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Research Paper

Chalcone-based derivatives as new scaffolds for *h*A_3 adenosine receptor antagonists

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Keywords
adenosine receptor; chalcone; coumarin-chalcone hybrids; structure–activity relationship

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Abstract

Objectives With the aim of finding new adenosine receptor (AR) ligands based on the chalcone scaffold, we report the synthesis of a new series of coumarin–chalcone hybrids and the pharmacological characterization of their actions at four subtypes of AR.

Methods The synthesized compounds **5–10** were characterized in radioligand binding (A_1, A_{2A} and A_3) and adenylyl cyclase activity assays (A_{2B}) to determine the affinity of the compounds for the four human AR (*h*AR) subtypes.

Key findings Coumarin–chalcone hybrids were found to be ligands with a novel structure, not reported thus far, that showed varying affinity and selectivity for AR subtypes.

Conclusions The coumarin–chalcone hybrids in which ring B of the chalcone scaffold was a thiophene (compounds **5** and **9**) were found to be the most potent compounds of the series. Compound **9**, in which ring A of the chalcone moiety was the phenyl ring of the coumarin, showed similar activity against *h*A_1, *h*A_{2A} and *h*A_3 ARs, while compound **5**, in which ring A of the chalcone was substituted by the benzopyrone ring of the coumarin moiety, showed similar activity only at the *h*A_3 AR and, therefore, was deemed to be selective (K_i (dissociation constant) = 5160 nM).

Introduction

Adenosine is a purine nucleoside produced by all metabolically active cells. It acts as an endogenous modulator controlling a wide range of physiological processes due to its interaction with four specific cell membrane G-protein-coupled receptors (GPCRs) classified as A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs).[1–3] Because of their ubiquitous presence in cells, ARs have been seen as promising targets in the field of medicinal chemistry. ARs are distributed along different tissues in mammalian systems and regulate diverse physiological functions by modulating cell signalling, being activated by endogenous adenosine and blocked by antagonists.[4,5] In the last two decades, a large number of ligands have been synthesized in the search for potent and selective agonists and antagonists for each AR subtype; selective A_{2B}AR agonists are among the most recently reported.[6] Targeting ARs has opened a new window for potential drug treatment of a variety of pathologies such as asthma, neurodegenerative disorders, cancer and inflammatory and ischaemic conditions.[7–12] More specifically, AR antagonists are involved in several pathological processes such as inflammation (A_{2A}),[13,14] heart and renal failure (A_1)[15] or neurological disorders like Parkinson's[16] and Alzheimer's disease (A_{2A} and/or A_1).[17]

AR antagonists developed recently present a chemical diversity, while the 1,3-dialkylxanthines are considered as derivatives of the classical scaffold.[18,19] Among the non-classical antagonists, the flavone and isoflavone derivatives have played a remarkable role, namely genistein, which has been described as a competitive antagonist at the A_1 AR in FRTL (thyroid) cells[20] or galangin, which was found to bind three subtypes of ARs, and it has been shown that this type of compound presents micromolar affinity for the A_3 AR.[21] (Figure 1)

Coumarins (chromone isosteres) and chalcones (flavone with an opened pyrone ring) are another class of benzopyran-related compounds of natural origin that

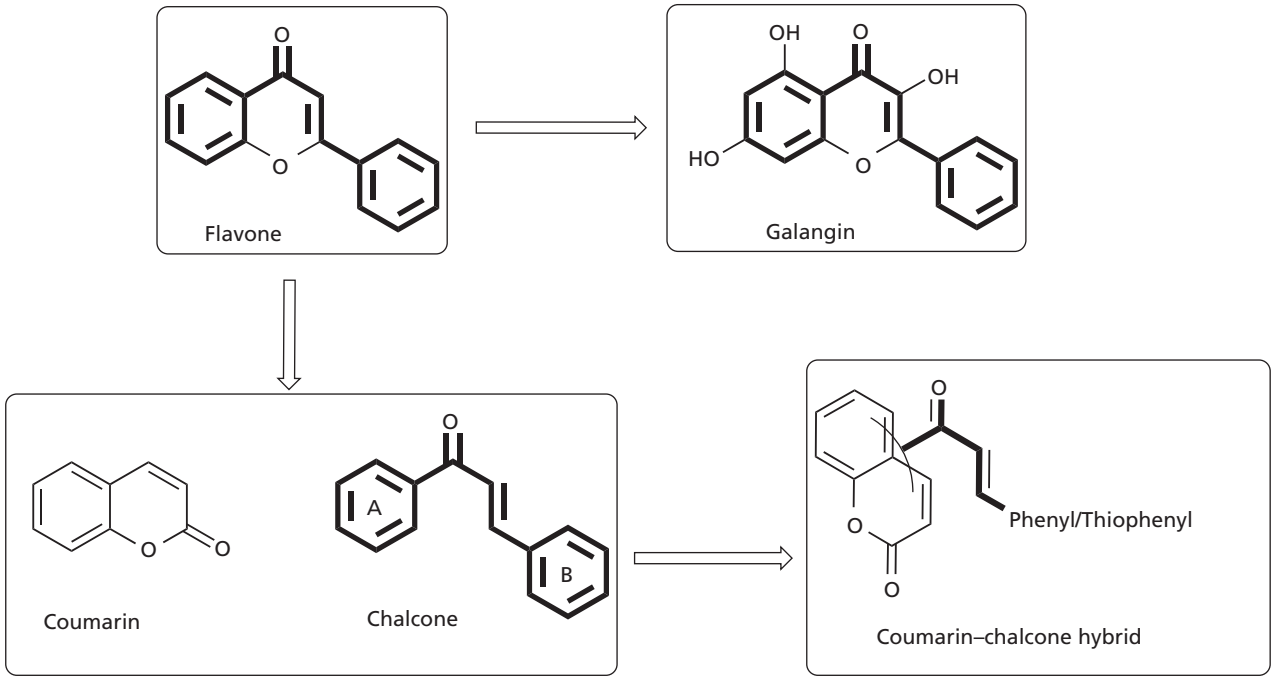

Figure 1 Rational design of the synthesized compounds based on flavonoid scaffolds.

present a wide range of pharmacological actions.[22–24] Coumarins have health value mainly due to their antimicrobial,[25] enzyme-inhibitory,[26–28] anticancer[29] and antioxidant[30] activity.

On the other hand, chalcones also present related biological activity as antimicrobial and anti-inflammatory,[24] anti-tumour,[29] antioxidant[31] or enzyme-inhibitory effects.[32] Thus, due to structural similarities between flavones, coumarins and chalcones, we decided to synthesize hybrid compounds bearing both scaffolds in the same molecule, and to evaluate their activity towards the four subtypes of human AR expressed in Chinese hamster ovary (CHO) cells. The designed compounds have the chalcone scaffold as the main structural core, and the different derivatives are mainly based on the substitution of one or both of the chalcone phenyl rings (A and/or B) for a coumarin and/or thiophene with different substituents (Figure 1). In addition, the drug-like properties of the hybrids were also evaluated.

Materials and Methods

Synthetic methodologies

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using tetramethylsilane as internal standard (chemical shifts in δ values, J in Hz). Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ± 0.4% of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography. Analytical thin-layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds was assessed by high-performance liquid chromatography (HPLC) coupled at diode array detector (DAD) on a Thermo Quest Spectrasystem (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a P4000 pump, a UV6000 UV-Vis diode array detector and an SN4000 interface for operation via a personal computer. Instrument software ChromQuest 5.0 (Thermo Fisher Scientific) was used for data acquisition. Different analytical columns and mobile phases (all solvents were HPLC grade) were tested. The mobile phase was H_2O–CH_3CN (70:30) and an Eclipse xdb C18 column (5 μm particle size, 0.46 mm i.d., 25 cm length; Agilent Technologies) was used. The purity of the compounds was found to be higher than 95%.

Chemistry

The chalcone-based derivatives **5–10** were efficiently synthesized according to the protocol outlined in Figure 2.

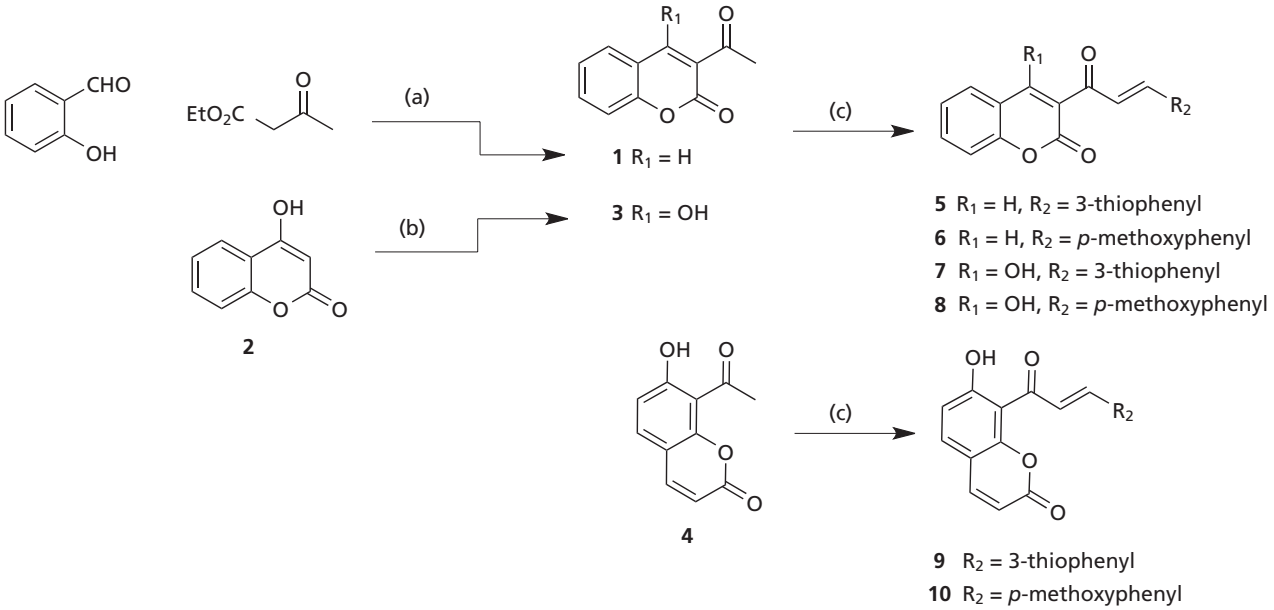

Figure 2 Protocol for synthesis of chalcone-based derivatives **5**–**10**. Reagents and conditions: (a) solvent-free, piperidine, rt, 10 min; (b) $POCl_3$, glacial acetic acid, reflux, 30 min; (c) appropriate aldehyde, EtOH, piperidine, reflux, 2–6 h.

Different starting materials were used in accordance with the substitution pattern of the final compounds. To synthesize compounds **5** and **6**,[33] we first prepared the 3-acetylcoumarin (**1**) by a Knoevenagel reaction using salicylaldehyde and ethyl acetoacetate without solvent and employing piperidine in a catalytic amount at room temperature.[34] To prepare compounds **7** and **8**,[35] 4-hydroxycoumarin (**2**) was used as the starting material. Acylation at the 3 position of compound **2**, using $POCl_3$ in glacial acetic acid under reflux, afforded precursor **3** (93%).[35] Compounds **9** and **10** were prepared from the commercially available 8-acetyl-7-hydroxycoumarin (**4**). Starting from precursors **1**, **3** and **4**, a Claisen-Schmidt condensation in EtOH, using piperidine as base, and under reflux mixed with the corresponding aromatic aldehydes, afforded the desired final compounds **5**–**10** in good yields (47–87%).

Synthesis of 3-(3-aryl)acryloylcoumarin (5–8) and 8-(3-aryl)acryloylcoumarin (9, 10) derivatives

The corresponding 3-acetylcoumarin (**1** or **3**, 1 mmol) or 8-acetylcoumarin (**4**) and the conveniently substituted aromatic aldehyde (1.1 mmol) were dissolved in EtOH (3 ml) and a catalytic amount of piperidine (0.05 ml) was added. The reaction mixtures were stirred for 2–6 h under reflux. After completion of reaction (followed by TLC), the solvent was evaporated under vacuum and the dry residue was purified by flash chromatography (hexane–ethyl acetate, 85:15) to give the desired products **5**–**10**.

Biological assays

The binding affinity of the compounds for the human AR subtypes hA_1, hA_{2A} and hA_3 was determined using radioligand competition experiments in CHO cells that had been stably transfected with the individual receptor subtypes.[36,37] The radioligands used were 1 nM [^{3}H]2-chloro-N6-cyclopentyladenosine (CCPA) for hA_1, 10 nM [^{3}H] N-ethylcarboxamidoadenosine (NECA) for hA_{2A} and 1 nM [^{3}H]2-(1-Hexynyl)-N-methyladenosine (HEMADO) for hA_3 receptors. Due to the lack of a suitable radioligand for hA_{2B} receptors the potency of antagonists at hA_{2B} receptor (expressed on CHO cells) was determined by inhibition of NECA-stimulated adenylyl cyclase activity.[36] The 50% inhibitory concentration (IC50) for inhibition of cAMP (cyclic adenosine monophosphate) production was determined and converted to a K_i value (dissociation constants) using the Cheng and Prusoff equation.[38] For all the tested compounds no measurable activity for the hA_{2B} AR (K_i > 10 000 nM) was detected.

Statistical methods

K_i values (dissociation constants) were determined in radioligand competition experiment with seven or eight different concentrations of test compound and each concentration was tested in duplicate. K_i values are given as geometric means of three independent experiments with 95% confidence intervals. For analysis of the competition curves the programme SCTFIT was used.[39]

Theoretical evaluation of absorption, distribution, metabolism and excretion properties

The absorption, distribution, metabolism and excretion (ADME) properties of the studied compounds were calculated using the Molinspiration property programme.[40] LogP was calculated using the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. Topological polar surface area (TPSA) was calculated based on the methodology published by Ertl *et al*. as a sum of fragment contributions.[42] Oxygen- and nitrogen-centred polar fragments were considered. Polar surface area (PSA) has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration. The method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting the sum of fragment contributions to 'real' three-dimensional (3D) volume for a training set of about 12 000, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semi-empirical AM1 method.

Results

Structural identification

(*E*)-3-(3-(Thiophen-3-yl)acryloyl)coumarin (**5**): Yield 61%. Mp 146–148°C ^{1}H NMR (300 MHz, $CDCl_3$) δppm 8.57 (s, 1H, H-4), 7.87 (d, J = 15.6 Hz, 1H, H_β), 7.74 (d, J = 15.7 Hz, 1H, H_α), 7.69–7.60 (m, 3H, H-2', H-4', H-5'), 7.43 –7.29 (m, 4H, H-5, H-6, H-7, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 186.5, 155.1, 148.1, 141.8, 138.4, 138.3, 134.2, 132.6, 130.7, 129.9, 126.9, 125.7, 124.9, 123.6, 118.6, 116.5. MS *m/z* (%) (ESI): 283 ($[M + 1]^+$, 8), 282 ($[M]^+$, 35), 254 (100), 226 (73), 137 (88), 109 (93). Anal. Calcd. for $C_{16}H_{10}O_3S$: C, 68.07; H, 3.57. Found: C, 68.11; H, 3.59

(*E*)-4-Hydroxy-3-(3-(thiophen-3-yl)acryloyl)coumarin (**7**): Yield 87%. Mp 192–194°C ^{1}H NMR (300 MHz, $CDCl_3$) δppm 8.25 (d, J = 15.6 Hz, 1H, H_β), 8.15–7.99 (m, 2H, H_α, H-5), 7.77–7.61 (m, 2H, H-2', H-5'), 7.52 (dd, J = 5.2, 1.3 Hz, 1H, H-4'), 7.43–7.23 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 192.5, 181.5, 160.1, 154.6, 140.5, 138.3, 135.9, 131.1, 127.1, 125.9, 125.6, 124.2, 122.1, 116.9, 116.2. MS *m/z* (%) (ESI): 299 ($[M + 1]^+$, 13), 298 ($[M]^+$, 68), 280 (38), 270 (46), 137 (100), 121 (99), 109 (86). Anal. Calcd. for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.38. Found: C, 64.39; H, 3.32

(*E*)-7-Hydroxy-8-(3-(thiophen-3-yl)acryloyl)coumarin (**9**): Yield 47%. Mp 169–171°C. ^{1}H NMR (300 MHz, $CDCl_3$) δppm 14.10 (bs, 1H, OH), 8.16 (d, J = 15.3 Hz, 1H, H_β), 7.98 (d, J = 15.1 Hz, 1H, H_α), 7.80–7.59 (m, 2H, H-4, H-5), 7.59–7.44 (m, 2H, H-6, H-2'), 7.39 (dd, J = 5.0, 2.8 Hz, 1H, H-4'), 6.93 (d, J = 8.7 Hz, 1H, H-5'), 6.30 (d, J = 9.6 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 193.2, 167.8, 159.4, 144.3, 139.6, 138.3, 134.4, 130.4, 127.3, 125.9, 125.7, 115.8, 111.9, 111.0, 109.6. MS *m/z* (%) (ESI): 299 ($[M + 1]^+$, 11), 298 ($[M]^+$, 28), 203 (25), 189 (100), 84 (89). Anal. Calcd. for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.38. Found: C, 64.44; H, 3.40.

(*E*)-7-Hydroxy-8-(3-(4-methoxyphenyl)acryloyl)coumarin (**10**): Yield: 68%. Mp 187–188°C. ^{1}H NMR (300 MHz, $CDCl_3$) δppm 14.00 (s, 1H, OH), 8.03 (d, J = 15.4 Hz, 1H, H_β), 7.82 (d, J = 15.5 Hz, 1H, H_α), 7.62–7.42 (m, 3H, H-4, H2', H6'), 7.34 (d, J = 8.8 Hz, 1H, H-5), 6.86–6.66 (m, 3H, H-6, H3', H-5'), 6.12 (d, J = 9.5 Hz, 1H, H-3), 3.70 (s, 3H, -OMe). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 192.85, 167.84, 162.22, 159.47, 155.44, 146.39, 144.28, 134.17, 131.05, 127.55, 123.42, 115.71, 114.59, 111.88, 111.01, 109.70, 55.41. MS *m/z* (%) (ESI): 323 ($[M + 1]^+$, 24), 322 ($[M]^+$, 47), 321 (100), 293 (10), 134 (44). Anal. Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.77; H, 4.35.

Binding affinity assays

The data obtained for the binding affinity assays from radioligand binding experiments for compounds **5**–**10** are

Table 1 The binding affinity of compounds **5**–**10** for the human adenosine receptor subtypes hA_1, hA_{2A} and hA_3

Compound	K_i (nM)		
	hA_1	hA_{2A}	hA_3
5	>30 000	>30 000	5 160 (3 000–8 900)
6	>10 000	>10 000	>10 000
7	>30 000	>30 000	31 500 (21 600–46 000)
8	>10 000	>10 000	>10 000
9	8 330 (7 510–9 230)	11 900 (7 790–18 300)	5 020 (3 260–7 730)
10	>10 000	>10 000	20 200 (14 600–28 100)

The binding affinity (K_i) for the human AR subtypes hA_1, hA_{2A} and hA_3 of compounds **5**–**10** was determined using radioligand competition experiments in Chinese hamster ovary (CHO) cells. Highest concentrations tested were different depending on the solubility of compounds under the respective assay conditions. Values are geometric means of three experiments and given with 95% confidence intervals in parentheses.

Table 2 Theoretical structural properties of the coumarin–chalcone hybrids

Compound	log*P*	Molecular weight	TPSA (Å^2)	*n*-OH acceptors	*n*-OHNH donors	Volume (Å^3)
5	3.22	282.32	47.28	3	0	237.11
6	3.87	306.32	56.52	4	0	271.94
7	2.93	298.32	67.51	4	1	245.12
8	3.58	322.32	76.54	5	1	279.96
9	3.19	298.32	67.51	4	1	245.12
10	3.84	322.32	76.74	5	1	279.96

n-OH, number of hydrogen acceptors; n-OHNH, number of hydrogen bond donors; TPSA, topological polar surface area. The data was determined with Molinspiration calculation software.

summarized in Table 1. Compound **9** showed binding affinity to hA_1 and hA_{2A} ARs in the micromolar range, comparable with the prototypical antagonist theophylline.[4,36] Compounds **5** and **9** bound with affinity in the low micromolar range to hA_3AR, with compound **5** being the one that showed selectivity for this subtype.

Theoretical evaluation of ADME properties

To better correlate the drug-like properties of the coumarin–chalcone hybrid compounds, the lipophilicity, expressed as the octanol/water partition coefficient and herein called logP, as well as other theoretical calculations such as the TPSA, the number of hydrogen acceptors and the number of hydrogen bond donors were calculated using the Molinspiration property programme.[40] From the data obtained, it was noticed that all the hybrid compounds, **5**–**10**, not only had logP values compatible with those required to cross membranes but also they did not break any point of the Lipinski's rule of five. Theoretical prediction of ADME properties of all compounds is summarized in Table 2.

Discussion

With the aim of finding novel and selective AR ligands, we have synthesized chalcone- and coumarin-containing hybrids in which one or both phenyl groups of the chalcone scaffold either retains the two phenyl groups corresponding to an original chalcone structure (rings A and B, Figure 1), or is isosterically substituted by heteroaromatic rings (pyrone ring of the coumarin and/or thiophene).

The data obtained in the binding affinity assays are shown in Table 1. From all the synthesized derivatives, compounds **5**, **7** and **9** exhibit significant binding affinity in the low micromolar range for one or more AR. The common feature of compounds **5**, **7** and **9** is the presentation of a thiophenyl substituent as the key isosterical change in ring B of the chalcone. However, only compound **5** displays a noteworthy selectivity for the hA_3 AR (K_i = 5160 nM). It is interesting to note that compound **9**, when compared with compound **5**, shows similar A_3 affinity; however, it also exhibits similar affinity for A_1 and A_{2A} ARs whereas compound **5** is devoid of measurable affinity for these subtypes. The configuration with both A and B rings replaced seems to be favourable for high A_3 affinity and selectivity. Comparing compounds **5**, **7** and **9** suggests that the 4-hydroxyl group in compound **7** and the corresponding 7-hydroxyl group in compound **9**, respectively, might abolish subtype selectivity. Compound **9** presents a binding affinity for the hA_3 AR (K_i = 5020 nM) and also for hA_1 AR (K_i = 8330 nM) and hA_{2A} AR (K_i = 11 900 nM). The A_1 and A_{2A} affinities are thus comparable to the affinity of the classical naturally occurring antagonist theophylline. [36]

Comparison of the structurally related compounds **5** and **7**, reveals that the only difference between them is the absence or presence of a hydroxyl group at position 4, respectively. One can conclude that the presence of the hydroxyl group seems to cause a marked decrease in the binding affinity for the hA_3 AR (K_i = 5160 and 31 500 nM, respectively).

Comparing another pair of 8-substituted derivatives, compounds **9** and **10**, that differ in the substitution of ring B of the chalcone (compound **10** has the original phenyl ring of the chalcone while compound **9** presents a thiophenyl ring as a result of an isosteric change), one can observe that compound **9** shows affinity with no selectivity and compound **10** is less potent at all three receptor subtypes. Due to the limited affinity close to the detection limit the degree of selectivity cannot be determined. From these preliminary structure–activity relationships, one can conclude that substitution of ring B of the chalcone (Figure 1) by a thiophenyl ring (compounds **5**, **7** and **9**) favoured binding affinity towards the AR. However, hA_3 AR selectivity is attained when ring B of the chalcone is substituted by a thiophene ring and when ring A is substituted for the pyrone ring contained in the coumarin moiety (compound **5**).

On the other hand, looking at the theoretical evaluation of ADME properties (Table 2), it can be observed that no violations of Lipinski's rule of five (molecular weight, logP, number of hydrogen donors and acceptors) were found, making these hybrid compounds promising leads for drug candidates.[41] TPSA, described as being a predictive

indicator of the drug capacity of membrane penetration, was also found to be positive.[42]

The remarkable results found for compounds **5** and **9** encourage us to continue our research looking for the optimization of these lead compounds with the aim of obtaining potent and selective chalcone-based hA_3 AR ligands.

Conclusions

Isosteric substitution of the phenyl rings of the chalcone moiety for one or two heteroaromatic rings (benzopyrone and/or thiophenyl) results in compounds (**5** and **9**) with binding affinity towards particular subtypes of ARs. A preliminary structure–activity relationship study of the synthesized derivatives allowed verification that hA_3 AR selectivity was achieved only when ring B of the chalcone was substituted for a thiophene and ring A was substituted for the pyrone ring included in the coumarin scaffold (compound **5**). Replacing ring B by a thiophenyl group but keeping a phenyl group in place of ring A, namely the benzene ring of coumarin (compound **9**), resulted in an increase of hA_1 and hA_{2A} AR affinity. As binding affinity remained similar for hA_3 AR, selectivity was, therefore, lost.

These findings encourage us to continue the efforts towards the optimization of the pharmacological profile for this type of hybrid as potent and selective ligands for the hA_3 ARs.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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