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Preliminary communication

Modulation of A_{2B} adenosine receptor by 1-Benzyl-3-ketoindole derivatives

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ABSTRACT

We have disclosed a series of 1-benzyl-3-ketoindole derivatives acting as either positive or negative modulators of the human A_{2B} adenosine receptor (A_{2B} AR) depending on small differences in their side chain. The new compounds were designed taking into account structural similarities between AR antagonists and ligands of the $GABA_A$/benzodiazepine receptor. All compounds resulted totally inactive at A_{2A} and A_3 ARs and showed small (**8a**,**b**) or none (**7a**,**b**, **8c** and **9a**,**b**) affinity for A_1 AR. When tested on A_{2B} AR-transfected CHO cells, **7a**,**b** and **8a** acted as positive modulators, whereas **8b**,**c** and **9a**,**b** acted as negative modulators, enhancing or weakening the NECA-induced increase of cAMP levels, respectively. Compounds **7**–**9** might be regarded as useful biological and pharmacological tools to explore the therapeutic potential of A_{2B} AR modulators, while their 3-ketoindole scaffold might be taken as a reference to design new analogs.

1. Introduction

Adenosine is an endogenous purine nucleoside that modulates a variety of physiological processes by triggering specific cell membrane G-protein-coupled receptors (GPCRs) known as adenosine receptors (ARs). ARs are widely distributed in mammalian tissues and have been classified into four subclasses: A_1, A_{2A}, A_{2B}, and A_3 [1–3]. A_{2B} AR is defined as the "low-affinity" subtype because requires high micromolar concentrations of adenosine to be activated [4–6]. It couples to Gs proteins, thus stimulating adenylate cyclase and cAMP accumulation, as well as Gq proteins, resulting in phospholipase C activation and enhancement of the inositol trisphosphate and diacylglycerol pathways [7]. A_{2B} AR regulates a number of physiological and pathological events that involve lungs, mast cells, eyes, the gastrointestinal tract, bladder, adipose tissue, brain, kidneys, liver, and other tissues [2,6]. This receptor is the least well-characterized among the ARs primarily due to the lack of suitable, specific ligands [3,8–10]. Recently, several potent and selective A_{2B} AR agonists have been identified, and a phenylpyridinesulfanyl acetamide derivative (BAY 60-6583, **1** in Chart 1), is currently in preclinical studies for the treatment of atherosclerosis and coronary artery disorders [10,11]. To the best of our knowledge, no allosteric modulators of A_{2B} AR have been described in the literature thus far [12–14]. Because of the involvement of A_{2B} AR in several physiological and pathological processes, including glucose metabolism [15], angiogenesis induction [16,17], the growth and development of some tumors [18], and inflammation [19,20], potent and selective A_{2B} AR antagonists are currently being developed as candidates for the treatment of diabetic retinopathy and cancer [21,22], colitis [23,24], and asthma [25–27]. Several classes of A_{2B} AR antagonists, including compounds **2**–**6** represented in Chart 1, have been described to date [3,8–10,26]: pyrrolopyrimidines (**2**) [28], pyrazolotriazolopyrimidines (**3**) [29], 2-aminopyrazines (**4**) [30], xanthines (**5**) [31], and triazinobenzimidazolones (**6**) [32].

Compound **6** was recently identified by a screening study of our "in house" collection of triazinobenzimidazolones, which were originally described as ligands of the $GABA_A$/benzodiazepine receptor (BzR) [33] and subsequently modified to obtain A_1 AR

Abbreviations: ADA, adenosine deaminase; AR, adenosine receptor; cAMP, 3′,5′-cyclic adenosine monophosphate; CHO, Chinese hamster ovary; Cl-IBMECA, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's Modified Eagle Medium; GPCRs, G-protein coupled receptors; [^{3}H]DPCPX, [^{3}H]8-cyclopentyl-1,3-dipropylxanthine; [^{3}H]NECA, [^{3}H]5′-*N*-ethylcarboxamideadenosine; [^{125}I]AB-MECA, [^{125}I]4-aminobenzyl-5′-*N*-methylcarboxamidoadenosine; SEM, standard error of mean.

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antagonists [34,35]. This strategy of designing AR antagonists from BzR ligands by simple structural modifications dates back to the discovery of the first non-xanthine AR antagonists [36]. Adopting this approach, we have recently used the indol-3-ylglyoxylamide **7**, which is the prototype of several indole derivatives that we previously reported to be BzR ligands [37–44], as a reference structure to design the following 3-ketoindoles as potential AR antagonists: the *N*-(indol-3-ylglyoxyl)amides **7a**,**b**, the 3-(arylglyoxylyl)indoles **8a**–**c** and the indol-3-ylcarboxamides **9a**,**b** (Chart 2).

These compounds can be regarded as open chain analogs of the triazinobenzimidazolone **6**. Moreover, each compound contains two structural features that are common to the majority of A_{2B} AR antagonists reported in Chart 1: a) three lipophilic moieties connected to a heterocyclic core scaffold, the fused benzene ring, the benzyl attached to the indole nitrogen and the side chain aryl ring, and b) a hydrophilic moiety capable of making hydrogen bonds, the COCONH, COCO and CONH fragments. These two features were hypothesized to be critical for the binding of compound **6** and its derivatives to A_{2B} AR because docking studies suggested that they are involved in hydrophobic contacts and in a hydrogen bond between the ligand carbonyl oxygen and the Asn-254 side chain of the receptor, respectively [32]. This last interaction was reported to be necessary for the affinity of A_{2B} AR antagonists based on X-ray crystallography [45] and mutagenesis data [46].

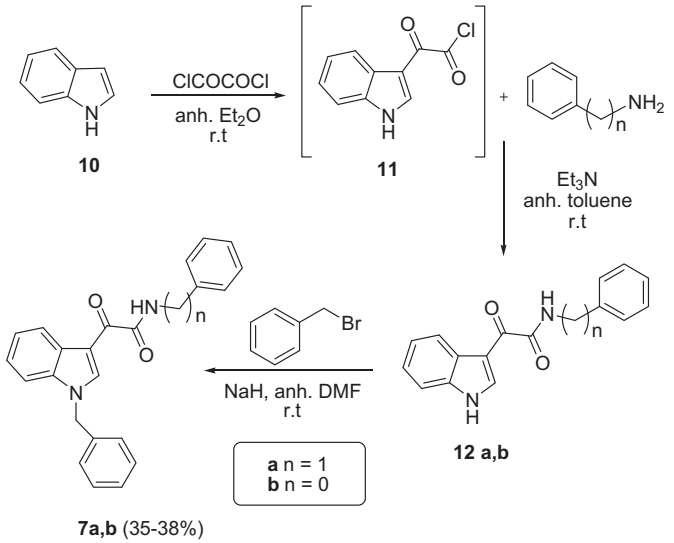
Here, we describe the synthesis and biological evaluation of the 3-ketoindoles **7**–**9** (Chart 2) on human A_1, A_{2A}, A_{2B} and A_3 ARs, which unexpectedly led to the identification of three compounds (**7a**,**b** and **8a**) as positive modulators and four compounds (**8b**,**c** and **9a**,**b**) as negative modulators of A_{2B} AR.

2. Chemistry

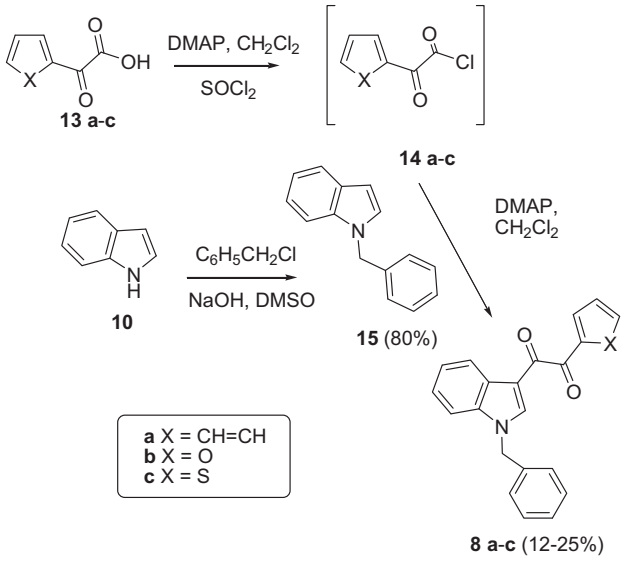
The general procedure employed to prepare compounds **7a**,**b** involved the acylation of commercially available indole **10** with oxalyl chloride to give the corresponding indol-3-ylglyoxyl chloride **11**, which was directly allowed to react with the appropriate amine in the presence of triethylamine in dry toluene solution, to obtain the amides **12a**,**b** (Scheme 1) [37]. Treatment of **12a**,**b** with sodium hydride and subsequent addition of benzyl bromide in dry DMF yielded the target derivatives **7a**,**b**.

The synthesis of compounds **8a**–**c** was achieved by the key intermediate **15**, as shown in Scheme 2. The suitable α-oxoacid (**13a**–**c**) reacted with $SOCl_2$ in the presence of DMAP in dichloromethane to provide the arylchloride (**14a**–**c**) that successively was treated with **15** using DMAP as a base to yield the desired α-diketo derivative **8a**–**c**.

Compounds **9a**,**b** are commercially available (Bionet).



Scheme 1. Synthesis of compounds **7a**,**b**.



Scheme 2. Synthesis of compounds **8a**–**c**.

3. Biological assays

The affinities of compounds **7**–**9** for human A_1, A_{2A} and A_3 ARs were evaluated by competition experiments assessing their respective abilities to displace [^{3}H]DPCPX, [^{3}H]NECA, or [^{125}I]AB-MECA binding from transfected CHO cells [35].

The functional activity of each compound at human A_1, and A_{2B} ARs was evaluated by cAMP assay, essentially following procedures previously described [32].

4. Results and discussion

The binding affinities of the 3-ketoindole derivatives **7**–**9** for human A_1, A_{2A} and A_3 ARs are summarized in Table 1, along with those of DPCPX, NECA, and Cl-IBMECA, which are used as the reference standards.

Table 1
Binding affinity of compounds **7**–**9** to human A_1, A_{2A}, and A_3 ARs.[a]

cpd	A_1 K_i (nM)[b]	A_{2A} K_i (nM)[c]	A_3 K_i (nM)[d]
7a	>10,000	>10,000	>1000
7b	>10,000	>10,000	>1000
8a	161.5 ± 17.4	>10,000	>1000
8b	343.0 ± 15.0	>10,000	>1000
8c	>10,000	>10,000	>1000
9a	>10,000	>10,000	>1000
9b	>10,000	>10,000	>1000
DPCPX	0.50 ± 0.03	337 ± 28	>1000
NECA	14 ± 4	16 ± 3	73 ± 5
Cl-IBMECA	890 ± 61	401 ± 25	0.22 ± 0.02

[a] Data are expressed as means ± SEM derived from an iterative curve-fitting procedure (Prism program, GraphPad, San Diego, CA); percentages refer to extent of inhibition of specific radioligand binding at 10 μM compound concentration.
[b] Displacement of specific [^{3}H]DPCPX binding in membranes obtained from human A_1 AR stably expressed in CHO cells.
[c] Displacement of specific [^{3}H]NECA binding in membranes obtained from human A_{2A} AR stably expressed in CHO cells.
[d] Displacement of specific [^{125}I]AB-MECA binding in membranes obtained from human A3 AR stably expressed in CHO cells.