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Original article

New chromene scaffolds for adenosine A_{2A} receptors: Synthesis, pharmacology and structure–activity relationships

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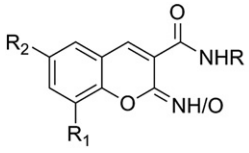
HIGHLIGHTS

- *In silico* screening identified a novel chromene scaffold for the human adenosine A_{2A} receptor.
- A focused library of 43 chromenes was synthesized to explore SAR.
- These compounds were tested in radioligand binding assays at human adenosine A_1, A_{2A}, A_{2B} and A_3 receptors.
- Eleven compounds were selective for the A_{2A} receptors at sub-micromolar concentration.

GRAPHICAL ABSTRACT



R_1- H, OH, OMe
R_2- H, Cl, Br
R- H, alkyl, cycloalkyl, heteroaromatic

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ABSTRACT

In silico screening of a collection of 1584 academic compounds identified a small molecule hit for the human adenosine A_{2A} receptor ($pK_i = 6.2$) containing a novel chromene scaffold (**3a**). To explore the structure–activity relationships of this new chemical series for adenosine receptors, a focused library of 43 2*H*-chromene-3-carboxamide derivatives was synthesized and tested in radioligand binding assays at human adenosine A_1, A_{2A}, A_{2B} and A_3 receptors. The series was found to be enriched with bioactive compounds for adenosine receptors, with 14 molecules showing submicromolar affinity ($pK_i \geq 6.0$) for at least one adenosine receptor subtype. These results provide evidence that the chromene scaffold, a core structure present in natural products from a wide variety of plants, vegetables, and fruits, constitutes a valuable source for novel therapeutic agents.

1. Introduction

Adenosine is an endogenous purinergic nucleoside, occurring in all cells of the body that modulates many physiologic and pathological conditions related to cardiovascular, immune, metabolic and neurological functions [1,2]. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3), belonging to the G protein-coupled receptor (GPCR) superfamily [3]. Therefore, it is of therapeutical interest to develop drugs active on adenosine receptors, as evidenced by the continuous patent claims on new compounds modulating adenosine receptors or new uses for selective ligands [4], with more than

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120 basic patent applications published in 2008–2009 related to adenosine receptor ligands [5]. Intensive efforts in medicinal chemistry in the adenosine receptor field yielded selective agonists and antagonists for the four receptor subtypes as well as allosteric modulators [3,5], some of them advancing to clinical trials or to FDA approval for diagnostic or therapeutic uses [2–6]. For example, A_1 ligands are under development for cardiovascular diseases, pain indications, and PET imaging agents [3–6]. Also, A_{2B} antagonists and dual A_{2B}/A_3 antagonists are being investigated for its use in asthma, diabetes, and cancer. In addition, A_3 agonists have been linked to inflammatory diseases, such as rheumatoid arthritis and psoriasis, liver cancer, hepatitis, and liver regeneration, and have shown efficacy in clinical trials for dry eye syndrome [3–6]. Finally, A_{2A} agonists are in clinical trials for cardiac imaging diagnostic and wound healing and some have been already approved for cardiac perfusion imaging [3–6], In particular, the A_{2A} receptor subtype is a very relevant biological target for drug discovery in neurodegenerative diseases, specifically antagonists for Huntington's [7], Alzheimer's [8], and Parkinson's [9–11] diseases, as well as PET imaging ligands [4,6]. Indeed, preladenant (SCH-420814), a pyrazolo[4,3-*e*]triazolo[1,5-*c*]pyrimidine derivative targeting these receptors, is already in phase III clinical trials for the treatment of Parkinson's disease [6,12]. The human A_{2A} receptor became the first non-rhodopsin, non-adrenergic GPCR for which an X-ray crystallographic structure was reported [13]. This progress can have a major effect on drug discovery in the A_{2A} research field together with the phenomenon of GPCR dimerization [3], as the well-established A_{2A} adenosine/D_2 dopamine receptor heterodimer is currently considered a target for the discovery of antagonists to treat Parkinson's disease [14].

The present work is part of an ongoing interdisciplinary project integrating organic and medicinal chemistry with *in silico* and *in vitro* pharmacology. In a previous study, we showed that *in silico* target profiling was able to identify the targets at which a chemical library of biologically-orphan molecules should be tested, leading to the identification of four novel antagonists for all four members of the adenosine receptor family [15]. Subsequently *in silico* target profiling of a library of 1584 compounds led to the identification of compound **3a** as a new hit for the A_{2A} receptor subtype containing a chromene scaffold (Fig. 1).

Chromene derivatives constitute an important class of compounds, widely present in plants, including edible vegetables and fruits [16]. Naturally occurring chromenes are now used as valuable leads for the design and synthesis of new active analogs with potential therapeutic applications, namely as anti-HIV, anti-tuberculosis, anti-inflammatory and antifungal agents [17]. Interestingly, there is overlapping activity between anti-infectious compounds and GPCR activity. One of the most recent examples is the antihistaminic astemizole that was reintroduced in the pharmaceutical market as an antimalarial agent [18].

In this work we introduce a new family of compounds based on the general structure of a 2*H*-chromene-3-carboxamide that was present in the original hit (**3a**). This chromene series shows a rich variety of affinity profiles for adenosine receptors, some with marked selectivity for the A_{2A} receptor subtype.

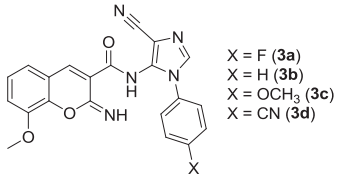


Fig. 1. Original chromene hit (**3a**) and related derivatives (**3b**–**d**).

2. Results and discussion

2.1. In silico target profiling

The increasing availability of drug–target interaction data has promoted the development of computational approaches that predict the affinity profile of small molecules across thousands of targets [19]. These methods represent an efficient means to prioritize those targets at which small molecules should be tested [15]. Accordingly, a collection of 1584 biologically-orphan molecules was profiled against ligand-based models available for 4643 proteins. This *in silico* target profiling identified four molecules (**3a**–**d**) containing a novel chromene scaffold for which affinity for adenosine receptors was predicted. The four compounds were then tested *in vitro* on all four adenosine receptors and micromolar affinity for the adenosine A_{2A} receptor subtype could be confirmed for compounds **3a** ($pK_i = 6.2 \pm 0.4$) and **3b** ($pK_i = 6.0 \pm 0.2$). These results set the basis for initiating a medicinal chemistry program around a chromene series.

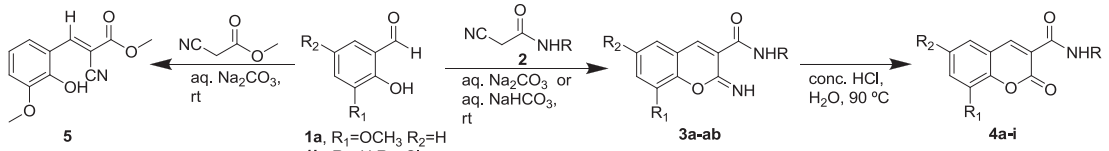
2.2. Chemistry

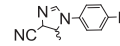
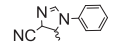
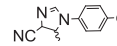
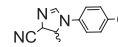
The synthesis of the target compounds is summarized in Schemes 1–3. To synthesize the 2-imino-2*H*-chromenes **3a**–**ab**, several 2-cyanoacetamides **2** were prepared by combining primary amines with 2-methylcyanoacetate [20] or by combining 5-amino-1-phenyl-1*H*-imidazole-4-carbonitrile [21] with 2-cyanoacetic acid. The Knoevenagel condensation of salicylaldehydes **1** with cyanoacetamides **2** was carried out at room temperature, using aqueous sodium carbonate or hydrogen carbonate solution. This eco-friendly procedure allowed the isolation of the 2-imino-2*H*-chromenes **3a**–**3ab** in 38–100% yield. The 2-oxo-2*H*-chromenes **4a**–**i** were isolated in 25–100% yield either from the corresponding 2-imino-2*H*-chromenes **3**, upon heating in an aqueous HCl solution, or after combining 2-cyanoacetamide **2** with salicylaldehyde **1** in aqueous base followed by addition of concentrated HCl and heating at 90 °C. When 2-methylcyanoacetate was combined with 8-methoxysalicylaldehyde **1a** in aqueous sodium carbonate solution, at room temperature, no condensation was observed and compound **5** was isolated after 20 min, in excellent yield (95%). When 1,3-dicarbonyl compounds **6** were reacted with 8-methoxysalicylaldehyde **1a** in water or in aqueous sodium carbonate solution and piperidine, at room temperature, compound **7a** (80% yield) or **7b** (69% yield) were isolated. The 2-cyanoacetamide resulting from the reaction of 2-methylcyanoacetate with the amino acids **8** (glycine and serine) could not be isolated. This amide was generated *in situ* and combined with salicylaldehydes **1a** and **1e** in aqueous sodium carbonate solution. The reaction mixture was stirred at room temperature, followed by addition of concentrated HCl and heating at 40 °C, leading to the isolation of compounds **9a**–**c** in 17–50% yield.

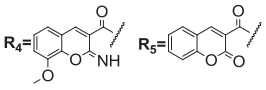
2.3. Pharmacology

All synthesized compounds were submitted to binding experiments with [^{3}H]-radioligands at human adenosine A_1, A_{2A}, A_{2B} and A_3 receptors heterologously expressed in mammalian cell lines. The inhibition percentage ($\%_{inhib.}$) was obtained for all the tested compounds at a concentration of 10 μM. The pK_i value was also calculated for those compounds presenting $\%_{inhib.}$ higher than 70% (Table 1).

Chromene **3e**, with no substitution in the 3-carboxamide moiety, presents a pK_i value of 7.1 ± 0.5 for adenosine A_{2A} receptors and lower affinities for A_1 (6.4 ± 0.3) and A_{2B} (6.5 ± 0.3) receptors. Fig. 2(a) shows a representative competition binding



Compd	R_1	R_2	R
3a	OCH_3	H	
3b	OCH_3	H	
3c	OCH_3	H	
3d	OCH_3	H	
3e	OCH_3	H	H
3f	OCH_3	H	$(CH_2)_2OCH_3$
3g	OCH_3	H	$(CH_2)_2OH$
3h	OCH_3	H	$(CH_2)_3OH$
3i	OCH_3	H	$(CH_2)_5OH$
3j	OCH_3	H	$(CH_2)_2Ph$(4-OH)
3k	OCH_3	H	$CH_2CH(Ph)OH$
3l	OCH_3	H	$C(CH_2OH)_3$
3m	OCH_3	H	$CH(CH_2CH_3)CH_2OH$
3n	OCH_3	H	
3o	OCH_3	H	
3p	OCH_3	H	$(CH_2)_3NHR_4$
3q	OCH_3	H	$(CH_2)_3N$  $N(CH_2)_3NHR_4$
3r	OCH_3	H	$(CH_2)_3O(CH_2)_4O(CH_2)_3NHR_4$
3s	OCH_3	H	$CH_2CH(OCH_2CH_3)_2$
3t	H	Cl	$(CH_2)_2OCH_3$
3u	H	Cl	$CH(CH_2CH_3)CH_2OH$
3v	OCH_3	Br	$(CH_2)_3OH$
3w	H	Br	$(CH_2)_2OH$
3x	H	Br	$(CH_2)_3OH$
3y	H	Br	$CH(CH_2CH_3)CH_2OH$
3z	H	Br	
3aa	H	Br	
3ab	H	Br	$CH_2CH(OH)CH_2OH$
4a	OCH_3	H	$(CH_2)_2OCH_3$
4b	OCH_3	H	$(CH_2)_2OH$
4c	OCH_3	H	$CH_2CH(OH)CH_2OH$
4d	H	H	$(CH_2)_3OH$
4e	H	H	
4f	H	H	$(CH_2)_3N$  $N(CH_2)_3NHR_5$
4g	OH	H	$(CH_2)_3OH$
4h	OH	H	$CH_2CH(OH)CH_2OH$
4i	H	Cl	$CH_2CH(OH)CH_2OH$

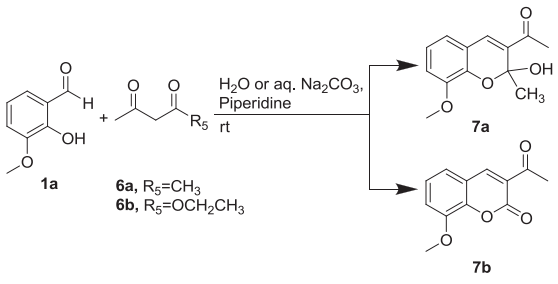


Scheme 1. Synthesis of compounds **3a**–**ab**, **4a**–**i**, and **5**.

curve for compound **3e** at A_{2A} receptors. This compound shows a higher affinity for A_{2A} receptors but also for the A_1 and A_{2B} receptors, reflecting a decrease in selectivity. These results indicate that the substitution in the 3-carboxamide moiety has an important role in the affinity profile of these compounds and also that the bulky *N*-(4-cyano-1-aryl-1*H*-imidazol-5-yl) group in compounds **3a** and **3b** contributes to the selectivity at A_{2A} receptors. However, when the substituent in the aryl moiety is the methoxyl (**3c**) or the cyano (**3d**) group, the affinity is considerably reduced probably due to steric hindrance in the binding site of A_{2A} receptors.

Comparing the affinity of chromene **3e** for the adenosine receptors with that of chromenes **7a** and **7b** that incorporate a ketone moiety in the 3-position of the chromene structure instead of the amide unit, a significant decrease in the affinities of these compounds can be observed. This suggests that the 3-carboxamide moiety has an important contribution for the affinity at adenosine receptors. The 2-imino substituent in the chromene structure is also a relevant feature for the activity of these compounds.

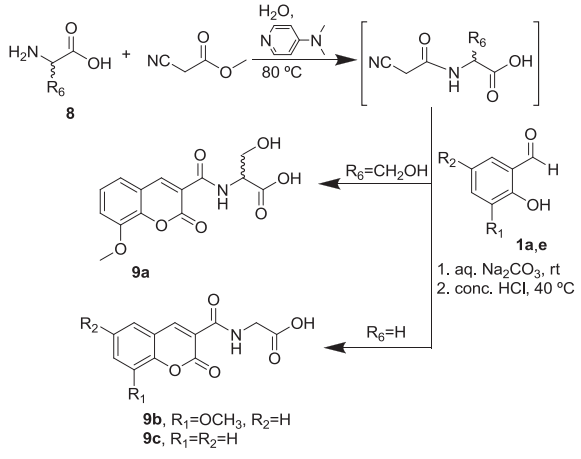
Chromene **3f** incorporates an alkyl chain with two methylene and a methoxyl group in the 3-carboxamide moiety and presented



Scheme 2. Synthesis of compounds **7a** and **b**.

pK_i values of 6.3 ± 0.2 for A_{2A} and 5.2 ± 0.4 for A_1 receptors. An increase in selectivity for A_{2A} over A_1 receptors was observed but, comparing with **3e**, a decrease in A_{2A} affinity was detected. For the same alkyl chain in the 3-carboxamide moiety, the 8-methoxyl group in the chromene ring was replaced by a 6-chlorine group (**3t**) and a decrease in affinity was obtained. The replacement of the 2-imino for a 2-oxo substituent (**4a**) also resulted in a decrease in affinity. This study showed the positive contribution of the 8-methoxyl group and again of the 2-imino moiety to the affinity of these chromenes for adenosine receptors.

For chromene **3g** the 2-hydroxyethyl group in the 3-carboxamide substituent led to a decrease in affinity for A_{2A} receptors (pK_i = 6.0 ± 0.2) when compared with the affinity of chromene **3e** at A_{2A} receptors, but selectivity at this receptor



Scheme 3. Synthesis of compounds **9a**–**c**.

Table 1
Binding affinities of the new compounds at human A_1, A_{2A}, A_{2B} and A_3 receptors (pK_i or $\%_{inhib}$ at 10 μM).

Compd.	Human adenosine receptors			
	hA_1	hA_{2A}	hA_{2B}	hA_3
3a	46 ± 3%	**6.2 ± 0.4**	30 ± 5%	20 ± 5%
3b	33 ± 4%	**6.0 ± 0.2**	9 ± 5%	40 ± 1%
3c	37 ± 2%	58 ± 1%	5 ± 5%	42 ± 4%
3d	18 ± 1%	41 ± 1%	10 ± 4%	1 ± 2%
3e	**6.4 ± 0.3**	**7.1 ± 0.5**	**6.5 ± 0.3**	33 ± 3%
3f	**5.2 ± 0.4**	**6.3 ± 0.2**	43 ± 6%	15 ± 5%
3g	47 ± 2%	**6.0 ± 0.2**	65 ± 2%	23 ± 5%
3h	47 ± 1%	**6.2 ± 0.3**	42 ± 5%	1 ± 5%
3i	44 ± 2%	**6.0 ± 0.1**	**5.9 ± 0.1**	13 ± 3%
3j	35 ± 3%	**6.9 ± 0.5**	**6.2 ± 0.2**	30 ± 2%
3k	55 ± 3%	**6.3 ± 0.2**	**6.0 ± 0.3**	8 ± 5%
3l	19 ± 1%	24 ± 2%	28 ± 1%	18 ± 5%
3m	24 ± 2%	66 ± 2%	39 ± 1%	6 ± 5%
3n	**5.5 ± 0.1**	**6.4 ± 0.3**	47 ± 5%	20 ± 4%
3o	40 ± 1%	**6.4 ± 0.2**	**4.7 ± 0.4**	16 ± 5%
3p	31 ± 5%	**6.4 ± 0.3**	**5.0 ± 0.2**	11 ± 4%
3q	**5.6 ± 0.2**	**6.5 ± 0.5**	**5.0 ± 0.2**	20 ± 4%
3r	30 ± 4%	**6.4 ± 0.1**	44 ± 5%	23 ± 5%
3s	40 ± 1%	**5.8 ± 0.2**	51 ± 1%	15 ± 3%
3t	17 ± 2%	10 ± 5%	9 ± 5%	13 ± 1%
3u	7 ± 2%	15 ± 5%	9 ± 0.3%	0%
3v	31 ± 1%	16 ± 2%	30 ± 2%	23 ± 1%
3w	26 ± 9%	6 ± 0.5%	15 ± 1%	0%
3x	32 ± 4%	14 ± 5%	25 ± 5%	17 ± 5%
3y	20 ± 3%	3 ± 1%	4 ± 5%	24 ± 2%
3z	17 ± 4%	5 ± 1.3%	28 ± 1%	0%
3aa	5 ± 2%	1 ± 5%	10 ± 5%	8 ± 5%
3 ab	11 ± 1%	8 ± 5%	4 ± 5%	10 ± 2%
4a	45 ± 10%	65 ± 0.2%	18 ± 4%	0%
4b	12 ± 6%	1 ± 4%	7 ± 4%	1 ± 5%
4c	14 ± 2%	7 ± 4%	25 ± 5%	1 ± 5%
4d	24 ± 4%	10 ± 4%	19 ± 3%	6 ± 3%
4e	16 ± 2%	1 ± 1%	18 ± 4%	20 ± 2%
4f	9 ± 1%	1 ± 2%	22 ± 5%	15 ± 1%
4g	18 ± 1%	1 ± 4%	24 ± 1%	8 ± 5%
4h	4 ± 0.3%	11 ± 2%	29 ± 6%	22 ± 1%
4i	21 ± 2%	2 ± 4%	23 ± 3%	1 ± 4%
5	**5.6 ± 0.4**	**6.5 ± 0.3**	**6.0 ± 0.3**	45 ± 1%
7a	6 ± 4%	10 ± 1%	24 ± 5%	30 ± 5%
7b	10 ± 1%	1 ± 5%	15 ± 1%	1 ± 5%
9a	12 ± 2%	6 ± 5%	24 ± 5%	3 ± 4%
9b	27 ± 6%	13 ± 2%	35 ± 5%	15 ± 5%
9c	1 ± 4%	2 ± 0.8%	9 ± 4%	0%

subtype was observed. Increasing the length of the hydroxylated chain by one methylene group (**3h**) led to a similar affinity for A_{2A} receptors ($pK_i = 6.2 \pm 0.3$), keeping the high selectivity. When an alkyl chain with five methylene groups (**3i**) was studied, the affinity at the A_{2A} receptors ($pK_i = 6.0 \pm 0.1$) was not significantly changed, but a decrease in selectivity was noticed as a comparable affinity at A_{2B} receptors was observed. Changing the 8-methoxyl group in the chromene ring to 8-methoxy-6-bromo or 6-bromo groups (compounds **3v**, **3w** and **3x**) led again to a decrease in affinity. For 2-oxochromenes **4b**, **4d** and **4g**, bearing the 2-hydroxyethyl/propyl group in the 3-carboxamide moiety and varying the chromene ring substitution, a decrease in affinity was again observed.

Chromenes **3l** and **3m** incorporate branched alkyl chains with hydroxyl groups in the 3-carboxamide moiety and presented very low affinities for all adenosine receptors subtypes. Chromene **3s** with a 2,2-diethoxyethyl group in the 3-carboxamide substituent presented a pK_i value of 5.8 ± 0.2 for A_{2A} receptors and a reasonable selectivity.

Chromenes **3n** and **3o**, with a cyclic alkyl group in the 3-carboxamide moiety presented pK_i values of 6.4 for A_{2A} receptors and the former was also active at A_1 while the later was active at A_{2B} receptors.

Chromene **3k**, with a 2-hydroxy-2-phenylethyl group in the 3-carboxamide moiety, presented pK_i values of 6.3 ± 0.2 for A_{2A} and 6.0 ± 0.3 for A_{2B} receptors, showing a low selectivity. However, in the case of chromene **3j**, where only the position of the OH group in the substituent is different, an increase in the selectivity was observed with pK_i values of 6.9 ± 0.5 for A_{2A} and 6.2 ± 0.2 for A_{2B} receptors were obtained.

Compounds **3p**, **3q** and **3r** are dimers where the two chromene units are connected by the 3-carboxamide moiety linked with an alkyl chain with oxygen/nitrogen heteroatoms. These 3 dimers have similar affinities for A_{2A} receptors (pK_i = 6.4–6.5) and showed a lower affinity for A_{2A} receptors when compared with chromene **3e**. They also show a higher selectivity, especially dimer **3r**. Dimeric chromene **4f** showed a very low affinity for all the adenosine receptors.

Compound **5**, a precursor of the chromene structure, was also tested and presented a pK_i value of 6.5 ± 0.3 for adenosine A_{2A} receptors and lower affinities for A_1 (5.6 ± 0.4) and A_{2B} (6.0 ± 0.3) receptors.

Chromenes **9a**, **b** and **c**, incorporating an amino acid unit in the 3-carboxamide moiety, presented low affinities for all the adenosine receptors.

From the most potent and/or selective compounds for A_{2A} receptors, 5 representative compounds were selected and tested in cyclic AMP assays to study their functional activity. The agonist/antagonist behaviour of compounds **3a**, **3e**, **3j**, **3o** and **3r** was examined by measuring their modulation of NECA-dependent intracellular cAMP formation. The results confirmed that the compounds were antagonists at A_{2A} receptors and their antagonist potency, expressed as pK_B values (Table 2) were in agreement with their affinity values (pK_i). Fig. 2 (b) shows the results of a representative experiment for the antagonist potency of compound **3e** at A_{2A} receptors.

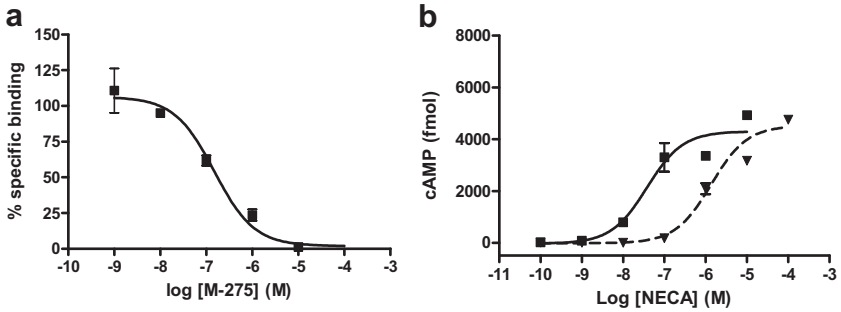


Fig. 2. (a) Competition binding curve for compound **3e** at A_{2A} receptors. (b) Concentration-response curves of NECA in the absence (■) and in the presence (▼) of 10 μM **3j** at human A_{2A} receptors expressed in CHO cells. Points represent the mean ± standard deviation (vertical bars) of duplicate measurements.

Table 2
Antagonistic potency (pK_B) of selected compounds at human A_{2A} receptors in cAMP assays.

Compounds	Potency (pK_B)
3a	6.02
3e	6.59
3j	6.54
3o	6.10
3r	6.36

3. Conclusion

In summary, several chromene-based compounds were designed, synthesized and pharmacologically tested for affinity for adenosine receptors. Compound **5** and chromenes **3a**, **3e**, **3f**, **3h**, **3j**, **3n**, **3o**, **3p**, **3q**, **3r** were selective for the A_{2A} receptors over the A_1, A_{2B} and A_3 receptors subtypes. This study showed the positive contribution of the 2-imino over the 2-oxo substituent in the chromene scaffold, the importance of the substituents in the 3-carboxamide moiety and the presence of the 8-methoxyl group in the chromene ring. These factors significantly affect the affinity of these chromenes towards adenosine receptors. Compound **3e** showed the highest affinity for A_{2A} receptors with no selectivity. The selectivity was improved in the other chromenes, with a linear/cyclic alkyl chain or substituted imidazole in the carboxamide moiety, with chromene **3r** as the most selective. In conclusion, a number of chromene-based compounds are herein described, some of them with selectivity for A_{2A} receptors at submicromolar concentration.

4. Experimental

4.1. Chemistry

Chemicals and organic solvents were purchased from commercial sources and used without further purification. All new compounds were fully characterized by elemental analysis and spectroscopic data. The NMR spectra were recorded at room temperature, on a Varian Unity Plus (^{1}H: 300 MHz, ^{13}C: 75 MHz) and Bruker Avance 3400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), including the ^{1}H-^{13}C correlation spectra (HMQC and HMBC) and deuterated DMSO was used as solvent. Chemical shifts (δ) were reported in parts per million (ppm) and the coupling constants, *J*, were reported in hertz (Hz). The melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. The purities of all tested compounds are higher than 95% by elemental analysis, which were performed on a LECO CHNS-932 instrument and were reported to be within 0.4% of calculated values. High resolution mass spectra (HRMS) were obtained from the C.A.C.T.I. – Universidade de Vigo. Compounds **2**, **3** and **4** were synthesized according to previously reported procedures [20]. Compound **2** with R = H is commercially available and compounds **2** bearing the imidazole substituent in the R position are new and were fully characterized in this publication. Chromenes **3a**–**d**, **3v**–**ab** and **4a**, **b**, **d**, **e** and **g** are also new and were fully characterized in this publication. Compound **7b** was previously reported [22].

4.1.1. General procedure for the preparation of compounds **2** bearing the imidazole substituent in the R position

5-Amino-1-phenyl-1*H*-imidazole-4-carbonitrile [22] (0.82 mmol) was added to a solution of 2-cyanoacetic acid (0.94 mmol) in acetic anhydride (1 mL) and the reaction mixture was heated in a water bath at 90 °C for 13 min to 4 h. The reaction mixture was cooled to room temperature, water (2 mL) was added and the solution heated in a water bath at 90 °C for 10–40 min. The solvents were removed in the rotary evaporator and the appropriate solvent was added to the crude mixture to precipitate the product identified as 2-cyano-*N*-(4-cyano-1-phenyl-1*H*-imidazol-5-yl)acetamide **2**. The solid was filtered and washed with the appropriate solvent.

*4.1.1.1. 2-Cyano-N-(4-cyano-1-(4-fluorophenyl)-1H-imidazol-5-yl) acetamide (**2a**).* Isolated as an off-white solid in 50% yield. Acetone and diethyl ether were used for solid precipitation. Mp 176–178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.94 (s, 2H), 7.34–7.47 (m, 2H), 7.47–7.56 (m, 2H), 8.14 (s, 1H), 10.69 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.70, 108.40, 114.17, 115.16, 116.69 (d, J = 23.2 Hz, 2C), 127.53 (d, J = 9.2 Hz, 2C), 129.46 (d, J = 2.6 Hz), 133.61, 138.10, 162.10, 162.88. Anal. Calcd for $C_{13}H_8N_5OF$: C, 57.99; H, 2.97; N, 26.02. Found: C, 57.86; H, 3.17; N, 25.74.

*4.1.1.2. 2-Cyano-N-(4-cyano-1-phenyl-1H-imidazol-5-yl)-acetamide (**2b**).* Isolated as an off-white solid in 88% yield. Dichloromethane was used for solid precipitation. Mp 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (s, 2H), 7.42–7.48 (m, 2H), 7.50–7.60 (m, 3H), 8.16 (s, 1H), 10.72 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.69, 108.73, 114.19, 115.16, 124.85 (2C), 129.26 (2C), 129.86, 133.40, 133.53, 138.05, 162.90. Anal. Calcd for $C_{13}H_9N_5O$: C, 62.15; H, 3.59; N, 27.89. Found: C, 61.89; H, 3.54; N, 27.70.

*4.1.1.3. 2-Cyano-N-(4-cyano-1-(4-methoxyphenyl)-1H-imidazol-5-yl)acetamide (**2c**).* Isolated as an off-white solid in 76% yield. After heating the reaction mixture in acetic anhydride and cooling to room temperature, the product precipitated, was filtered and washed with water. Mp 195–197 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H), 3.93 (s, 2H), 7.09 (d, J = 9.0 Hz, 2H), 7.36 (d, J = 9.0 Hz, 2H), 8.07 (s, 1H), 10.64 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.69, 55.60, 108.38, 114.88 (2C), 114.29, 115.20, 126.21 (2C), 126.51, 133.62, 138.16, 159.72, 162.91. Anal. Calcd for $C_{14}H_{11}N_5O_2.0.02H_2O$: C, 59.71; H, 3.92; N, 24.88. Found: C, 59.82; H, 4.19; N, 24.61.

*4.1.1.4. 2-Cyano-N-(4-cyano-1-(4-cyanophenyl)-1H-imidazol-5-yl) acetamide (**2d**).* Isolated as an orange solid in 67% yield. After heating in acetic anhydride the reaction mixture was stirred at room temperature for 54 h and then water was added, following the above described procedure. The desired product precipitated from water. Mp 165–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (s, 2H), 7.70 (dd, J = 6.8, 2.0 Hz, 2H), 8.06 (dd, J = 6.8, 2.0 Hz, 2H), 8.23 (s, 1H), 10.81 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.79, 108.62, 111.77, 114.00, 115.14, 117.94, 125.70 (2C), 133.59, 134.00 (2C), 137.35, 137.80, 162.85. Anal. Calcd for $C_{14}H_8N_6O.0.7H_2O$: C, 58.21; H, 3.26; N, 29.11. Found: C, 58.33; H, 3.27; N, 28.83.

4.1.2. Chromenes **3a**–**d**, **3v**–**ab** and **4a**, **b**, **d**, **e** and **g**

These compounds were synthesized according to previously reported procedures [21].

*4.1.2.1. N-(4-Cyano-1-(4-fluorophenyl)-1H-imidazol-5-yl)-2-imino-8-methoxy-2H-chromene-3-carboxamide (**3a**).* Compound **3a** was isolated as a yellow solid in 94% yield. Mp 146–149 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.88 (s, 3H), 7.21 (t, J = 7.8 Hz, 1H), 7.26–7.48 (m, 4H), 7.55–7.68 (m, 2H), 8.05 (s, 1H), 8.53 (s, 1H), 9.31 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.04, 105.33, 115.11, 116.46 (d, J = 30.5 Hz, 2C), 116.84, 118.36, 118.76, 121.54, 124.30, 128.18 (d, J = 12.2 Hz, 2C), 129.84 (d, J = 3.9 Hz), 134.96, 136.76, 142.73, 143.30, 145.89, 155.36, 162.16 (d, J = 327.2 Hz). Anal. Calcd for $C_{21}H_{14}N_5O_3F.H_2O$: C, 59.86; H, 3.80; N, 16.63. Found: C, 59.85; H, 3.77; N, 16.58.

*4.1.2.2. N-(4-Cyano-1-phenyl-1H-imidazol-5-yl)-2-imino-8-methoxy-2H-chromene-3-carboxamide (**3b**).* Compound **3b** was isolated as a yellow solid in 94% yield. Mp 208–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.86 (s, 3H), 7.18 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 7.44–7.58 (m, 5H), 8.09 (s, 1H), 8.50 (s, 1H), 9.26 (s, 1H), 13.16 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.00, 106.00, 115.04, 116.33, 118.34, 118.72, 121.51, 124.27, 125.47 (2C), 129.38, 129.78 (2C), 133.51, 134.57, 136.82, 142.73, 143.32, 145.85, 155.26, 160.34. Anal. Calcd for $C_{21}H_{15}N_5O_3.0.6H_2O$: C, 63.67; H, 4.09; N, 17.69. Found: C, 63.67; H, 3.98; N, 17.62.

*4.1.2.3. N-(4-Cyano-1-(4-methoxyphenyl)-1H-imidazol-5-yl)-2-imino-8-methoxy-2H-chromene-3-carboxamide (**3c**).* Compound **3c** was isolated as a yellow solid in 97% yield. Mp 184–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.80 (s, 3H), 3.86 (s, 3H), 7.07 (d, J = 9.0 Hz, 1H), 7.19 (t, J = 8.1 Hz, 1H), 7.26–7.36 (m, 2H), 7.44 (s, 1H), 7.99 (s, 1H), 8.52 (s, 1H), 9.28 (s, 1H), 13.19 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.52, 56.03, 105.18, 114.89 (2C), 115.24, 116.36, 118.38, 118.76, 121.54, 124.30, 126.05, 127.18 (2C), 134.77, 136.85, 142.76, 143.28, 145.88, 155.23, 159.78, 160.14. Anal. Calcd for $C_{22}H_{17}N_5O_4.1.5H_2O$: C, 59.73; H, 4.52; N, 15.84. Found: C, 59.48; H, 4.49; N, 15.81.

*4.1.2.4. N-(4-Cyano-1-(4-cyanophenyl)-1H-imidazol-5-yl)-2-imino-8-methoxy-2H-chromene-3-carboxamide (**3d**).* Compound **3d** was isolated as an off-white solid in 86% yield. Mp 213–216 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.88 (s, 3H), 7.23 (t, J = 6.0 Hz, 1H), 7.31–7.37 (m, 2H), 7.79 (dd, J = 8.8, 2.0 Hz, 1H), 8.04 (dd, J = 6.8, 2.0 Hz, 1H), 8.15 (s, 1H), 9.45 (s, 1H), 13.35 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.06, 105.76, 111.97, 114.97, 116.44, 118.04, 118.44, 118.82, 121.54, 124.41, 126.37 (2C), 133.94 (2C), 135.32, 136.42, 137.46, 142.64, 143.42, 145.92, 155.76, 160.37. Anal. Calcd for $C_{22}H_{14}N_6O_3.1.5H_2O$: C, 60.41; H, 3.89; N, 19.22. Found: C, 60.66; H, 5.90; N, 19.30.

*4.1.2.5. 6-Bromo-N-(3-hydroxypropyl)-2-imino-8-methoxy-2H-chromene-3-carboxamide (**3v**).* Compound **3v** was isolated as a yellow solid in 99% yield. Mp 173–175 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64 (q, J = 7.2 Hz, 2H), 3.32–3.34 (m, 2H), 3.44 (t, J = 6.0 Hz, 2H), 3.88 (s, 3H), 4.51 (s, 1H), 7.38 (d, J = 2.0 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 8.31 (d, J = 1.2 Hz, 1H), 9.16 (s, 1H), 10.24 (t, J = 5.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 32.12, 36.16, 56.42, 58.31, 115.13, 117.78, 120.36, 121.53, 122.87, 139.46, 142.00, 146.66, 154.39, 161.07. Anal. Calcd for $C_{14}H_{15}N_2O_4Br.0.5H_2O$: C, 46.15; H, 4.40; N, 7.69. Found: C, 46.15; H, 4.44; N, 7.63.

*4.1.2.6. 6-Bromo-N-(2-hydroxyethyl)-2-imino-2H-chromene-3-carboxamide (**3w**).* Compound **3w** was isolated as a yellow solid in 81% yield. Mp 160–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.30–3.40 (m, 2H), 3.48–3.51 (m, 2H), 4.70–4.80 (brs, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.66 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 8.01 (d, J = 2.0 Hz, 1H), 8.38 (s, 1H), 9.10 (s, 1H), 10.33 (t, J = 5.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.91, 59.58, 115.32, 117.05, 120.55, 121.43, 131.78, 134.94, 139.39, 152.52, 154.61, 161.11. Anal. Calcd for $C_{12}H_{11}N_2O_3Br.0.5H_2O$: C, 45.00; H, 3.75; N, 8.75. Found: C, 44.90; H, 3.74; N, 8.73.

*4.1.2.7. 6-Bromo-N-(3-hydroxypropyl)-2-imino-2H-chromene-3-carboxamide (**3x**).* Compound **3x** was isolated as a yellow solid in 94% yield. Mp 161–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64 (q, J = 6.9 Hz, 2H), 3.25–3.39 (m, 2H), 3.40–3.49 (m, 2H), 4.20–4.80 (brs, 1H), 7.15 (d, J = 9.0 Hz, 1H), 7.66 (dd, J = 8.7 Hz, 2.4 Hz, 1H), 8.01 (d, J = 2.4 Hz, 1H), 8.37 (s, 1H), 9.11 (s, 1H), 10.22 (t, J = 5.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 32.12, 36.17, 58.31, 115.34, 117.06, 120.56, 121.42, 131.78, 134.95, 139.33, 152.50, 154.79, 161.07. HRMS-FAB (m/z): [M + H]+ calcd. for $C_{13}H_{13}N_2O_3Br$: 327.01682, found: 327.01573.

*4.1.2.8. 6-Bromo-N-(1-hydroxybutan-2-yl)-2-imino-2H-chromene-3-carboxamide (**3y**).* Compound **3y** was isolated as a yellow solid in 95% yield. Mp 134–136 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42–1.60 (m, 5H), 3.27–3.32 (m, 1H), 3.40 (t, J = 6.0 Hz, 2H), 4.41 (s, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.66 (dd, J = 7.6 Hz, 2.4 Hz, 1H), 8.01 (d, J = 2.4 Hz, 1H), 8.37 (s, 1H), 9.10 (s, 1H), 10.22 (t, J = 5.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.62, 29.98, 39.64, 60.12, 115.33, 117.10, 120.55, 121.42, 131.76, 134.94, 139.33, 152.48, 154.85, 160.94. Anal. Calcd for $C_{14}H_{15}N_2O_3Br.0.1H_2O$: C, 49.40; H, 4.46; N, 8.22. Found: C, 49.17; H, 4.54; N, 8.05.

*4.1.2.9. 6-Bromo-N-cyclopentyl-2-imino-2H-chromene-3-carboxamide (**3z**).* Compound **3z** was isolated as an off-white solid in 96% yield. Mp 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36–1.48 (m, 2H), 1.49–1.72 (m, 4H), 1.84–1.95 (m, 2H), 4.08–4.21 (m, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.65 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 8.01 (d, J = 2.0 Hz, 1H), 8.36 (s, 1H), 9.06 (s, 1H), 10.30 (d, J = 7.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.26 (2 C), 32.55 (2 C), 50.72, 115.33, 117.04, 120.55, 121.33, 131.75, 134.91, 139.58, 152.46, 154.94, 160.35. Anal. Calcd for $C_{15}H_{15}N_2O_2Br$: C, 53.73; H, 4.48; N, 8.36. Found: C, 53.74; H, 4.32; N, 8.28.

*4.1.2.10. 6-Bromo-N-cyclohexyl-2-imino-2H-chromene-3-carboxamide (**3aa**).* Compound **3aa** was isolated as a white solid in 100% yield. Mp 207–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20–1.40 (m, 5H), 1.45–2.00 (m, 5H), 3.76–3.78 (m, 1H), 7.16 (d, J = 8.8 Hz, 1H), 7.67 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 8.02 (d, J = 2.4 Hz, 1H), 8.38 (s, 1H), 9.10 (s, 1H), 10.31 (d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 (2 C), 25.14, 32.00 (2 C), 115.34, 117.05, 120.58, 121.40, 131.78, 134.95, 139.43, 152.48, 154.96, 159.92. Anal. Calcd for $C_{16}H_{17}N_2O_2Br$: C, 55.01; H, 4.87; N, 8.02. Found: C, 53.03; H, 4.78; N, 7.94.

*4.1.2.11. 6-Bromo-N-(2,3-dihydroxypropyl)-2-imino-2H-chromene-3-carboxamide (**3ab**).* Compound **3ab** was isolated as a yellow solid in 88% yield. Mp 166–168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.10–3.22 (m, 1H), 3.24–3.39 (m, 2H), 3.46–3.68 (m, 1H), 4.62 (d, J = 5.2 Hz, 1H), 4.89 (d, J = 4.8 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.66 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 8.01 (d, J = 2.4 Hz, 1H), 8.38 (d, J = 2.4 Hz, 1H), 9.10 (d, J = 1.2 Hz, 1H), 10.31 (t, J = 5.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.16, 63.71, 70.01, 115.31, 117.04, 120.56, 121.44, 131.78, 134.94, 139.39, 152.53, 154.60, 161.18. Anal. Calcd for $C_{13}H_{13}N_2O_4Br$: C, 45.75; H, 3.81; N, 8.21. Found: C, 45.53; H, 3.84; N, 8.34.

*4.1.2.12. 8-Methoxy-N-(2-methoxyethyl)-2-oxo-2H-chromene-3-carboxamide (**4a**).* Compound **4a** was isolated as a yellow solid in 88% yield. Mp 151–153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.28 (s, 3H), 3.47–3.48 (m, 4H), 3.91 (s, 3H), 7.33 (t, J = 7.8 Hz, 1H), 7.40 (dd, J = 8.1 Hz, 1.5 Hz, 1H), 7.49 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 8.81 (d, J = 1.2 Hz, 1H), 8.79 (brs, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.87, 58.02, 70.29, 116.00, 118.99, 121.16, 125.07, 143.18, 146.23, 147.85, 160.20, 161.02. Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.65; H, 5.42; N, 5.05. Found: C, 60.61; H, 5.34; N, 5.30.

*4.1.2.13. N-(2-Hydroxyethyl)-8-methoxy-2-oxo-2H-chromene-3-carboxamide (**4b**).* Compound **4b** was isolated as an off-white solid in 55% yield. Mp 194–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.39 (q, J = 5.4 Hz, 2H), 3.53 (q, J = 5.4 Hz, 2H), 3.92 (s, 3H), 4.86 (t, J = 4.8 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.40 (dd, J = 8.2 Hz, 1.8 Hz, 1H), 7.49 (dd, J = 7.6 Hz, 1.5 Hz, 1H), 8.83 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.84, 56.14, 59.43, 115.95, 118.76, 121.15, 125.05, 143.15, 146.22, 147.77, 160.18, 160.97. Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.32; H, 4.94; N, 5.32. Found: C, 59.15; H, 4.83; N, 5.41.

*4.1.2.14. N-(3-Hydroxypropyl)-2-oxo-2H-chromene-3-carboxamide (**4d**).* Compound **4d** was isolated as a yellow solid in 63% yield. Mp 132–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67 (q, J = 6.4 Hz, 2H), 3.38 (q, J = 6.0 Hz, 2H), 3.47 (q, J = 6.0 Hz, 2H), 4.55 (s, 1H), 7.42 (td, J = 7.4 Hz, 1.2 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.73 (td, J = 7.6 Hz, 1.6 Hz, 1H), 7.97 (dd, J = 7.8 Hz, 1.2 Hz, 1H), 8.75 (s, 1H), 8.84 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.99, 36.63, 58.55, 116.10, 118.47, 119.05, 125.09, 130.21, 133.99, 147.29, 153.83, 160.33, 161.02. Anal. Calcd for $C_{13}H_{13}NO_4.0.05H_2O$: C, 62.93; H, 5.28; N, 5.65. Found: C, 62.65; H, 5.28; N, 6.08.

*4.1.2.15. N-Cyclopentyl-2-oxo-2H-chromene-3-carboxamide (**4e**).* Compound **4e** was isolated as an off-white solid in 55% yield. Mp 142–144 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40–1.74 (m, 6H), 1.84–2.00 (m, 2H), 4.15–4.27 (m, 1H), 7.42 (td, J = 7.5 Hz, 0.9 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.73 (td, J = 8.2 Hz, 1.5 Hz, 1H), 7.96 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 1H), 8.81 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.35 (2 C), 32.46 (2 C), 50.80, 116.12, 118.47, 119.15, 125.12, 130.18, 133.98, 147.09, 153.79, 160.55 (2 C). Anal. Calcd for $C_{15}H_{14}NO_3.0.1H_2O$: C, 69.82; H, 5.51; N, 5.43. Found: C, 69.93; H, 5.82; N, 5.61.

*4.1.2.16. 8-Hydroxy-N-(3-hydroxypropyl)-2-oxo-2H-chromene-3-carboxamide (**4g**).* Compound **4g** was isolated as a yellow solid in 64% yield. Mp 196–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76 (q, J = 6.3 Hz, 2H), 3.32–3.42 (m, 2H), 3.47 (q, J = 4.2 Hz, 2H), 4.56 (s, 1H), 7.17–7.23 (m, 2H), 7.37 (dd, J = 6.6 Hz, 3.3 Hz, 1H), 8.77 (s, 2H), 10.42 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 32.03, 36.61, 58.56, 118.80, 119.44, 120.05 (2 C), 125.09, 142.53, 144.44, 147.80, 160.30, 161.14. Anal. Calcd for $C_{13}H_{13}NO_50.2H_2O$: C, 58.52; H, 5.03; N, 5.25. Found: C, 58.54; H, 4.85; N, 5.22.

*4.1.3. Procedure for the preparation of methyl 2-cyano-3-(2-hydroxy-3-methoxyphenyl)prop-2-enoate **5***

Methyl 2-cyanoacetate (0.08 g, 0.81 mmol) was added to a solution of 8-methoxysalylaldehyde **1a** (0.15 g, 0.98 mmol) in aq. Na_2CO_3 (0.05 M, 3 mL) and the reaction mixture was stirred at room temperature, for 25 min. The yellow solid was filtered and washed with water (1 mL) and a few drops of diethyl ether (0.17 g, 0.77 mmol, 95%). Mp 113–115 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.85 (s, 3H), 3.84 (s, 3H), 6.94 (t, J = 8.1 Hz, 1H), 7.21 (dd, J = 8.1 Hz, 1.5 Hz, 1H), 7.72 (dd, J = 8.2 Hz, 1.5 Hz, 1H), 8.66 (s, 1H), 10.21 (brs, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.22, 56.08, 100.21, 115.90, 116.54, 118.41, 119.03, 119.44, 148.10, 148.41, 149.01, 162.80. Anal. Calcd for $C_{12}H_{11}NO_4.0.1H_2O$: C, 61.31; H, 4.77; N, 5.96. Found: C, 61.23; H, 4.79; N, 6.09.

*4.1.4. General procedure for the preparation of 8-methoxy-2H-chromenes **7a** and **7b***

1,3-Dicarbonyl compound **6** (1.20 mmol) was added to a solution of 8-methoxysalicylaldehyde **1a** (0.09 g, 0.59 mmol) in water (1 mL, for R_5 = CH_3) or aq. Na_2CO_3 (0.05 M, 1 mL, for R_5 = OCH_2CH_3) and piperidine (1 equiv, 60 μL). The reaction mixture was stirred at room temperature, for 4–30 h. The solid was filtered and washed with water (1 mL) and a few drops of diethyl ether.

*4.1.4.1. 1-(2-Hydroxy-8-methoxy-2-methyl-2H-chromen-3-yl)ethanone (**7a**).* Compound **7a** was isolated as a white solid in 80% yield. Mp 172–173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83 (s, 3H), 2.38 (s, 3H), 3.77 (s, 3H), 6.92 (t, J = 8.1 Hz, 1H), 6.95 (s, 1H), 6.99–7.05 (m, 2H), 7.66 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.15, 27.31, 55.66, 97.73, 114.81, 120.08, 120.66, 120.76, 133.88, 134.55, 142.21, 147.80, 196.45. Anal. Calcd for $C_{13}H_{14}O_4$: C, 66.67; H, 5.98. Found: C, 66.44; H, 6.13.

*4.1.5. General procedure for the preparation of 8-methoxy-2-oxo-2H-chromenes **9a–c***

Amino acid **8** (2.95 mmol) was added to a solution of methyl 2-cyanoacetate (0.29 g, 2.93 mmol) and 4-(dimethylamino)pyridine (0.36 g, 2.95 mmol) in water (0.6 mL) and the reaction mixture was stirred in a water bath at 80 °C for 3 h. An aqueous solution of Na_2CO_3 (0.05 M, 5 mL) and aldehyde **1** (0.45 g, 2.96 mmol) were added to the reaction mixture that was stirred at room temperature for 17 h. Concentrated HCl (3.7 equiv, 0.9 mL) was added and the solution was heated in a water bath at 40 °C for 30 min. The solid was filtered and washed with water (1 mL) and a few drops of diethyl ether.

*4.1.5.1. N-[(8-methoxy-2-oxo-2H-chromen-3-yl)carbonyl]-serine (**9a**).* Compound **9a** was isolated as a yellow solid in 50% yield. Mp 249–251 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.75 (dd, J = 11.0 Hz, 3.6 Hz, 1H), 3.88 (dd, J = 11.2 Hz, 3.9 Hz, 1H), 3.93 (s, 3H), 4.55 (q, J = 3.3 Hz, 1H), 5.26 (brs, 1H), 7.35 (t, J = 8.4 Hz, 1H), 7.42 (dd, J = 8.1 Hz, 1.5 Hz, 1H), 7.52 (dd, J = 7.8 Hz, 1.5 Hz, 1H), 8.88 (s, 1H), 9.23 (d, J = 7.5 Hz, 1H), 12.95 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.85, 56.16, 61.10, 116.22, 118.12, 118.95, 121.29, 125.15, 143.29, 146.25, 148.42, 160.28, 160.62, 171.54. Anal. Calcd for $C_{14}H_{13}NO_7$: C, 54.72; H, 4.23; N, 4.56. Found: C, 54.74; H, 3.96; N, 4.65.

*4.1.5.2. N-[(8-methoxy-2-oxo-2H-chromen-3-yl)carbonyl]-glycine (**9b**).* Compound **9b** was isolated as an off-white solid in 35% yield. Mp 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.93 (s, 3H), 4.03 (d, J = 5.6 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.44 (dd, J = 8.4 Hz, 1.6 Hz, 1H), 7.53 (dd, J = 7.8 Hz, 1.6 Hz, 1H), 8.87 (s, 1H), 9.03 (t, J = 5.6 Hz, 1H), 12.78 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.58, 56.19, 116.20, 118.33, 118.96, 121.27, 125.09, 143.30, 146.25, 148.26, 160.05, 161.07, 170.74. Anal. Calcd for $C_{13}H_{11}NO_6.1H_2O$: C, 52.88; H, 4.41; N, 4.75. Found: C, 52.63; H, 4.29; N, 4.64.

*4.1.5.3. N-[(2-oxo-2H-chromen-3-yl)carbonyl]glycine (**9c**).* Compound **9c** was isolated as an orange solid in 17% yield. Mp 249–251 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.05 (d, J = 5.6 Hz, 1H), 7.44 (td, J = 7.6 Hz, 1.2 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.75 (td, J = 7.8 Hz, 1.6 Hz, 1H), 7.99 (dd, J = 8.0 Hz, 1.6 Hz, 1H), 8.90 (s, 1H), 9.02 (t, J = 5.6 Hz, 1H), 12.80 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.48, 116.15, 118.17, 118.40, 125.14, 134.27, 130.39, 153.97, 148.08, 160.35, 161.14, 170.77. Anal. Calcd for $C_{12}H_9NO_5$: C, 58.30; H, 3.64; N, 5.67. Found: C, 58.23; H, 3.67; N, 5.64.

4.2. Radioligand binding assays

The inhibition percentage of the compounds was assayed at the concentration of 10 μM at all adenosine receptors following the conditions stated above. Competition binding curves at all receptors were carried out by assaying 6 different concentrations (range from 10 nM to 100 μM) for all the compounds showing an inhibition percentage above 70%. The −log of the inhibition constant (pK_i) of each compound was calculated by the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_D)$, where IC_{50} is the concentration of compound that displaces the binding of the radioligand by 50%, [L] is the free radioligand concentration and K_D is the dissociation constant of each radioligand. IC_{50} values were obtained by non-linear regression fitting the data, using Prism 2.1 software (GraphPad, San Diego, CA).

4.2.1. Human A_1 receptors

Adenosine A_1 receptor competition binding experiments were carried out in membranes from CHO-A_1 cells (Euroscreen, Gosselies, Belgium). On the day of assay, membranes were defrosted and re-suspended in incubation buffer 20 mM Hepes, 100 mM NaCl,

10 mM $MgCl_2$, 2 UI/ml adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 15 μg of protein, 2 nM [^{3}H]DPCPX and test compound. Non-specific binding was determined in the presence of 10 μM (*R*)-PIA. The reaction mixture was incubated at 25 °C for 60 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

4.2.2. Human A_{2A} receptors

Adenosine A_{2A} receptor competition binding experiments were carried out in membranes from HeLa-A_{2A} cells. On the day of assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris–HCl, 1 mM EDTA, 10 mM $MgCl_2$ and 2 UI/mL adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 10 μg of protein, 3 nM [^{3}H]ZM241385 and test compound. Non-specific binding was determined in the presence of 50 μM NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

4.2.3. Human A_{2B} receptors

Adenosine A_{2B} receptor competition binding experiments were carried out in membranes from HEK-293-A_{2B} cells (Euroscreen, Gosselies, Belgium) prepared following the provider's protocol. On the day of assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris–HCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.1 mM benzamidine, 10 μg/mL bacitracine and 2 UI/mL adenosine deaminase (pH = 6.5). Each reaction well prepared in duplicate, contained 18 μg of protein, 35 nM [^{3}H]DPCPX and test compound. Non-specific binding was determined in the presence of 400 μM NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered through a multiscreen GF/C microplate and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

4.2.4. Human A_3 receptors

Adenosine A_3 receptor competition binding experiments were carried out in membranes from HeLa-A_3 cells. On the day of assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris–HCl, 1 mM EDTA, 5 mM $MgCl_2$ and 2 UI/mL adenosine deaminase (pH = 7.4). Each reaction well of a GF/B multiscreen plate (Millipore, Madrid, Spain), prepared in triplicate, contained 90 μg of protein, 30 nM [^{3}H]NECA and test compound. Non-specific binding was determined in the presence of 100 μM (*R*)-PIA. The reaction mixture was incubated at 25 °C for 180 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

4.3. Cyclic AMP assay

The adenylyl cyclase activity mediated by A_{2A} receptors was evaluated in CHO cells stably expressing the receptors, by measuring the levels of cAMP accumulated in response to increasing concentrations of NECA in the absence or presence of test compounds. Briefly, cells grown in 96 well plates with growth medium containing dialyzed fetal bovine serum, were washed twice with DMEM F-12 nutrient mixture medium containing 25 mM HEPES pH 7.4 and 30 μM of the phosphodiesterase inhibitor rolipram (incubation buffer). Then antagonists were preincubated for 15 min in assay medium and after this time, different concentrations (0.1 nM-1 mM) of the agonist NECA were added to each well. The incubation was continued for 15 min and cAMP was quantified by an enzymeimmunoassay (Perkin Elmer).

NECA concentration-response curves in cAMP assays were fitted to the following equations with Prism 2.1 (Graph Pad, San Diego, CA) and Kaleidagraph software (Synergy Software, Reading, PA), respectively:

$$E = E_{max} \times [A]^s / (EC_{50}^s + [A]^s)$$

where E_{max}, [*A*], and *s* represent the maximum response, agonist concentration, and curve slope, respectively. EC_{50} is the concentration of agonist that produces 50% of the maximal response. The EC_{50} values are given as means ± S.E.M. (standard error of the mean). The antagonist potency was expressed as pK_B (−log of the dissociation constant, K_B), calculated for one concentration of antagonist following the equation:

$$K_B = ((EC_{50'}/EC_{50}) - 1)/[B]$$

where $EC_{50'}/EC_{50}$ is the ratio of concentrations of agonist giving equal responses (50% of the maximal effect) in the presence and in the absence of a given concentration [*B*] of the antagonist, respectively.

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