A New Series of 2-Alkoxy(aralkoxy)-[1,2,4]triazolo[1,5-*a*]quinazolin-5-ones as Adenosine Receptor Antagonists

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This research was carried out to study the pharmacological activity of a newly synthesized series of 2-alkoxy-[1,2,4]triazolo[1,5-*a*]quinazolin-5-ones as adenosine receptor antagonists. These compounds have been tested in radioligand binding assays on cloned Chinese hamster ovary (CHO) cells transfected with A_1, A_{2A}, A_{2B} and A_3 receptors. In particular, among the triazoloquinazolines (1—11), the dialkoxy derivative (7b) was found to have the highest affinity at A_1 subtype receptor, and its radioligand binding activity together with 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) was studied. Finally, the structure–activity relationship (SAR) studies on the titled compounds provide some new insights about steric hindrance and lipophilic requirements for anchoring to the adenosine receptors recognition site.

Key words 1,2,4-triazoloquinazoline; adenosine receptor; radioligand binding affinity

Adenosine, an important regulator of homeostasis in the brain, heart, kidney and other organs, interacts with at least four cell surface receptor subtypes classified as A_1, A_{2A}, A_{2B} and A_3. These adenosine receptors (ARs) belong to the super family of seven transmembrane G-protein coupled receptors: A_1 and A_3 subtypes inhibit adenylate cyclase (AC) *via* Gi protein, whereas A_{2A} and A_{2B} activate AC *via* Gs protein.[1,2] In addition, coupling with other messenger systems, such as calcium and potassium channels (A_1 receptor) or phospholipase C (A_1, A_{2B} and A_3 receptors) and D (A_3 receptor), has been described.[3—6] The A_1 and A_{2A} receptors are high affinity receptors, while A_{2B} and A_3 are low affinity ARs.[7,8] The naturally occurring xanthines such as caffeine and theophylline were the initial prototypic AR antagonists.[9,10] The many attempts to improve their potency and selectivity have resulted in the preparation of a large number of xanthine derivatives, and much is known now in terms of their structure activity and structure selectivity relationships, as well as about their pharmacological activity.[11—13] However, most of these xanthine derivatives showed poor water solubility and a high metabolic rate (specially due to their interaction with cytochrome P450 family) which strongly limitate its drug ability profile.

The extensive research in this topic has also led to the discovery of different classes of non-xanthine AR antagonists, being most of them nitrogen-containing heterocyclic compounds.[14—22] Some of the early described tricyclic adenosine antagonists were identified from collections of compounds initially designed as ligands of the benzodiazepine receptors.[14—18] Several elegant examples of the pharmacomodulation of these prototypes have allowed the development of new selective adenosine antagonists. Within the huge number of tricyclic heteroaromatic systems tested as adenosine antagonists (Fig. 1) those containing a 1,2,4-triazoloquinoxaline scaffold have been extensively explored, showing to be an extremely versatile motif during the identification of new valuable and selective AR ligands.[23—33]

In our previous papers,[34—36] we have described the synthetic methodology to obtain a new series of 2-alkoxy-[1,2,4]triazolo[1,5-a]quinazolin-5-ones and their derivatives

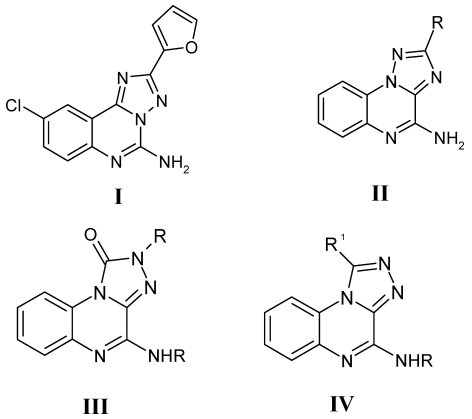

Fig. 1. 1,2,4-Triazolo Annelated Quinoxaline(quinazoline) as Adenosine Antagonists

1—**11**, Chart 1. In this scenario, and as a part of our interest in the search for novel adenosine receptor antagonists, we herein report the pharmacological characterization of our compounds (**1**—**11**) at all adenosine receptor subtypes.

Results and Discussion

The binding results of compounds **2**—**11** are shown in Table 1 together with those of their parent compounds **1a**—**d**. Moreover, the binding data of theophylline, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and some selective adenosine receptors, included as antagonist reference compounds in Table 2, are also reported.[37—47] Examination of the binding results listed in Table 1 indicated that, the data obtained confirmed the structural modifications carried out on the 5-position of the tricyclic system **1** produces a remarkable modification of the adenosine receptorial profile in terms of affinity and/or selectivity.

The AR binding affinities of the parent compounds **1a**—**d** indicate that, these compounds are almost selective at A_1 AR with K_i values of 2.4—6.8 μM, and showed slight affinity at A_{2A} subtype. The modifications carried out on the parent structures **1a**—**d** have different effects on A_1, A_{2A} and A_{2B} affinities. For example, alkylation of lactam moiety in the

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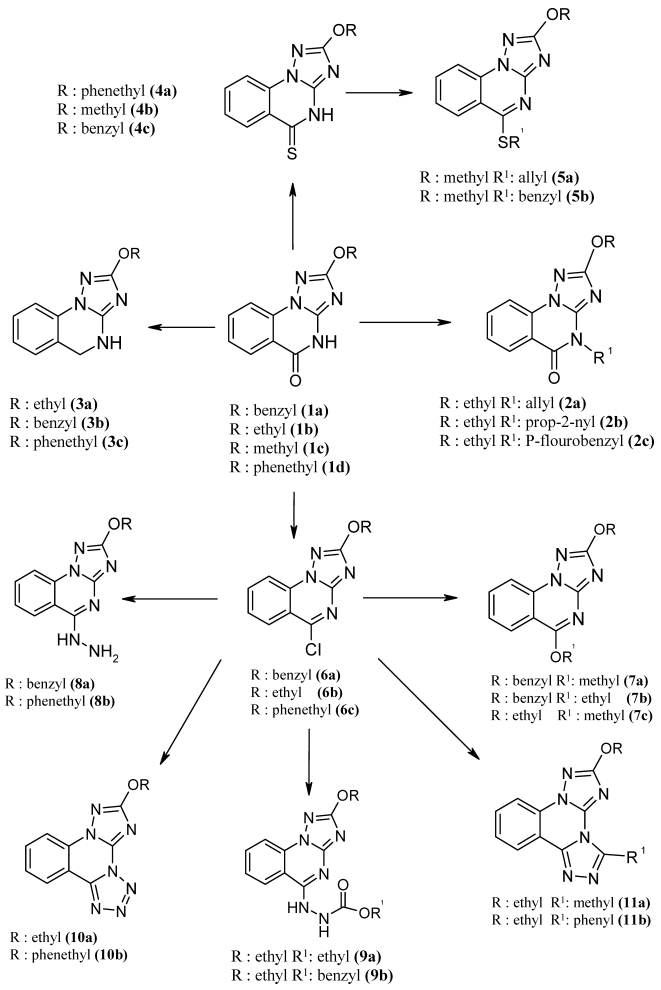

Chart 1. Structure Modifications on the 2-Alkoxy-[1,2,4]triazoloquinazolin-5-ones **1a**—**d**

parent compound **1b** furnished *N*-alkylated products **2a**—**c**, that were also tested in binding experiments, in particular affinity to A_1 and A_{2A} subtypes with respect to **1b**. In addition, variation in the substituted alkyl groups have demonstrated remarkable binding affinities at A_1, A_{2A}, A_{2B} receptors, such as compound **2b** was displayed a significant K_i value of 1.1 μM at A_1 in regard to the parent **1b** (2.4 μM). Furthermore, **2b** was found to possess the highest affinity at A_{2B} (IC_{50}=2.3 μM), although its parent **1b** does not emerge to exhibit effect in the A_{2B} binding affinity. The reduction products **3a**—**c** of **1a**, **b**, **d** showed remarkable attenuated binding affinity at A_1, where **3a** gave K_i value of 6 μM with respect to 2.4 μM for **1b**, despite these compounds possess enhanced lipophilicity comparable to that parent compounds **1a**, **b**, **d**.

Thionation of **1a**, **c**, **d** into **4a**—**c** was accomplished with significantly different changes in the terms of affinity. In particular, **4a** showed good A_1 potency of 4.4 μM with respect to the parent **1d** (6.8 μM), whereas slightly decrease in the affinity was noted in case of **4c** (5 μM) in regard to **1a** (4.25 μM). These results could suggest that, lactam or thiolactam moieties in **1** and **4** did not play an important role for anchoring their parent compounds to the ARs, but have been used also as suitable precursors for elaborating more derivatives with high affinity terms. Consequently, the thioether derivatives **5a**, **b** of compound **4b** showed remarkable significant improvement the affinity towards A_1, A_{2B} and A_3 subtypes. In particular, **5a** was found to exhibit high affinity at A_1 (3.4 μM) with respect to **4b** (>10 μM), and was advantageous for A_{2B} receptor–ligand interaction comparable to that of the parent **4b**.

It has been demonstrated that introduction of electron withdrawing atoms or groups in triazoloquinazoline rings enhances strongly the binding affinity towards benzodiazepine and adenosine A_1, A_{2A} receptors.[48,49] Within our work, conversion of the lactam moiety in **1a**, **b**, **d** into an imidoyl chloride function **6a**—**c** has not influenced positively effects on the affinity profiles towards A_1 and A_{2A}. However, compounds **6a**, **c** have emerged almost the same behavior in the terms of affinity at A_1 subtype (4, 6.8 μM) with their parent compounds **1a**, **d** (4.25, 6.8 μM), whereas **6b** was less potent than **1b**. Furthermore, the presence of chloride functional group in 5-position of **6a**—**c** does seem to offer slightly advantageous for A_{2B} and A_3 affinity. Nevertheless, the significantly A_1 AR affinity of triazoloquinzolines **6a**—**c** bearing at the 5-position a chlorine atom indicates that, not only chloro lipophilic factor, but also the steric effect are important for anchoring the ARs recognition. Thus, replacement of the chlorine atom in **6a**—**c** by different nucleophiles provided access to a variety of derivatives with a variable affinity towards adenosine receptor subtypes. For instance, the dialkoxy triazoloquinazolines **7a**—**c** derivatives represent the most populated set of compounds obtained during this study, particularly **7b** was found to display the highest affinity at A_1 receptor with a K_i value of 0.068 μM in regard to the parent **6a** (4 μM). Moreover, the best results in terms of affinity which has shown in **7a**, **b** may be attributed to the presence of steric hindrance of alkoxy groups in 5-position. This indicates, such functionalization in **7a**, **b** is favorable for the interaction with the A_1 adenosine receptor subtype (K_i=0.154 and 0.068 μM for **7a** and **7b** respectively). However, it is worth noting that the dialkoxy triazoloquinazoline **7b** possesses the highest A_1 AR affinity among the herein reported compounds. Furthermore, it has been found that, **7b** was proved to be a potent A_1 AR antagonist (IC_{50}=108 nM) in regard to the DPCPX. On the contrary, a dramatic drop of affinity was observed by hydrazines **8a**, **b** and carbazides **9a**, **b**; this behavior confirmed that such functionalization hindered the interaction with adenosine receptors due to decrease of the lipophilicity. Finally, the transformation of the tricyclic systems **6b**, **c** into tetracyclcic systems **10a**, **b** and **11a**, **b** does not offer advantageous in the affinity terms at A_1. However, compound **10b** showed better potency (1.75 μM) than its parent **6c** (6.8 μM). As well as, these results indicate that, the presence of fused ring is not well tolerated for anchoring to the ARs recognition site.

In conculsion, structure modifications on the lead compound **1** have afforded derivatives with a variety of ARs affinity terms. A comparison study was reported between the tested 1,2,4-triazolo[1,5-*a*]quinazoline derivatives and some adenosine antagonists references using xanthine and non-xanthine compounds. Most of the tested compounds have been disclosed as A_1 AR antagonists with different K_i values. Among of them, compounds **7a**, **b** were displayed potent A_1 antagonist activity with respect to DPCPX, while compound **2b** was nonselective and being potent antagonist at A_1 and A_{2B} subtypes with regard to the DPCPX and MRS-1754.

Table 1. The Binding Affinities of Compounds (**1**—**11**)

Compounds	A_1 K_i (μM)	Affinity (%) with 10 μM	A_{2A} K_i (μM)	Affinity (%) with 10 μM	A_{2B} IC_{50} (μM)	Affinity (%) with 10 μM	A_3 IC_{50} (μM)	Affinity (%) with 10 μM
1a	4.25	(49)	>10	(14)	—	—	—	—
1b	2.4	(62)	—	(10)	—	—	—	—
1c	5	(44)	—	(7)	—	—	—	—
1d	6.8	(45)	—	(3)	—	(1)	—	—
2a	2.2	(72)	>10	(33)	—	—	—	—
2b	1.1	(84)	2.5	(57)	2.3	(95)	—	(7)
2c	15.1	(33)	3	(55)	—	—	—	—
3a	6	(43)	—	(1)	—	—	—	—
3b	6	(48)	>10	(1)	—	—	—	—
3c	>10	(30)	—	(7)	—	—	—	—
4a	4.4	(47)	>10	(1)	—	—	—	—
4b	>10	(42)	—	(1)	—	—	—	—
4c	5	(43)	>10	(28)	>10	(37)	—	—
5a	3.4	(58)	>10	(26)	3	(62)	—	(75)
5b	5.1	(40)	>10	(12)	>10	(31)	—	—
6a	4	(61)	>10	(1)	—	(31)	—	(48)
6b	3.1	(51)	>10	(10)	—	(21)	—	(37)
6c	6.8	(40)	>10	(6)	—	(33)	—	(21)
7a	0.154	(89)	>10	(1)	>10	(23)	>10	(14)
7b	0.068	(95)	>10	(1)	>10	(37)	>10	(52)
7c	7.5	(40)	—	(1)	—	—	—	—
8a	>10	(28)	—	(1)	—	—	—	—
8b	>10	(19)	—	—	—	—	—	—
9a	>10	(31)	—	(4)	—	—	—	—
9b	—	(9)	—	(1)	—	—	—	—
10a	>10	(36)	—	(1)	—	—	—	—
10b	1.75	(67)	—	(8)	—	—	—	—
11a	>10	(36)	—	(22)	—	(45)	—	(13)
11b	—	(51)	—	(3)	—	—	—	—

Table 2. K_i-Values of Xanthine and Non-xanthine Adenosine Antagonists

Adenosine receptors subtype	Compound	K_i-value (nM)			
		A_1AR	A_{2A}AR	A_{2B}AR (IC_{50}, nM)	A_3AR
Non selective	Theophylline	6770	1710	9200	86400
	Coffeine	55	48	10400	N.D.
A_1	DPCPX	3.9	129	56	3980
	BG-9928	29	4720	690	42110
A_{2A}	KW-6002	2830	36	1800	>3000
	CGS-15943	3.5	0.15	71	50.8
A_{2B}	MRS-1754	403	503	2.0	570
	OSIP-339391	37	328	0.410	450
A_3	MRS-1220	305	52	N.D.	0.65
	MRS-1067	>1000	>1000	N.D.	560

Moreover, the structure–activity relationship (SAR) study of compounds **1**—**11** gave us some useful insights about the characteristics requirements for the optimal anchoring of the targeted compounds to the ARs recognition site, which may be taken into consideration in the design of new ARs antagonists.

Experimental

Radioligand Binding Assay The cells were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 without nucleosides at 37 °C in 5% CO_2 95% air. The standard assay medium was prepared by FBS (fetal bovine serum), P/S (Penicillin/Streptomycin) and Geneticin. The cells were washed with phosphate-buffered saline and scrapped off flasks in ice cold hypotonic buffer (50 mM Tris–HCl, 100 mM ethylenediaminetetraacetic acid (EDTA), water, pH 7.4 at 4 °C). The cell suspension was homogenized with a Polytron and the homogenate was centrifuged for 30 min at 18000 rpm. The membrane pellet was resuspended in 50 mM Tris–HCl buffer at pH 7.4 for A_1 receptors, in 50 mM Tris–HCl, 100 mM $MgCl_2$, at pH 7.4 for A_{2A} receptors. The [^{3}H]ZM241385 and [^{3}H]DPCPX[50,51] were utilized in radioligand binding assay to membranes prepared from Chinese hamster ovary (CHO) cells expressing the appropriate adenosine receptors on their surfaces.[52] Adenosine-deaminase (ADA) was present during the preparation of the membranes, in a preincubation of 30 min at 37 °C, and during the incubation with the radioligands. All nonradioactive compounds were initially dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer to the final concentration, where the amount of DMSO never exceeded 2%. Bound and free radioactivity were separated by rapid filtration through Whatman GF/B glass-fiber filters which were washed three times with ice cold buffer. The filter bound radioactivity was counted in a Beckman LS-1800 spectrometer (efficiency 55%). Inhibitory binding constant, K_i, and IC_{50} were calculated according to the Cheng–Prusoff equation ($K_i = IC_{50}/(1+[*DPCPX]/K_d)$. Where [*DPCPX] is the concentration of the radioligands (1 nM) and dissociation constant (K_d) of radioligands for *DPCPX, and *ZM are 1 and 2 nM respectively. Binding experiments at human A_{2B} and A_3 receptors were performed on crude membranes obtained from CHO cells and the procedure was carried out as described previously with minor modifications.[52] The cells were dissolved with 2 ml of tyrpsine and washed with phosphate-buffered saline. The cell suspension was centrifuged for (10 min, 12000 rpm, 40 °C). The membrane pellet was resuspended in 5 ml cAMP puffer at 37 °C. To the preparation medium was added RO-20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinon) as phosphodiesterase-inhibitor and NECA (5-(6-amino-9*H*-purin-9-yl)-*N*-ethyl-3,4-dihydroxytetrahydrofuran-2-carboxamide) as non selective adenosine agonist. IC_{50} values for concentration-dependent inhibition of NECA-stimulated adenylyl cyclase caused by antagonists were calculated according to Cheng and Prusoff equation. The protein concentration was determined according to a Bio-Rad method.[53] The K_i values for antagonists were then calculated with the Cheng and Prusoff equation.[54]

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