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SYNTHESIS OF NEW PYRAZOLO[4,3-e]1,2,4-TRIAZOLO[1,5-c] PYRIMIDINE AND 1,2,3-TRIAZOLO[4,5-e]1,2,4-TRIAZOLO[1,5-c] PYRIMIDINE DISPLAYING POTENT AND SELECTIVE ACTIVITY AS A_{2a} ADENOSINE RECEPTOR ANTAGONISTS.

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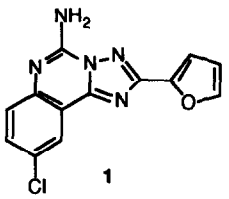
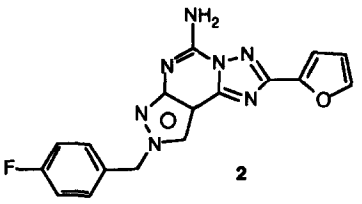
Abstract. A series of pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidines and 1,2,3-triazolo[4,5-e]1,2,4-triazolo[1,5-c]pyrimidines were prepared and evaluated for their activity as adenosine A_{2a} receptor antagonists. In the present study, 5-amino-7-(phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine **7d (SCH 58261)** was identified as potent and selective adenosine A_{2a} antagonist in binding assays (Ki = 2.3 nM, Ki ratio: A_1/A_{2a} = 52.6).

Adenosine affects many biological actions in mammals through the modulation of specific cell surface receptors, known as A_1, A_2, A_3 and A_4 receptors[1a,b]. The A_2 receptor has been subdivided into A_{2a} and A_{2b} receptor subtypes on the basis of biochemical studies[2] and molecular cloning[3].

Synthetic efforts made over several years have led to the discovery of a series of adenosine analogs which possess agonist properties at A_1 or A_2 receptors[1b]. As for antagonists, a large number of xanthine derivatives have been synthesized in an attempt to improve affinity and selectivity of the natural compounds caffeine and theophylline. Thus, several 8-substituted xanthines have been found to be potent and selective antagonists at A_1 receptors and one of them, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) is currently used as a reference A_1 antagonist[4]. More recently, some 8-styrylxanthines have been found to possess A_{2a} antagonist properties[5a,b]. However, these compounds undergo rapid isomerization when diluted and exposed to natural light[6]. A number of non-xanthine heterocyclic derivatives have been found to be potent but weakly selective for A_{2a} receptors. Examples of such compounds are the triazoloquinazoline CGS 15943 **1**[7], the triazoloquinoxaline CP 66,713[8].



1



2

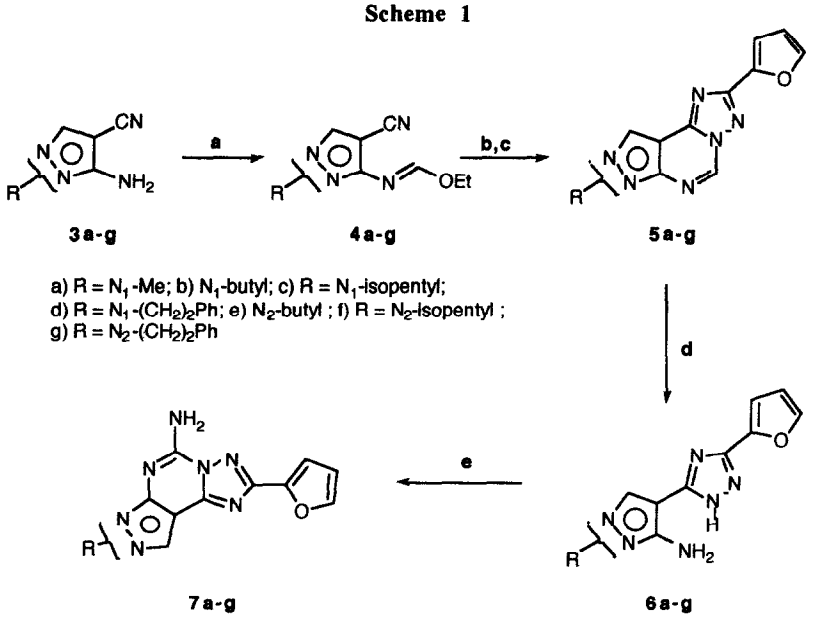
Recently, we have reported a series of CGS 15943 analogs, in which the phenyl group was replaced by an heterocyclic ring such as pyrazole and imidazole[9]. For example, 5-amino-8-(4-fluorobenzyl)-2-(2-furyl)-

pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (8FB-PTP) **2** possess competitive A_{2a} antagonist properties in functional assays but low selectivity[9,10]. In this communication, we wish to report the drug design studies made on the structure **2**, leading to the discovery of a potent and selective A_{2a} antagonist, the pyrazole analog **7d (SCH 58261)**.

In order to investigate the structure-activity relationships of the lead **2** and to improve markedly its A_{2a} selectivity, we undertook a program of analog synthesis. The importance of the hydrophobic, electronic and steric parameters of the substituent at the pyrazole nitrogen of **2** was evaluated by synthesizing a series of N_7 and N_8 derivatives according to the decision Topliss tree for aliphatic substituents[11]. We also applied the typical bioisosteric replacement of the pyrazole ring in the lead structure **2** , with a triazole ring (Table 1). CGS 15943 and our designed prototypes **2**, **7a-g**, **11a-f** may also be regarded as 3-deaza-5-aza-adenines bearing at C_2 and C_3 carbons (adenine numbering) a fusion with another ring, such as benzene, pyrazole, and triazole moieties. They are also structurally related to the family of 2-substituted adenosines which possess potent and selective agonistic properties for A_{2a} receptors[12-14]. Furthermore, SAR studies of 2-alkoxyadenosines[13] and N-substituted 2-aminoadenosines[14] have shown that, in the C_2 position of adenine a chain of two or three carbon atoms followed by a phenyl ring or a cycloalkyl moiety is essential to obtain selective and potent activity at A_{2a} receptors.

The synthesized compounds **7a-g** and **11a-f** were prepared following the Schemes 1 and 2. Alkylation of 4-cyano-5-amino pyrazole with the appropriate alkyl halide in DMF in the presence of anhydrous potassium carbonate led to an approximately 1:1 mixture of N_1-isomers **3b-d** and N_2-isomers **3e-g**, easily separable by crystallization or column chromatography[15]. Pyrazole **3a** was prepared starting from commercially available ethoxymethylenemalondinitrile and methylhydrazine following a well known procedure[16] (Scheme 1).

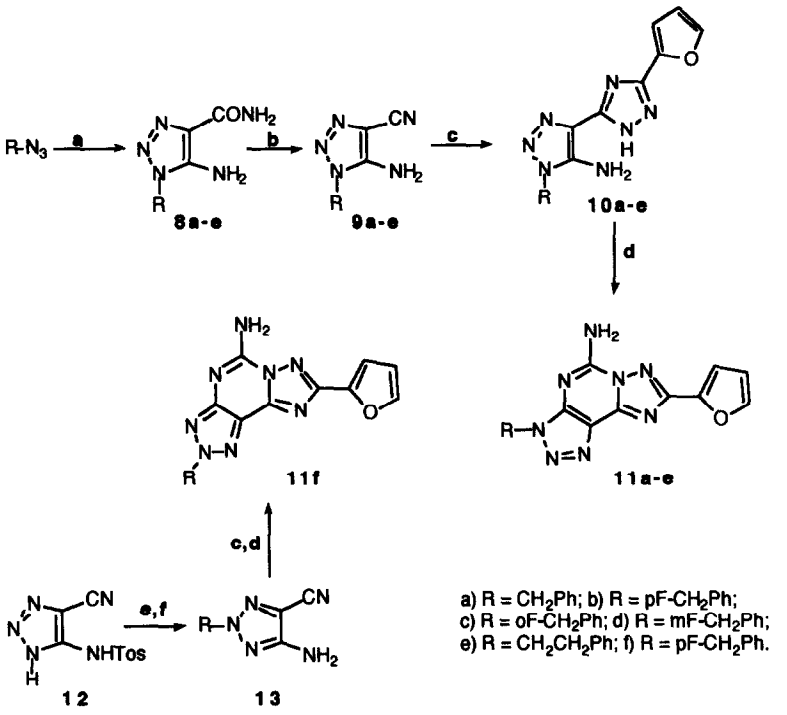
Scheme 1



Reagents : **a**) $HC(OEt)_3$, reflux ; **b**) Furoic hydrazide, $MeO(CH_2)_2OH$; **c**) Ph_2O , 260°C ; **d**) HCl , reflux ; **e**) NH_2CN , pTsOH, 140°C.

The designed compounds **7a-g** were synthesized taking advantage from the general synthetic methodology to pyrazolo[4,3-e]1,2,4-triazolo[1,5-c] tricyclic system developed by Gatta et al.[9]. Thus, imidates **4a-g** obtained by refluxing **3a-g** in triethylorthoformate, were reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[3,4-d]pyrimidine intermediates. The latter compounds were converted through a thermally induced cyclization in diphenylether to the derivatives **5a-g** in good overall yield. Treatment of **5a-g** with dilute hydrochloric acid at reflux temperature induced pyrimidine ring opening to furnish the 5-amino-4-(1H-1,2,4-triazol-5-yl)-pyrazoles **6a-g** in good yield. These derivatives were converted into the final compounds **7a-g**[17] by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140°C. The preparation of the bioisosters **11a-f**, containing the 1,2,3-triazole ring in place of pyrazolic one, were easily obtained following a more direct route, also developed by Gatta et al.[9]. (Scheme 2).

Scheme 2



Reagents : **a**) $NCCH_2CONH_2$, K_2CO_3, DMSO; **b**) $POCl_3$; **c**) furoic acid hydrazide, Ph_2O; 260°C ; **d**) NH_2CN, pTsOH, 140°C; **e**) RX, K_2CO_3, DMF ; **f**) H_2SO_4 .

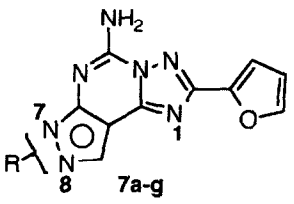
Thus, 1-substituted-4-cyano-5-amino-1,2,3-triazoles **9a-e** were prepared in two steps involving: i) reaction of the corresponding azides with cyanoacetamide in DMSO[18] in the presence of potassium carbonate to give the triazole carboxamides **8a-e**; ii) their dehydration with phosphoryl choride.

Treatment of **9a-e** with 2-furoic acid hydrazide in diphenyl ether at 260°C for 30 min led to a mixture which, after chromatographic purification, furnished **10a-e** in good yield. Finally, reaction of **10a-e** with

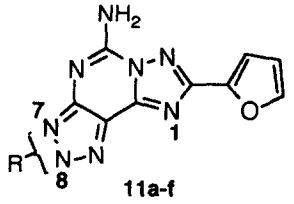
an excess of cyanamide in 1-methyl-2-pyrrolidone at 140°C gave the desired derivatives **11a-e** in low yield. Compound **11f** was prepared in a similar manner starting from **13**, in turn obtained from 4-cyano-5-tosylamino-1,2,3-triazole **12** by alkylation and tosyl group deprotection[19].

A_1 and A_{2a} receptor binding assays were performed on rat whole brain and striatum using [^{3}H]-N_6-cyclohexyladenosine ([^{3}H]CHA)[20] and [^{3}H] 2-[4-(2-carboxyethyl)-phenylethylamino]-5'-N-ethylcarboxamidoadenosine ([^{3}H]CGS 21680)[21] as radioligands, respectively. Ki values were calculated from the Cheng-Prusoff equation[22] using 1.0 and 16.0 nM as for Kd values in A_1 and A_{2a} binding assays, respectively. The results show that many of the tested compounds have affinity at A_{2a} receptors in the low nanomolar range with different degrees of selectivity (Table 1).

Table 1. Biological activity of compounds 7a-g and 11a-f



7a-g



11a-f

Compound	R	Binding assays[a]		Selectivity
		A_1	A_{2a}	A_1/A_{2a}
		Ki (nM)		
DPCPX		1.5 (1.3-1.7)	706 (540-924)	0.002
1(CGS15943)		6.4 (6.2-6.6)	1.2 (1.1-1.3)	5.3
2(8FB-PTP)		3.3 (2.9-6.6)	1.2 (0.9-1.4)	2.8
7a	N7-Me	651 (486-872)	101 (94-109)	6.4
7b	N7-nButyl	236 (205-272)	8.9 (7.9-10.0)	26.5
7c	N7-Isopentyl	116 (79-171)	12.0 (7.6-19.0)	9.7
7d(SCH 58261)	N7-$(CH_2)_2$Ph	121 (103-143)	2.3 (2.0-2.7)	52.6
7e	N8-Butyl	30.4 (21.5-43.0)	2.4 (2.3-2.5)	12.7
7f	N8-Isopentyl	5.6 (4.0-7.9)	1.9 (1.4-2.4)	2.9
7g	N8-$(CH_2)_2$Ph	4.7 (4.1-5.5)	1.4 (0.9-2.3)	3.4
11a	N7-Benzyl	6.1 (4.1-9.1)	4.6 (3.3-6.3)	1.3
11b	N7-pF-Benzyl	61.2 (52.1-71.9)	13.7 (11.5-16.3)	4.5
11c	N7-oF-Benzyl	13.7 (9.0-20.9)	10.4 (8.1-13.2)	1.3
11d	N7-mF-Benzyl	19.0 (17.7-20.4)	4.9 (3.8-6.4)	3.9
11e	N7-$(CH_2)_2$Ph	42.4 (37.2-48.5)	6.9 (5.2-9.2)	6.1
11f	N8-pF-Benzyl	18.3 (14.1-23.8)	2.5 (1.9-3.4)	7.3

[a] Inhibition of [^{3}H]CHA binding (A_1) in rat whole brain homogenates or [^{3}H]CGS 21680 binding (A_{2a}) in rat striatal homogenates; data are expressed as geometric mean, with 95% confidence limits in parenthesis, of at least three separate experiments.

In particular, 5-amino-7-(phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine **7d** (**SCH 58261**) was the most potent compound in inhibiting [^{3}H]CGS 21680 binding with a Ki value of

2.3 nM and a 53-fold selectivity for A_{2a} vs. A_1 receptors. The selectivity of **SCH 58261** was also confirmed in binding studies carried out on bovine brain tissues (Ki at A_{2a} receptors = 2.0 nM; A_1/A_{2a} ratio = 102).

Results from binding studies showed that: a) comparing N_7 and N_8 substituted pyrazole derivatives (i.e. **7b,c,d** and **7e,f,g**, respectively), in agreement with the trend observed for **2** (**8FB-PTP**) and the corresponding N_7 derivative[9], N_8 substitution always induces an increase in the affinity associated with the concomitant decrease in the A_{2a} vs. A_1 selectivity ; b) conversely, comparing N_7 and N_8 substituted triazole derivatives (i.e. **11b** and **11f** respectively), an increase in both affinity and A_{2a} vs. A_1 selectivity was observed; c) finally, comparing the two different heterocyclic species, namely pyrazole and triazole-derivatives, (i.e. **2** and **11f** and N_7-isomer of **2** and **11b**, respectively) a moderate decrement in the A_{2a}-affinity and a concomitant slight increment in the selectivity were observed for **2** and **11f**. On the contrary, a decrement in either A_{2a}-affinity and selectivity was observed in the case of the N_7-isomer of **2** and **11b.** Moreover, within the N_7 and N_8 pyrazole series, respectively, A_{2a}-affinity increased (Ki trend: **7d<7b≤7c<<7a** and **7g≤7f≤7e**) with the increasing lipophilicity, in agreement with the Topliss tree for aliphatic substituents[11].Accordingly, within the N_7-triazole series, the best selectivity was achieved with the compound **11e**.

In conclusion, our data provide important information to understand the structure-activity relationships of adenosine receptors and show **SCH 58261** as the first potent and selective non-xanthine A_{2a} adenosine antagonist in binding assays. Preliminary functional assays, here not reported, confirm the activity of **SCH 58261** in A_{2a}-mediated responses. The compound seems therefore, suitable for investigation of the biological function of A_{2a} receptors and the therapeutic potential of A_{2a} adenosine antagonists, according to the current knowledge on the possible role of A_{2a} receptors in neurodegenerative disorders such as Parkinson's disease[22].

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15. **3d**: mp 172-173°C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.04 (t, 2H, J=6.8 Hz); 4.12 (t, 2H, J=6.8 Hz); 5.85 (bs, 2H); 7.21-7.30 (m, 5H); 7.41 (s, 1H). **3g**: mp 98-100°C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 3.07 (t, 2H, J=6.8 Hz); 4.10 (t, 2H, J= 6.8Hz); 4.23 (bs, 2H); 7.00-7.28 (m, 5H); 7.17 (s,1H).
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17. **7d**: mp 225-226°C (DMF-Et_2O); ^{1}H NMR (DMSO-d_6) δ: 3.21 (t, 2H, J = 6.8 Hz); 4.51 (t, 2H, J = 6.8 Hz); 6.65 (s, 1H); 7.1-7.44 (m, 6H); 7.78 (s, 1H); 7.89 (bs, 2H); 8.07 (s, 1H). **7g**: mp 212-213°C (DMF-Et_2O); ^{1}H NMR (DMSO-d_6) δ: 3.21 (t, 2H, J = 6.8 Hz); 4.53 (t, 2H, J = 6.8 Hz); 6.7 (s, 1H); 7.1-7.40 (m, 6H); 7.65 (bs, 2H); 7.93 (s, 1H); 8.45 (s, 1H).
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