H); 7.49 (m, 5H); 7.35 (m, 3H);	5.83 (s, 2H)	5.27 (m, 1H, CHN); 3.82 (s, 3H, OCH_3); 3.65 (s, 3H, OCH_3); (t, 2H, CH_2–CO); 2.30 (m, 2H, CH_2)	6.91 (1H)	460 (M^+, 12); 387 (22); 287 (10); 91 (100)
8	8.52 (m, 2H); 7.52 (m, 5H); 7.35 (m, 3H)	5.83 (s, 2H)	5.33 (m, 1H, CH); 3.81 (s, 3H, OCH_3); 2.68 (t, 2H, CH_2–S); 2.38 (m, 2H, CH–CH_2); 2.12 (s, 3H, S–CH_3)	6.92 (1H)	374 (4); 287 (6); 91 (100); 61 (54)
9	8.51 (m, 2H); 7.50 (m, 5H); 7.35 (m, 3H)	5.82 (s, 2H)	5.15 (m, 1H, CH); 3.79 (s, 3H, OCH_3); 1.98 (m, 2H, CH_2); 1.50 (m, 2H, CH_2); 0.99 (t, 3H, CH_3)	6.64 (1H)	357 (5); 302 (1); 91 (100)
10	8.51 (m, 2H); 7.51 (m, 5H); 7.35 (m, 3H)	5.82 (s, 2H)	5.12 (m, 1H, CH); 3.79 (s, 3H, OCH_3); 2.00 (m, 2H, CH_2); 1.42 (m, 4H, CH_2–CH_2); 0.91 (t, 3H)	6.66 (1H)	371 (6); 302 (2); 91 (100)
11	8.51 (m, 2H); 7.50 (m, 5H); 7.35 (m, 3H)	5.83 (s, 2H)	5.14 (m, 1H, CH–N); 3.80 (s, 3H, OCH_3); 2.42 (m, 1H, CH); 1.14 (d, 3H, CH_3); 1.11 (d, 3H, CH_3)	6.66 (1H)	357 (3); 302 (3.5); 91 (100)

group on the carbon bound to N^6 cannot give positive interactions with the receptors.

Acknowledgements

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