Synthesis and Structure Activity Relationships of Chalcone based Benzocycloalkanone Derivatives as Adenosine A_1 and/or A_{2A} Receptor Antagonists

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ABSTRACT

Adenosine A_1 and/or A_{2A} receptor antagonists hold promise for the potential treatment of neurological conditions, such as Parkinson's disease. Herein, a total of seventeen benzocycloalkanone derivatives were synthesised and evaluated for affinity towards adenosine receptors (A_1 and A_{2A} AR). The obtained results allowed for the conclusion that affinity and/or selectivity of the 2-benzylidene-1-indanone and -tetralone derivatives toward A_1 and/or A_{2A} ARs may be modulated by the nature of the substituents (either -OH, -OCH_3 or morpholine) attached at position C4 of the 1-indanone core and C5 of the 1-tetralone core as well as the *meta* (C3') and/or *para* (C4') position(s) on ring B. Several compounds (**2a–b**, **3b–c** and **4a–b**) possessed affinity for the A_1 and/or A_{2A} AR below 10 μM. Additionally, compounds **2a**, **3b** and **4a** were A_1 AR antagonists. These results, once again, confirmed the importance of C4 methoxy-group substitution on ring A in combination with *meta* (C3') and/or *para* (C4') hydroxyl-group substitution ring B of the 2-benzylidene-1-indanone scaffold leading to drug-like compounds **1h** and **1j** with affinity in the nanomolar-range.

Introduction

Ensuing almost 100 years of research on adenosine and its receptors (A_1, A_{2A}, A_{2B} and A_3) as well as the ligands that bind to these adenosine receptors (ARs) and, subsequently, modulate the adenosine system, the potential of the said system as a drug discovery target is evident [1].

Despite the amount of research on adenosine and its receptors, there are only two United States Food and Drug Administration (USFDA) approved AR agonist drugs, namely, adenosine and regadenoson. Furthermore, the first in class selective A_{2A} AR antagonist, istradefylline (**1a**) has been approved for manufacturing and marketing as Nouriast® in Japan only since 2013 as a novel antiparkinsonian agent for wearing-off phenomena associated with levodopa treatment [2]. A post-marketing surveillance study conducted in Japan proved istradefylline (**1a**) safe and effective; it is generally well tolerated, despite dyskinesias and hallucinations as common adverse effects [3]. Recently, the USFDA approved istradefylline (**1a**) as add-on drug to treat off episodes in adults with Parkinson's disease (PD) [4].

The xanthine derivative istradefylline (**1a**) not only improves wearing-off phenomena but also some non-motor symptoms of PD, such as the common mood disorder in PD, namely depression

[5, 6]. This is, however, yet to be confirmed by a double-blind placebo-controlled trial [6]. Furthermore, istradefylline (**1a**) also improves daytime sleepiness and bladder dysfunction, also non-motor symptoms associated with PD [7, 8]. It is, unfortunately, difficult to demonstrate neuroprotection by istradefylline (**1a**) in humans, because there is, to date, no way to assess neurodegeneration or neuroprotection in humans [9]. Although, in animal models of PD istradefylline (**1a**) protected against dopaminergic neurodegeneration [10, 11].

Consequently, no approved drug is available for the prevention of neuronal cell loss in patients suffering from PD; although, the multifactorial aetiology of PD and, thus, the underlying molecular causes of the disease are more clear – yet, the approach to drug discovery is not multifaceted [12]. For that reason, it may be said that the idea of "one protein, one target" is outdated [12]. In contrast, a robust (and, indeed, a multi-target approach) may be the restoration of neurotransmitter levels by means of a combined inhibition of cholinesterases, monoamine oxidases and, of note, A_{2A} and A_1 ARs [12].

It is not yet clear which is the better approach towards treating the symptoms of PD between treatment with a selective A_{2A} AR antagonist or a dual A_{2A} and A_1 AR antagonist [1]. Based on published preclinical and clinical data, it seems that most researchers are searching for selective A_{2A} AR antagonists in order to minimize possible cardiovascular effects caused by interaction with the A_1 AR [13]. Other researchers consider A_1 AR antagonism a desirable feature to address unmet needs in PD treatment (such as a decline in cognitive function) [14, 15]. For example, the dual A_{2A} and A_1 AR antagonists ASP5854 (**1b**) and JNJ-40255293 (**1c**), with K_i values in the nanomolar-range (▶ **Fig. 1**), showed promising effects in animal models of PD neurodegeneration (A_{2A}) and motor symptoms (A_{2A}), as well as cognition (A_1) [16].

Of interest to the current study is dual A_{2A} and A_1 AR antagonist compound **1c** (▶ **Fig. 1**)[16]; which contains the 1-indanone moiety. The 1-indanone moiety is associated with wide-ranging biological activity and considered a privileged scaffold in medicinal chemistry [17, 18]. In fact, it is present in therapeutic agents, such as the acetylcholinesterase inhibitor donepezil (Aricept®) (**1d**) (▶ **Fig. 2**), indicated for the symptomatic treatment of mild to moderate dementia in Alzheimer's disease (AD) [19, 20].

The 2-benzylidene-1-indanone (or arylidene indanone) scaffold consists of 1-indanone core (fused 6- and 5-membered rings, namely ring A and ring C), as well as phenyl ring B, linked to the 1-indanone core by an exocyclic double bond. This leads to a planar chemical structure that permits transmission of electron donating substituent effects through the exocyclic double bond onto the carbonyl functional group of the 1-indanone core [21, 22]. Numerous 2-benzylidene-1-indanone derivatives (**1e–j**, ▶ **Fig. 3**) were previously synthesised and evaluated as A_{2A} and A_1 AR antagonists [23, 24]. (Other ring A and benzylidene ring B substitutions such as halogens were also previously explored.) The methoxy substituted 2-benzylidene-1-indanone derivatives **1h** and **1j** (▶ **Fig. 3**) proved to be potent dual A_{2A} and A_1 AR antagonists with affinity in the nanomolar-range. However, the presence of liable reactive functional groups in compounds **1h** and **1j** (namely α,β-unsaturated ketone (**1h** & **1j**) and catechol (**1j**)) have raised questions [23]. A series of the structurally related 2-benzylidene-1-tetralone derivatives (**1k–q**, ▶ **Fig. 3**)) were also previously synthesised and evaluated as A_{2A} and A_1 AR antagonists [25, 26].

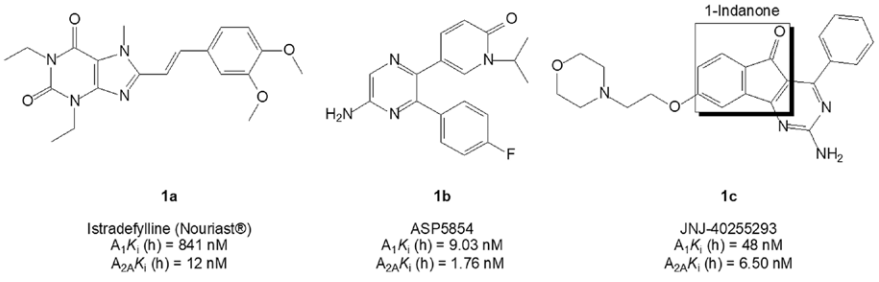
The term chalcone generally refers to chemicals with an α,ß-unsaturated carbonyl system; thus, the chalcone family has extensive structural diversity [27, 28]; as seen with the aforesaid compounds, that may be considered hybrid chalcones.

In order to further investigate the structure activity relationships (SARs) of this class of compounds (benzocycloalkanones) that govern A_1 and A_{2A} AR affinity, two series of arylidene indanone and one series of arylidene tetralone derivatives were designed.

Materials and Methods

Chemistry

Unless otherwise noted, all starting materials and solvents were procured from Sigma-Aldrich and used without further purification. TLC silica gel 60 F_{254} aluminium sheets from Merck was used to monitor reaction progress. Melting point range was measured with a Buchi B545 m.p. apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively, using either $CDCl_3$ or DMSO-d6 as solvent and TMS as reference. Chemical shifts were reported in parts per million (δ) in relation to the solvent peak ($CDCl_3$: residual CH at 7.26 ppm and DMSO-d6: residual CH_3 at 2.50 ppm for ^{1}H NMR.) Spin multiplicities were indicated as follows: singlet (s), doublet (d), dd (doublet



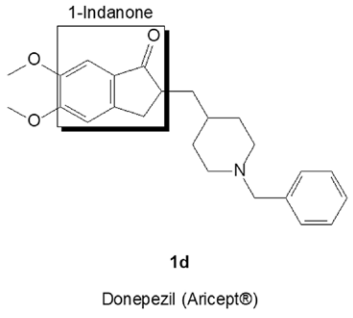
▶ **Fig. 1** The structure and K_i values for the binding affinity of **1a–c** against human (h) A_1 and A_{2A} ARs.

of doublets), triplet (t) and multiplet (m). *J* values were reported in Hz. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric chemical ionisation (APCI) mode. HPLC analyses were done on an Agilent 1100 HPLC system.

General procedure for the synthesis of 2a–h

(2*E*)-4-hydroxy-2-(3-hydroxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2a)**

4-Hydroxy-1-indanone (0.500 g, 3.375 mmol) and 3-hydroxybenzaldehyde (0.412 g, 3.375 mmol) were suspended in HCl (32 %, 6 mL) and MeOH (4 mL) and mechanically stirred at 120 °C under reflux for 24 h. Upon completion, the reaction mixture was cooled down to room temperature, quenched with ice (20 g), filtered, dried (30 °C) and recrystallized from a suitable solvent (either EtOH or MeOH) to yield **2a** as dark brown powder (0.390 g, 46 %): Rf: 0.09 (DCM/EtOAc 10:1); mp: 280.2–281.5 °C; ^{1}H NMR (600 MHz, DMSO) δ 10.14 (s, 1H), 9.72 (s, 1H), 7.42 (s, 1H), 7.31 (dd, *J* = 7.8, 7.3 Hz, 2H), 7.23 (dd, *J* = 23.1, 7.0 Hz, 3H), 7.10 (d, *J* = 7.7 Hz, 1H), 6.87 (dd, *J* = 7.4, 1.7 Hz, 1H), 3.90 (s, 2H); ^{13}C NMR (151 MHz, DMSO) δ 193.64, 157.72, 154.83, 138.92, 136.35, 136.10, 134.85, 133.04, 130.05, 129.10, 122.25, 120.39, 117.14, 116.73, 114.15, 29.17. APCI-HRMS *m/z* calcd. for $C_{16}H_{12}O_3$ (MH +): 253.0859, found: 253.0866. Purity (HPLC): 100 %.



▶ **Fig. 2** The structure of Donepezil (**1d**).

(2*E*)-4-hydroxy-2-(4-hydroxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2b)**

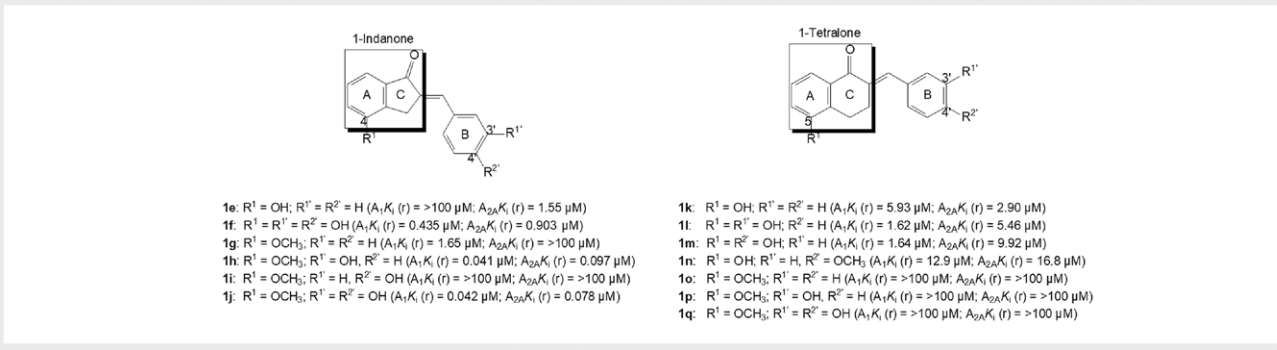
Prepared as for **2a** from 4-hydroxy-1-indanone (0.500 g, 3.375 mmol) and 4-hydroxybenzaldehyde (0.412 g, 3.375 mmol) to yield **2b** as brown powder (0.690 g, 81 %): Rf: 0.13 (DCM/EtOAc 10:1); mp: 276.3–278.6 °C; ^{1}H NMR (600 MHz, DMSO) δ 10.23 (s, 1H), 10.15 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 2H), 7.44 (s, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.3 Hz, 1H), 7.07 (dd, *J* = 7.8, 0.7 Hz, 1H), 6.90 (t, *J* = 5.7 Hz, 2H), 3.85 (s, 2H); ^{13}C NMR (151 MHz, DMSO) δ 193.82, 159.56, 154.88, 139.42, 136.23, 133.60, 133.16, 131.71, 129.19, 126.20, 120.32, 116.27, 114.26, 29.28. APCI-HRMS *m/z* calcd. for $C_{16}H_{12}O_3$ (MH +): 253.0859, found: 252.0793. Purity (HPLC): 99.5 %.

(2*E*)-4-hydroxy-2-(3-methoxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2c)**

Prepared as for **2a** from 4-hydroxy-1-indanone (0.300 g, 2.025 mmol) and 3-methoxybenzaldehyde (0.276 g, 2.025 mmol) to yield **2c** as black powder (0.350 g, 65 %): Rf: 0.60 (DCM/EtOAc 10:1); mp: 226.4–239.4 °C; ^{1}H NMR (600 MHz, DMSO) δ 10.10 (s, 1H), 7.50 (t, *J* = 1.8 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.40–7.28 (m, 3H), 7.25 (d, *J* = 7.1 Hz, 1H), 7.10 (dd, *J* = 7.8, 0.7 Hz, 1H), 7.05 (dd, *J* = 8.0, 2.1 Hz, 1H), 3.94 (d, *J* = 1.3 Hz, 2H), 3.83 (s, 3H); ^{13}C NMR (151 MHz, DMSO) δ 193.62, 159.55, 154.82, 138.85, 136.33, 136.26, 135.34, 132.81, 130.09, 129.12, 123.04, 120.53, 116.05, 115.55, 114.20, 55.25, 29.06. APCI-HRMS *m/z* calcd. for $C_{17}H_{14}O_3$ (MH +): 267.1016, found: 267.1018. Purity (HPLC): 99.5 %.

(2*E*)-4-hydroxy-2-(4-methoxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2d)**

Prepared as for **2a** from 4-hydroxy-1-indanone (0.500 g, 3.375 mmol) and 4-methoxybenzaldehyde (0.460 g, 3.375 mmol) to yield **2d** as brown powder (0.600 g, 67 %): Rf: 0.44 (DCM/EtOAc 10:1); mp: 251.7–271.8 °C; ^{1}H NMR (600 MHz, DMSO) δ 10.09 (s, 1H), 7.75 (d, *J* = 8.6 Hz, 2H), 7.49 (s, 1H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.23 (d, *J* = 7.4 Hz, 1H), 7.08 (dd, *J* = 8.1, 4.6 Hz, 3H), 3.88 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (151 MHz, DMSO) δ 193.57, 160.66, 154.81, 139.18, 136.15, 132.87, 132.72, 132.60, 129.04, 127.58, 120.23, 114.65, 114.10, 55.43, 29.11. APCI-HRMS *m/z* calcd. for $C_{17}H_{14}O_3$ (MH +): 267.1016, found: 267.1016. Purity (HPLC): 97.8 %.



▶ **Fig. 3** The structures and K_i values for the binding affinity of **1e–q** against rat (r) A_1 and A_{2A} ARs.

(2*E*)-2-(3,4-dimethoxybenzylidene)-4-hydroxy-2,3-dihydro-1*H*-inden-1-one **(2e)**

Prepared as for **2a** from 4-hydroxy-1-indanone (0.500 g, 3.375 mmol) and 3,4-dimethoxybenzaldehyde (0.561 g, 3.375 mmol) to yield **2e** as brown powder (0.630 g, 63 %): Rf: 0.53 (PE/EtOAc 1:1); mp: 224.6–226.2 °C; ^{1}H NMR (600 MHz, DMSO) δ 10.06 (s, 1H), 7.50 (s, 1H), 7.42–7.35 (m, 2H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 7.1 Hz, 1H), 7.12–7.07 (m, 2H), 3.92 (d, *J* = 1.0 Hz, 2H), 3.85 (d, *J* = 16.9 Hz, 6H); ^{13}C NMR (151 MHz, DMSO) δ 193.49, 154.76, 150.49, 148.81, 139.16, 136.05, 133.33, 132.63, 129.01, 127.72, 124.59, 120.26, 114.08, 113.98, 111.92, 55.64, 55.60, 28.93. APCI-HRMS *m/z* calcd. for $C_{18}H_{16}O_4$ (MH +): 297.1121, found: 297.1121. Purity (HPLC): 99.6 %.

(2*E*)-4-methoxy-2-(3-methoxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2f)**

Prepared as for **2a** from 4-methoxy-1-indanone (0.500 g, 3.083 mmol) and 3-methoxybenzaldehyde (0.420 g, 3.083 mmol) to yield **2f** as light brown powder (0.640 g, 74 %): Rf: 0.39 (PE/EtOAc 4:1); mp: 125.9–299.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (t, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.39 (dt, *J* = 11.1, 7.9 Hz, 2H), 7.32 (d, *J* = 7.7 Hz, 1H), 7.24–7.20 (m, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 6.96 (dd, *J* = 8.1, 2.0 Hz, 1H), 3.96–3.91 (m, 5H), 3.87 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 194.66, 159.92, 156.79, 139.52, 138.52, 136.80, 135.05, 134.09, 130.01, 129.27, 123.38, 116.43, 116.20, 115.25, 115.16, 55.64, 55.51, 29.44. APCI-HRMS *m/z* calcd. for $C_{18}H_{16}O_3$ (MH +): 281.1172, found: 281.1182. Purity (HPLC): 98.1 %.

(2*E*)-4-methoxy-2-(4-methoxybenzylidene)-2,3-dihydro-1*H*-inden-1-one **(2g)**

Prepared as for **2a** from 4-methoxy-1-indanone (0.500 g, 3.083 mmol) and 4-methoxybenzaldehyde (0.420 g, 3.083 mmol) to yield **2g** as golden brown powder (0.380 g, 44 %): Rf: 0.77 (DCM/EtOAc 10:1); mp: 117.1–296.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.5 Hz, 2H), 7.63 (s, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.06 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 2H), 3.94 (s, 3H), 3.89 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 194.66, 160.97, 156.73, 139.82, 138.28, 134.03, 132.76, 132.42, 129.16, 128.26, 116.13, 114.90, 114.56, 55.65, 55.52, 29.46. APCI-HRMS *m/z* calcd. for $C_{18}H_{16}O_3$ (MH +): 281.1172, found: 281.1172. Purity (HPLC): 97.3 %.

(2*E*)-2-(3,4-dimethoxybenzylidene)-4-methoxy-2,3-dihydro-1*H*-inden-1-one **(2h)**

Prepared as for **2a** from 4-methoxy-1-indanone (0.500 g, 3.083 mmol) and 3,4-dimethoxybenzaldehyde (0.512 g, 3.083 mmol) to yield **2h** as brown powder (0.520 g, 54 %): Rf: 0.20 (PE/EtOAc 4:1); mp: 173.8–175.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (t, *J* = 1.8 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.42–7.33 (m, 2H), 7.20 (d, *J* = 1.9 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 3.98–3.92 (m, 9H), 3.91 (d, *J* = 1.4 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 194.58, 156.72, 150.69, 149.16, 139.77, 138.22, 134.32, 132.71, 129.22, 128.53, 124.64, 116.14, 114.95, 113.82, 111.38, 56.16, 56.12, 55.64, 29.33. APCI-HRMS *m/z* calcd. for $C_{19}H_{18}O_4$ (MH +): 311.1278, found: 311.1278. Purity (HPLC): 100 %.

General procedure for the synthesis of 3a–f

4-[2-(morpholin-4-yl)ethoxy]-2,3-dihydro-1*H*-inden-1-one hydrochloride **(3a)**

A mixture of 4-hydroxy-1-indanone (1.21 g, 8.17 mmol), 4-(2-chloroethyl)morpholine hydrochloride (1.22 g, 8.17 mmol) and K_2CO_3 (2.53g, 40.85 mmol) in acetone (40 mL) were mechanically stirred at 120 °C under reflux for approximately 24 h. The reaction mixture was then cooled down to room temperature, filtered over SiO_2 and the filtrate conc. to yield a dark brown oil. The said oil was dissolved in EtOAc (20 mL) and acetyl chloride (6 mL) was added to the solution and mechanically stirred at room temperature until a precipitate formed. The resulting precipitate was filtered and dried at 30 °C to yield **3a** as a beige powder (0.92 g, 43 %): Rf: 0.17 (PE/EtOAc 3:1); mp: 223.2–223.6 °C; ^{1}H NMR (600 MHz, DMSO) δ 11.77 (d, *J* = 1.9 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.28 (dd, *J* = 21.2, 7.7 Hz, 2H), 4.58 (s, 2H), 4.00–3.82 (m, 4H), 3.62–3.46 (m, 4H), 3.24 (dd, *J* = 3.6, 2.3 Hz, 2H), 3.07–2.97 (m, 2H), 2.68–2.59 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 206.22, 155.18, 143.81, 138.36, 129.05, 116.53, 115.25, 63.20, 62.82, 54.81, 51.76, 39.52, 35.73, 22.35. APCI-HRMS *m/z* calcd. for $C_{15}H_{19}NO_3 \cdot HCl$ (MH +): 262.1438, found: 262.1458. Purity (HPLC): 74.4 %.

(2*E*)-2-(3-hydroxybenzylidene)-4-[2-(morpholin-4-yl) ethoxy]-2,3-dihydro-1*H*-inden-1-one **(3b)**

Prepared as for **2a** from **3a** (0.300 g, 1.007 mmol) and 3-hydroxybenzaldehyde (0.123 g, 1.007 mmol) to yield **3b** as black powder (0.030 g, 8 %): Rf: 0.11 (PE/EtOAc/MeoH 5:1:1); mp: 213.1–214.5 °C; ^{1}H NMR (600 MHz, DMSO) δ 9.74 (s, 1H), 7.47–7.43 (m, 2H), 7.38–7.30 (m, 3H), 7.21–7.16 (m, 2H), 6.88 (d, *J* = 7.9 Hz, 1H), 4.28 (t, *J* = 5.9 Hz, 2H), 4.11 (dd, *J* = 10.3, 5.1 Hz, 1H), 3.90 (s, 2H), 3.60–3.55 (m, 4H), 3.17 (d, *J* = 5.1 Hz, 2H), 2.78 (t, *J* = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 193.31, 157.69, 155.78, 138.72, 138.30, 136.00, 134.50, 133.32, 130.06, 129.41, 122.14, 117.37, 117.19, 116.72, 115.39, 66.23, 56.84, 53.67, 48.60, 28.93. APCI-HRMS *m/z* calcd. for $C_{22}H_{23}NO_4 \cdot HCl$ (MH +): 366.1700, found: 366.1731. Purity (HPLC): 99.5 %.

(2*E*)-2-(3,4-dihydroxybenzylidene)-4-[2-(morpholin-4-yl) ethoxy]-2,3-dihydro-1*H*-inden-1-one **(3b)**

Prepared as for **2a** from **3a** (0.300 g, 1.007 mmol) and 3,4-dihydroxybenzaldehyde (0.139 g, 1.007 mmol) to yield compound **3c** as dark green powder (0.070 g, 18 %): Rf: 0.15 (EtOAc 100 %); mp: 161.4–184.4 °C; ^{1}H NMR (600 MHz, DMSO) δ 11.20 (d, *J* = 9.2 Hz, 1H), 9.78 (s, 1H), 9.32 (s, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.34 (dd, *J* = 9.1, 4.9 Hz, 2H), 7.14 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 4.59 (s, 2H), 4.03–3.98 (m, 2H), 3.95 (s, 2H), 3.88 (t, *J* = 11.8 Hz, 2H), 3.65 (s, 2H), 3.58 (d, *J* = 11.3 Hz, 2H), 3.29 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 193.09, 154.85, 148.18, 145.67, 139.30, 138.23, 134.19, 130.98, 129.29, 126.30, 124.43, 117.68, 116.98, 116.12, 115.95, 63.25, 62.69, 54.83, 51.77, 29.23. APCI-HRMS *m/z* calcd. for $C_{22}H_{23}NO_5 \cdot HCl$ (MH +): 382.1649, found: 382.1680. Purity (HPLC): 74.7 %.

5-[2-(morpholin-4-yl)ethoxy]-2,3-dihydro-1*H*-inden-1-one hydrochloride **(3d)**

Prepared as for **2a** from 5-hydroxy-1-indanone (1.000 g, 6.75 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.260 g,

6.75 mmol) to yield compound **3d** as light brown powder (0.930 g, 53 %): Rf: 0.11 (PE/EtOAc 3:1); mp: 199.9–200.8 °C; ^{1}H NMR (600 MHz, DMSO) δ 11.73 (d, *J* = 1.1 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 1.6 Hz, 1H), 7.03 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.60–4.54 (m, 2H), 3.98–3.83 (m, 5H), 3.57 (s, 2H), 3.48 (d, *J* = 12.0 Hz, 2H), 3.20 (d, *J* = 9.1 Hz, 2H), 3.08–3.04 (m, 2H), 2.62–2.58 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 204.35, 162.88, 158.19, 130.44, 124.63, 115.74, 111.02, 63.12, 62.69, 54.59, 51.61, 36.05, 25.49. APCI-HRMS *m/z* calcd. for $C_{15}H_{19}NO_3 \cdot HCl$ (MH +): 262.1438, found: 262.1456. Purity (HPLC): 100 %.

(2*E*)-2-(3-hydroxybenzylidene)-5-[2-(morpholin-4-yl) ethoxy]-2,3-dihydro-1*H*-inden-1-one **(3e)**

Prepared as for **2a** from **3d** (0.300 g, 1.007 mmol) and 3-hydroxybenzaldehyde (0.123 g, 1.007 mmol) to yield **3e** as dark brown powder (0.150 g, 41 %): Rf: 0.07 (PE/EtOAc/MeoH 5:1:1); mp: 150.6–153.0 °C; ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.35 (s, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.19 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.14 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.86 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.34–4.16 (m, 3H), 4.05–3.99 (m, 3H), 3.60 (s, 6H), 2.73 (d, *J* = 16.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 191.57, 157.69, 152.85, 136.20, 135.39, 131.75, 129.94, 128.65, 125.45, 124.53, 121.74, 116.82, 115.72, 115.64, 110.88, 67.40, 66.04, 59.75, 53.45, 32.04. APCI-HRMS *m/z* calcd. for $C_{22}H_{23}NO_4 \cdot HCl$ (MH +): 366.1700, found: 366.1718. Purity (HPLC): 95.5 %.

(2*E*)-2-(3,4-dihydroxybenzylidene)-5-[2-(morpholin-4-yl) ethoxy]-2,3-dihydro-1*H*-inden-1-one **(3f)**

Prepared as for **2a** from **3d** (0.089 g, 0.300 mmol) and 3,4-dihydroxybenzaldehyde (0.041 g, 0.300 mmol) to yield compound **3f** as brown powder (0.100 g, 88 %): Rf: 0.17 (EtOAc 100 %); mp: 151.8–151.9 °C; ^{1}H NMR (600 MHz, DMSO) δ 11.21 (s, 1H), 9.72 (s, 1H), 9.28 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.31 (s, 1H), 7.24 (d, *J* = 1.0 Hz, 1H), 7.20 (d, *J* = 1.9 Hz, 1H), 7.11–7.07 (m, 2H), 6.87 (d, *J* = 8.2 Hz, 1H), 4.58 (s, 2H), 3.99 (s, 2H), 3.96 (s, 1H), 3.83 (t, *J* = 12.0 Hz, 2H), 3.61 (d, *J* = 1.2 Hz, 2H), 3.52 (d, *J* = 12.2 Hz, 2H), 3.22 (dd, *J* = 9.7, 1.3 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 191.58, 162.70, 152.46, 147.85, 145.61, 132.74, 131.71, 131.50, 126.47, 125.25, 123.89, 117.55, 116.06, 115.63, 111.10, 63.16, 62.61, 54.75, 51.66, 32.09. APCI-HRMS *m/z* calcd. for $C_{22}H_{23}NO_5 \cdot HCl$ (MH +): 382.1649, found: 382.1674. Purity (HPLC): 75.5 %.

General procedure for the synthesis of 4a–e

(2*E*)-5-hydroxy-2-(3-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one **(4a)**

5-Hydroxy-1-tetralone (0.500 g, 3.083 mmol) and 3-methoxybenzaldehyde (0.420 g, 3.083 mmol) were suspended in HCl (32 %, 6 mL) and MeOH (4 mL) and mechanically stirred at 120 °C under reflux for 24 h. Upon completion, the reaction mixture was cooled down to room temperature, extracted with EtOAc (3 × 100 mL), combined organic extracts dried ($MgSO_4$), filtered and conc. to yield **4a** as dark brown powder (0.540g, 62 %): Rf: 0.88 (PE/EtOAc 1:1); mp: 328.2–328.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.04 (dd, *J* = 10.8, 3.9 Hz, 2H), 6.96 (s, 1H), 6.91 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.06 (d, *J* = 6.0 Hz, 1H), 3.84 (s, 3H), 3.11 (td, *J* = 6.6, 1.6 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.63, 159.60, 152.92, 137.23, 136.85, 135.75, 134.79, 130.33, 129.61, 127.37, 122.46, 120.61, 119.84, 115.48, 114.27, 55.46, 26.65, 21.71. APCI-HRMS *m/z* calcd. for $C_{18}H_{16}O_3$ (MH +): 281.1172, found: 281.1181. Purity (HPLC): 99.5 %.

(2*E*)-2-(3,4-dimethoxybenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one **(4b)**

Prepared as for **4a** from 5-hydroxy-1-tetralone (0.500 g, 3.083 mmol) and 3,4-dimethoxybenzaldehyde (0.512 g, 3.083 mmol) to yield **4b** as dark brown powder (0.670 g, 70 %): Rf: 0.57 (PE/EtOAc 1:1); mp: 252.5–253.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.11–7.01 (m, 2H), 6.98 (s, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.04 (d, *J* = 0.4 Hz, 1H), 3.91 (d, *J* = 12.6 Hz, 6H), 3.18–3.05 (m, 2H), 2.94 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.43, 152.86, 149.71, 148.82, 137.19, 134.97, 133.81, 130.05, 128.74, 127.32, 123.47, 120.54, 119.68, 113.38, 111.04, 56.08, 56.07, 26.70, 21.55. APCI-HRMS *m/z* calcd. for $C_{19}H_{18}O_4$ (MH +): 311.1278, found: 311.1293. Purity (HPLC): 91.6 %.

(2*E*)-5-methoxy-2-(3-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one **(4c)**

Prepared as for **4a** from 5-methoxy-1-tetralone (0.500 g, 2.806 mmol) and 3-methoxybenzaldehyde (0.382 g, 2.806 mmol) to yield **4c** as golden brown powder (0.730 g, 88 %): Rf: 0.67 (PE/EtOAc 1:1); mp: 114.1–143 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82–7.74 (m, 2H), 7.37–7.29 (m, 2H), 7.04 (dd, *J* = 10.6, 3.5 Hz, 2H), 6.97 (d, *J* = 1.9 Hz, 1H), 6.91 (dd, *J* = 8.1, 2.4 Hz, 1H), 3.86 (d, *J* = 18.1 Hz, 6H), 3.09 (td, *J* = 6.5, 1.8 Hz, 2H), 2.92 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.29, 159.62, 156.43, 137.37, 136.32, 135.89, 134.55, 132.49, 129.57, 127.31, 122.43, 120.02, 115.45, 114.46, 114.15, 55.87, 55.44, 26.72, 21.66. APCI-HRMS *m/z* calcd. for $C_{19}H_{18}O_3$ (MH +): 295.1329, found: 295.1333. Purity (HPLC): 69.4 %.

(2*E*)-5-methoxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one **(4d)**

Prepared as for **4a** from 5-methoxy-1-tetralone (0.500 g, 2.806 mmol) and 4-methoxybenzaldehyde (0.382 g, 2.806 mmol) to yield **4d** as dark brown powder (0.810 g, 98 %): Rf: 0.77 (PE/EtOAc 1:1); mp: 99.4–185.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.75 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.47–7.34 (m, 2H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.04 (dd, *J* = 8.1, 0.8 Hz, 1H), 6.97–6.86 (m, 2H), 3.89–3.84 (m, 6H), 3.10 (td, *J* = 6.6, 1.7 Hz, 2H), 2.92 (t, *J* = 6.6 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.24, 160.02, 156.36, 136.51, 134.74, 133.67, 132.24, 131.88, 128.59, 127.23, 120.00, 114.31, 114.06, 55.87, 55.48, 26.67, 21.48. APCI-HRMS *m/z* calcd. for $C_{19}H_{18}O_3$ (MH +): 295.1329, found: 295.1329. Purity (HPLC): 83.4 %.

(2*E*)-2-(3,4-dimethoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(2*H*)-one **(4e)**

Prepared as for **4a** from 5-methoxy-1-tetralone (0.500 g, 2.806 mmol) and 3,4-dimethoxybenzaldehyde (0.466 g, 2.806 mmol) to yield **4e** as dark brown powder (0.750 g, 82 %): Rf: 0.39 (PE/EtOAc 1:1); mp: 117.7–154.6 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (s, 1H),

7.77–7.72 (m, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.07 (ddd, *J* = 24.4, 6.4, 1.1 Hz, 2H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 3.92 (d, *J* = 10.4 Hz, 6H), 3.87 (s, 3H), 3.12 (td, *J* = 6.6, 1.7 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.18, 156.36, 149.64, 148.83, 136.68, 134.71, 133.96, 132.23, 128.85, 127.27, 123.39, 120.00, 114.35, 113.36, 111.02, 56.08, 56.06, 55.88, 26.74, 21.48. APCI-HRMS *m/z* calcd. for $C_{20}H_{20}O_4$ (MH +): 325.1434, found: 325.1457. Purity (HPLC): 79.9 %.

Biology

All reagents were commercially available and purchased from various manufacturers: radioligands [^{3}H]DPCPX (specific activity 120 Ci/mmol) and [^{3}H]NECA (specific activity 21.1 Ci/mmol) as well as filter-count from PerkinElmer and Whatman GF/B 25 mm diameter filters from Merck. Radio activity was counted by a Packard Tri-CARB 2810 liquid scintillation counter.

Adenosine A_1 and A_{2A} receptor radioligand binding assays

The degree of binding affinity that the test compounds (**2a–h**, **3a–f** & **4a–e**) possess towards rat A_1 and A_{2A} ARs were determined via radioligand binding assays, as described previously [29–32]. The collection of tissue samples for the A_1 and A_{2A} radioligand binding assays was approved by the Research Ethics Committee of the North-West University (NWU) (application number NWU - 00260–17-A5). Sigmoidal dose response curves, from which IC_{50} values were calculated, were obtained by plotting the specific binding against the logarithm of the test compounds' concentrations. Subsequently, the IC_{50} values were used to calculate the K_i values for the competitive inhibition of [^{3}H]DPCPX (K_d = 0.36 nM) against rat whole brain membranes (expressing the A_1 AR) and [^{3}H]NECA (K_d = 15.3 nM) against rat striatal membranes (expressing the A_{2A} AR) by the test compounds by means of the Cheng-Prusoff equation. Also, concurrent radioligand binding assays allow for the calculation of the degree of selectivity that the test compounds (**2a–h**, **3a–f** & **4a–e**) possess towards either rat (r) A_1 or A_{2A} ARs (selectivity index (SI)). These results, expressed as K_i values (μM), are summarized in ▶ **Tables 1–3**.

GTP shift assay

The type of binding affinity that test compounds **2a, 3b** and **4a** exhibited at the rat A_1 AR was determined via a GTP shift assay, as described previously [31, 33, 34]. GTP shifts were calculated by dividing the K_i values of compounds reported in the presence of GTP by the K_i values obtained in the absence of GTP and the results are summarized in ▶ **Table 4**.

SwissADME

SwissADME (http://www.swissadme.ch), a free web tool, was used to evaluate key parameters of small molecules, such as pharmacokinetics, drug-likeness and medicinal chemistry friendliness [35].

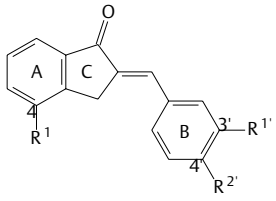
Results, Discussion

Chemistry

A total of seventeen test compounds (**2a–h**, **3b–c, 3e–f** & **4a–e**), of which eleven are either novel 2-benzylidene-1-indanone (**2f–h**,

▶ **Table 1** K_i values for the binding affinity of 2-benzylidene-1-indanone derivatives against rat A_1 and A_{2A} ARs.

2-Benzylidene-1-indanone derivatives



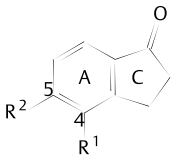
2a-h

#	Ring A	Ring B		K_i ± SEM (μM)[a] (Specific binding (%))[b]		SI[d]
	4	3'	4'	A_1[c] vs [^{3}H]DPCPX	A_{2A}[c] vs [^{3}H]NECA	A_1/A_{2A}
	R^1	$R^{1'}$	$R^{2'}$			
2a	OH	OH	H	0.7921 ± 0.0301[a]	0.4345 ± 0.0171[a]	1.823
2b	OH	H	OH	2.897 ± 0.2126[a]	1.051 ± 0.2480[a]	2.756
2c	OH	OCH_3	H	(55.5)[b]	(23)[b]	-
2d	OH	H	OCH_3	(44.5)[b]	(30.5)[b]	-
2e	OH	OCH_3	OCH_3	(42)[b]	(26)[b]	-
2f	OCH_3	OCH_3	H	(29)[b]	(46.5)[b]	-
2g	OCH_3	H	OCH_3	(79.5)[b]	(45.5)[b]	-
2h	OCH_3	OCH_3	OCH_3	(27)[b]	(23.5)[b]	-

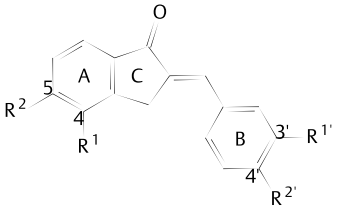
[a] All K_i values were determined in triplicate and expressed as mean ± standard error of the mean (SEM) in μM. [b] Specific binding (%) of the radioligand at a maximum tested concentration of 100 μM were determined in duplicate and expressed as the mean in %. [c] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes). [d] Selectivity index (SI) for the A_{2A} AR isoform calcd. as a ratio of $A_1K_i/A_{2A}K_i$.

▶ **Table 2** K_i values for the binding affinity of 2-benzylidene-1-indanone derivatives against rat A_1 and A_{2A} ARs.

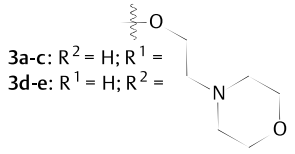
2-Benzylidene-1-indanone derivatives



3a & 3d



3b-c & 3e-f



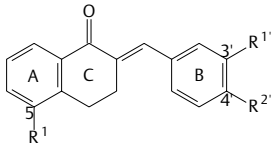
morpholine

#	Ring A		Ring B		K_i ± SEM (μM)[a] (Specific binding (%))[b]		SI[d]
	4	5	3'	4'	A_1[c] vs [^{3}H]DPCPX	A_{2A}[c] vs [^{3}H] NECA	A_1/A_{2A}
	R^1	R^2	$R^{1'}$	$R^{2'}$			
3a		H	-	-	(91)[b]	(99)[b]	-
3b		H	OH	H	6.398 ± 0.2344[a]	(51.5)[b]	-
3c		H	OH	OH	12.35 ± 1.058[a]	(55)[b]	-
3d	H		-	-	(98)[b]	(91)[b]	-
3e	H		OH	H	(29.5)[b]	(35.5)[b]	-
3f	H		OH	OH	(56.5)[b]	(83)[b]	-

[a] All K_i values were determined in triplicate and expressed as mean ± standard error of the mean (SEM) in μM. [b] Specific binding (%) of the radioligand at a maximum tested concentration of 100 μM were determined in duplicate and expressed as the mean in %. [c] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes). [d] Selectivity index (SI) for the A_{2A} AR isoform calcd. as a ratio of $A_1K_i/A_{2A}K_i$.

▶ **Table 3** K_i values for the binding affinity of 2-benzylidene-1-tetrealone derivatives against rat A_1 and A_{2A} ARs.

2-Benzylidene-1-tetralone derivatives



4a-e

#	Ring A	Ring B		K_i ± SEM (μM)[a] (Specific binding (%))[b]		SI[d]
	5	3'	4'	A_1[c] vs [^{3}H]DPCPX	A_{2A}[c] vs [^{3}H]NECA	A_1/A_{2A}
	R^1	$R^{1'}$	$R^{2'}$			
4a	OH	OCH_3	H	4.343 ± 0.4174[a]	(39)[b]	-
4b	OH	OCH_3	OCH_3	6.843 ± 1.118[a]	7.054 ± 2.752 [a]	0.9701
4c	OCH_3	OCH_3	H	(38)[b]	(45.5)[b]	-
4d	OCH_3	H	OCH_3	(32)[b]	(54)[b]	-
4e	OCH_3	OCH_3	OCH_3	(29)[b]	(32.5)[b]	-

[a] All K_i values were determined in triplicate and expressed as mean ± standard error of the mean (SEM) in μM. [b] Specific binding (%) of the radioligand at a maximum tested concentration of 100 μM were determined in duplicate and expressed as the mean in %. [c] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes). [d] Selectivity index (SI) for the A_{2A} AR isoform calcd. as a ratio of $A_1K_i/A_{2A}K_i$.

3b–c & **3e–f**) or –tetralone derivatives (**4a–c** & **4e**), were synthesised via acid-catalysed aldol condensation reactions, as described in literature and outlined in ▶ **Fig. 4b**, [23, 25, 26]. As previously described, the key starting material for **2a–e** was prepared by rearrangement of 3,4-dihydrocoumarin to yield 4-hydroxy-1-indanone [24] and the key starting material for **2f–h** by methylation of 4-hydroxy-1-indanone to yield 4-methoxy-1-indanone [23]. Compound **3a**, the key starting material for **3b–c**, was prepared by reacting the previously synthesised phenol 4-hydroxy-1-indanone [24] with 4-(2-chloroethyl)morpholine in the presence of K_2CO_3 in

acetone (similar to a method used by [36]) to give the ethers **3b–c** (▶ **Fig. 4a**). Demethylation of 5-methoxy-1-indanone to yield 5-hydroxy-1-indanone provided the starting material for compound **3d** [36]. Compound **3d**, the key starting material for **3e–f**, was prepared as for compound **3a**. All synthesized reagents and test compounds (**2a–h**, **3b–c, 3e–f** & **4a–e**) were obtained in fair to poor yields, purified by recrystallization (except synthesized reagents) from a suitable solvent (either MeOH or EtOH) and, in each instance, the structure, molecular mass and/or purity of these compounds were verified by ^{1}H NMR, ^{13}C NMR, MS and/or HPLC.

The 2-benzylidene-1-indanone (**2a–h**, **3b–c** & **3e–f**) and –tetralone derivatives (**4a–e**) were always obtained in the (*E*)-configuration, as seen in the ^{1}H NMR spectra. On the ^{1}H NMR spectra of the said derivatives the signal of the vinyl proton of the α,β-unsaturated carbonyl group was observed at approximately 7.42–7.81 ppm. The same trend was previously observed with the characterization of benzylidene indanones [23, 24] and tetralones [25, 26, 37, 38]. Theoretically, these derivatives may be either (*E*)- or (*Z*)-isomers [39]. The (*E*)-configuration is favourable for thermodynamic reasons, due to steric interaction between the carbonyl and aryl groups in the case of (*Z*)-isomers [34, 39]. As a result of the diamagnetic anisotropy (deshielding of proton due to local diamagnetic current) of the carbonyl functional group, the vinyl proton of the (*E*)-isomer gives a signal at a greater chemical shift than the vinyl proton of the (*Z*)-isomer, as the latter is more remote from the carbonyl functional group [40].

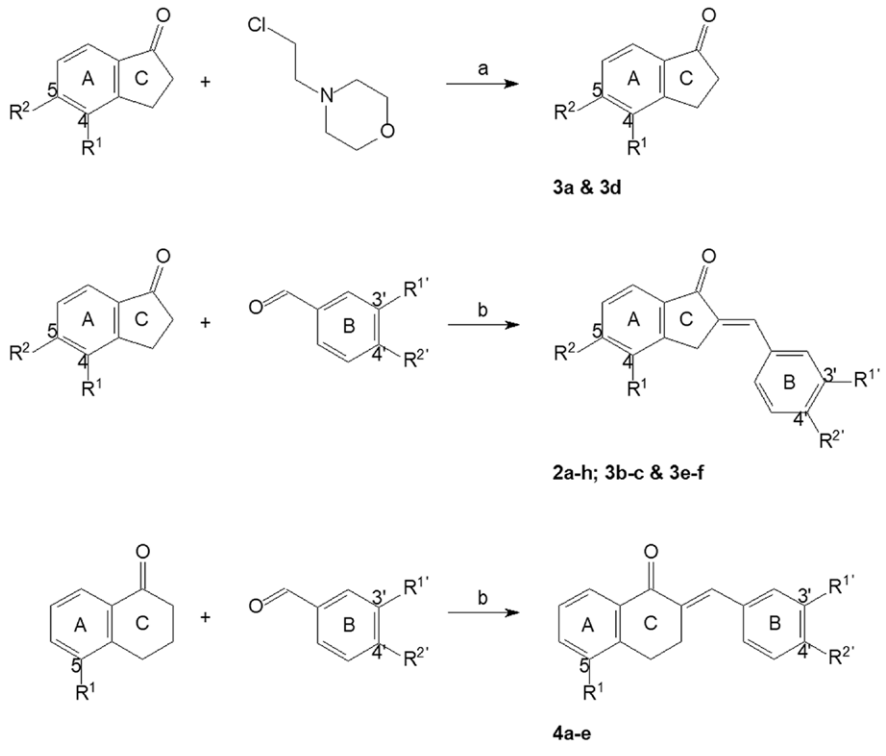
The ^{1}H NMR spectra of the C4 and C5 morpholine substituted 2-benzylidene-1-indanone derivatives **3a–f** showed that the protons present in the morpholine moiety correspond to previously synthesised fluorinated heteroaryl chalcones containing a morpholine moiety [41].

Also, the ^{13}C NMR spectra displayed a prominent signal for the carbonyl group at approximately 191.57–194.66 ppm (**2a–h**, **3b–**

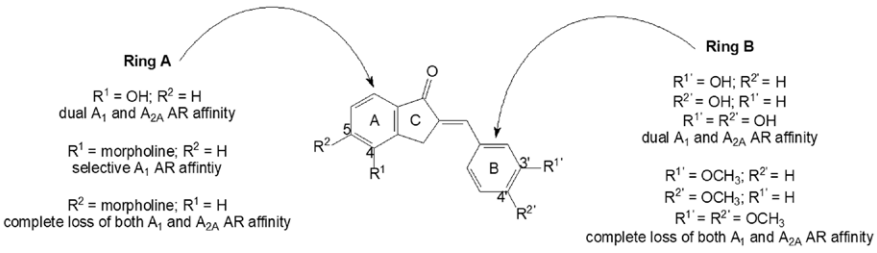
▶ **Table 4** A_1K_i values (in the absence and presence of GTP) and calcd. GTP shifts of **2a**, **3b** & **4a**.

#	K_i ± SEM (μM)[a]		GTP shift[d]
	A_1[b] vs [^{3}H]DPCPX	A_1[b] + GTP[c] vs [^{3}H]DPCPX	
2a	0.7921 ± 0.0301[a]	0.9467 ± 0.0998	1.195
3b	6.398 ± 0.2344[a]	5.945 ± 0.5055[a]	0.9293
4a	4.343 ± 0.4174[a]	5.561 ± 0.8727[a]	1.281

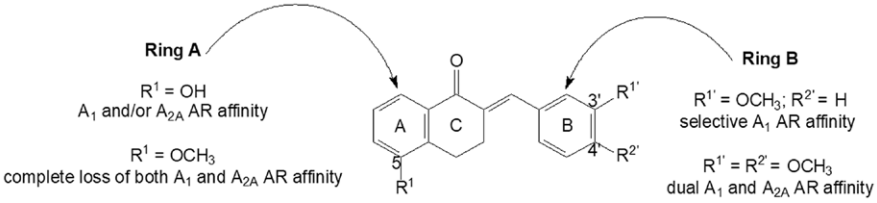
[a] All inhibition constant (K_i) values were determined in triplicate and expressed as mean ± standard error of the mean (SEM) in μM. [b] Rat receptors were used (A_1: rat whole brain membranes). [c] Addition of 100 μM GTP to A_1 AR radioligand binding assay. [d] GTP shift calcd. by dividing K_i value in the presence of 100 μM GTP by K_i value in the absence of 100 μM GTP.



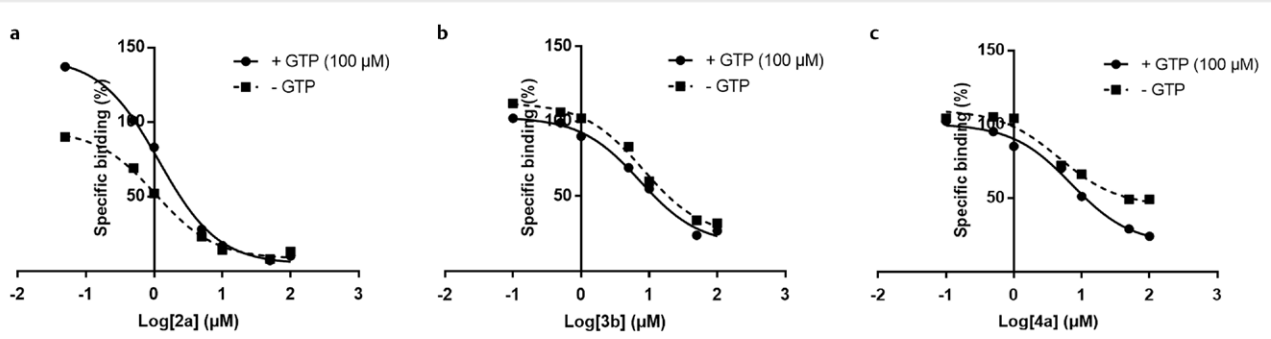
▶ **Fig. 4** Synthesis **3a** & **3d**. Reagents and conditions: a) K_2CO_3, Acetone (40 mL), 120 °C (24 h); Synthesis of **2a–h**, **3b–c, 3e–f** & **4a–e**. b) HCl (32 %, 6 mL), MeOH (4 mL), 120 °C (24 h)



▶ **Fig. 5** Structural requirements of the 2-benzylidene-1-indanone scaffold for A_1 and/or A_{2A} AR affinity.



▶ **Fig. 6** Structural requirements of the 2-benzylidene-1-tetralone scaffold for A_1 and/or A_{2A} AR affinity.



▶ **Fig. 7** The binding curves of compounds **2a** (A), **3b** (B) and **4a** (C) are examples of A_1 AR antagonistic action determined via a GTP shift assay (with and without 100 µM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. Calcd. GTP shift of: 1.195 (**2a**), 0.9293 (**3b**) 1.281(**4a**).

c, 3e–f), 204.22–206.22 ppm (**3a** & **3d**) and 188.18–188.63 ppm (**4a–e**). Signals for the CH_2-group present in the 1-indanone core were observed at 28.93–29.46 ppm (**2a–h**, **3b–c**) and 32.04–32.09 (**3e–f**), whereas the $(CH_2)_2$-groups present in the compounds **3a** & **3d** (starting material for **3b–c** & **3e–f**, respectively) were observed at 35.73–36.05 ppm and 22.35–25.49 ppm. The $(CH_2)_2$-groups present in the 1-tetralone core (**4a–e**) were observed at 26.65–26.74 ppm and 21.48–21.71 ppm.

Mass spectra of all chalcone based benzocycloalkanone derivatives showed intensive molecular ions in accordance with the formulations depicted.

Biology

In vitro evaluation

The SAR studies of the 2-benzylidene-1-indanone derivatives (**2a–h**) for the A_1 and A_{2A} ARs, summarized in ▶ **Table 1** (▶ **Fig. 5**), illustrated that the two compounds with both A_1 and A_{2A} AR affinity, with slight selectivity toward the A_{2A} AR, contain only strong electron donating OH-groups on both ring A and phenyl ring B.

Comparison of compound **2a** to the previously synthesised and evaluated unsubstituted compound **1e** showed that *meta* (C3') OH-group substitution on ring B led to A_1 and A_{2A} AR affinity below 1 µM. Compound **1e** possessed selective A_{2A} AR affinity ($A_{2A}K_i$ (r) =

▶ **Table 5** K_i values for the binding affinity of reference compounds against rat A_1 and A_{2A} ARs.

#	K_i ± SEM (μM)[a]			SI[d] A_1/A_{2A}	GTP shift[e]
	A_1[b] vs [^{3}H]DPCPX	A_{2A}[b] vs [^{3}H]NECA	A_1[b] + GTP[c] vs [^{3}H]DPCPX		
CPA (A_1 agonist)	0.0057 ± 0.0015[a] (0.0068)[f] (0.015)[g] (0.0079)[h]	0.4004 ± 0.1715[a] (0.163)[f] (0.331)[g]	0.0990 ± 0.0150[a] (0.099)[f] (0.099)[g]	0.0142[d] (0.0417)[d] (0.0453)[d]	17.36[e] (15)[f] (14)[g]
DPCPX (A_1 antagonist)	0.0005 ± 0.00003[a] (0.0004)[f] (0.0005)[g] (0.0003)[i]	0.2338 ± 0.0328[a] (0.545)[f] (0.530)[g] (0.340)[i]	0.0006 ± 0.00003[a] (0.0004)[f] (0.0004)[g]	0.0021[d] (0.0007)[d] (0.0009)[d] (0.0008)[d]	1.2[e] (1)[f] (1.25)[g]
Istradefylline (1a) (A_{2A} antagonist)	0.1924 ± 0.0131[a] (0.2300)[j]	0.0014 ± 0.0003[a] (0.0022)[j]	0.1514 ± 0.0178[a]	137.4[d] (104.5)[d]	0.7869[e]

[a] All inhibition constant (K_i) values were determined in triplicate and expressed as mean ± standard error of the mean (SEM) in μM. [b] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes). [c] Addition of 100 μM GTP to A_1 AR radioligand binding assay. [d] Selectivity index (SI) for the A_{2A} AR isoform calcd. as a ratio of $A_1K_i/A_{2A}K_i$. [e] GTP shift calcd. by dividing K_i value in the presence of 100 μM GTP by K_i value in the absence of 100 μM GTP. [f] Literature value obtained from [25][g] Literature value obtained from [28][h] Literature value obtained from [26][i] Literature value obtained from [30][j] Literature value obtained from [46].

▶ **Table 6** Physiochemical properties of compounds ▶ **2a–b**, **3b–c** & **4a–b**

#	Physiochemical properties								Lipophilicity	Water solubility
	Molecular formula	Molecular weight (g/mol)	Fraction Csp3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar refractivity	TPSA (Å^2)	Consensus $logP_{o/w}$	Consensus logS
2a	$C_{16}H_{12}O_3$	252.26	0.06	1	3	2	73.15	57.53	2.62	−4.08
2b	$C_{16}H_{12}O_3$	252.26	0.06	1	3	2	73.15	57.53	2.64	−4.08
3b	$C_{22}H_{23}NO_4$	365.42	0.32	5	5	1	107.43	59.00	2.87	−4.60
3c	$C_{22}H_{23}NO_5$	381.42	0.32	5	6	2	109.46	79.23	2.58	−4.37
4a	$C_{18}H_{16}O_3$	280.32	0.17	2	3	1	82.42	46.53	3.38	−4.69
4b	$C_{19}H_{18}O_4$	310.34	0.21	3	4	1	88.91	55.76	3.27	−4.80

▶ **Table 7** Drug-likeness and medicinal chemistry friendliness of compounds **2a–b**, **3b–c** & **4a–b**

#	Drug-likeness					Lead-likeness
	Num. violations					
	Lipinski[a]	Ghose[b]	Veber[c]	Egan[d]	Muegge[e]	Teague[f]
2a	0	0	0	0	0	0
2b	0	0	0	0	0	0
3b	0	0	0	0	0	1
3c	0	0	0	0	0	1
4a	0	0	0	0	0	1
4b	0	0	0	0	0	1

[a] Lipinski: MW < 500, MLOGP < 4.15, N or O < 10, NH or OH < 5 [39][b] Ghose: 160 < MW < 480, -04 < WLOGP < 5.6, 40 < MR < 130, 20 < atoms < 70 [40][c] Veber: Num. rotatable bonds < 10, TPSA < 140 [41][d] Egan: WLOGP < 5.88, TPSA < 131.6 [42][e] Muegge: 200 < MW < 600, -2 < XLOGP < 5, TPSA < 150, num. rings < 7, num. carbon > 4, num. heteroatoms > 1, num. rotatable bonds < 15, num. H-bond acceptors < 10, num. H-bond donors < 5 [43][f] Teague: 250 < MW < 350, XLOGP3 < 3.5, num. rotatable bonds < 7 [44].

1.55 μM), whereas **2b** gained A_1 (A_1K_i (r) = 0.7921 μM) and increased A_{2A} ($A_{2A}K_i$ (r) = 0.4345 μM) AR affinity and, thus, attained dual A_1 and A_{2A} AR affinity. *Meta* (C3') OH-group substitution on ring B was preferred to *para* (C4') substitution, as seen upon comparison of compound **2a** with **2b** (A_1K_i (r) = 2.897 μM; $A_{2A}K_i$ (r) = 1.051 μM). Yet, *meta* (C3') and *para* (C4') diOH-group substitution on ring B was favoured over either *meta* (C3') or *para* (C4') substitution regarding the A_1 AR (**1f**: A_1K_i (r) = 0.435 μM > **2a**: A_1K_i (r) =

0.7921 µM > **2b:** A_1K_i (r) = 2.897 µM). The almost exact opposite is true regarding the A_{2A} AR, as *meta* (C3') OH-group substitution was favoured over *meta* (C3') and *para* (C4') disubstitution (**2a**: $A_{2A}K_i$ (r) = 0.4345 µM > **1f**: $A_{2A}K_i$ (r) = 0.903 µM > **2b:** A2A_1K_i (r) = 1.051 µM).

Compounds **2c–h** lack A_1 and A_{2A} AR affinity and *meta* (C3') and/or *para* (C4') OCH_3-group substitution on ring B may well be detrimental to AR affinity (**2c–h**: A_1 & $A_{2A}K_i$ (r) > 100 µM versus **2a–b**, **1f** & **1h–j**). This was, however, specific to 2-benzylidene-1-indanone derivatives, as the first in class selective A_{2A} AR antagonist istradefylline (**1a**) also possessed *meta* (C3') and *para* (C4') diOCH_3-group substitution on the phenyl ring.

Interestingly, *para* (C4') OH- or OCH_3- group substitution on phenyl ring B appears to diminish both A_1 and A_{2A} AR affinity, e. g. compounds **2d** and **1i** - except for **2b**. Although **2b** has A_1 and A_{2A} AR affinity, these affinities are much lower than **2b**'s *meta* (C3') and/or *para* (C4') substituted counterparts that retain C4 OH-group substitution on ring A **(2a** & **1f**). The *para* substituted compounds possess lower affinity than the *meta* and/or *para* counterparts.

2-Benzylidene-1-indanone derivatives holding *meta* (C3') and/or *para* (C4') OCH_3-group substitution on ring B were specifically designed to point out the importance of hydroxyl group substitution, e. g. compounds **2c**, **2e**, **2f** & **2h** versus **2a**, **1f**, **1h** & **1j**. A previous study identified compounds **1h** and **1j** as potent A_1 and A_{2A} AR antagonists, but questions were raised due to the presence of a catechol group in compound **1j**. A catechol group, a known reactive functional group, is considered a liability, although widespread in literature as a potential starting point to further explore SARs. The absence of a catechol group, unfortunately, also led to the absence of A_1 and A_{2A} AR affinity; **2e**: A_1 & $A_{2A}K_i$ (r) > 100 µM versus **1f**: A_1K_i (r) = 0.435 µM; $A_{2A}K_i$ (r) = 0.903 µM and **2h**: A_1 & $A_{2A}K_i$ (r) > 100 µM versus **1j**: A_1K_i (r) = 0.042 µM; $A_{2A}K_i$ (r) = 0.078 µM. Further analysis points out that affinity is not only due to hydroxyl group substitution, as insinuated, but that the methoxy group at position C4 on ring A is pivotal to A_1 and A_{2A} AR affinity. Therefore, it may be said that the A_1 and A_{2A} ARs desire either C4 OH- or OCH_3-group substitution on ring A in combination with either *meta* (C3') or *meta* (C3') and *para* (C4') OH-group substitution on phenyl ring B (as exemplified with compounds **2a–b**, **1f**, **1g**, **1h** & **1j**). The presence of a OCH_3-group on phenyl ring B, no matter the substitution pattern on ring A, led to a loss of both A_1 and A_{2A} AR affinity (as exemplified with compounds **2c–h**).

Furthermore, compounds **3b–c** and **3e–f** were designed (see ▶ **Table 2**) – the morpholine analogues of the 2-benzylidene-1-indanone derivatives **1h** and **1i**, respectively. Inclusion of the morpholine moiety was inspired by JNJ-40255293 (**1c**), as it gave optimal *in vitro* as well as *in vivo* activity and had an ED_{50} value of < 0.1 mg/kg in the mouse catalepsy model [42].

The parent scaffolds of the present series **3a** and **3d** (unsubstituted at position C2 of the 1-indanone core) lack both A_1 and A_{2A} AR affinity. The radioligand binding assay results of compounds **3b–c** and **3e–f** were also disappointing; compounds **3b–c** showed more than 155- and 290-fold decrease in A_1 AR affinity, respectively, and a complete loss of A_{2A} AR affinity upon comparison to the C4 OCH_3-group substituted compounds **1h** and **1j**. Before, *meta* (C3') and/or *para* (C4') OH-group substitution on ring B gave similar A_1K_i values (for example **1h** (A_1K_i (r) = 0.041 µM) and **1j** (A_1K_i (r) = 0.042 µM)). Now, *meta* monosubstitution (**3b**) is favoured over *meta* and *para* disubstitution (**3b**) (**3b**: A_1K_i (r) = 6.398 µM versus **3c**: A_1K_i (r) = 12.35 µM). Additionally, the C5 substituted compounds **3e–f** did not retain either A_1 or A_{2A} AR affinity.

Comparison of **3b–c** to **3e–f**, respectively, again demonstrates that the C4 position is preferred to the C5 position on ring A [23]. (For selective A_1 AR affinity: C4 morpholine substitution > C5 morpholine substitution.)

Fortunately, all was not lost as another observation could be made. Comparison of compounds **3b–c** with **1h** and **1j**, respectively, highlighted the importance of C4 OCH_3-group substitution on ring A in order to attain dual A_1 and A_{2A} AR affinity. Moreover, the aforementioned catechol group (previously considered to be problematic) is now in the clear (**3c** versus **1j**); because it may now be said that *meta* (C3') and *para* (C4') diOH-group substitution on ring B is not solely responsible for the nanomolar dual A_1 and A_{2A} AR affinity possessed by compound **1j**.The structurally related 2-benzylidene-1-tetralone derivatives (**4a–e**, ▶ **Table 3**) were also evaluated in accordance with previous research (▶ **Fig. 6**) [25, 26].

The A_1 and A_{2A} ARs did not tolerate C5 OCH_3-group substitution on ring A, as seen with compound **4c** (A_1K_i (r) > 100 µM) as opposed to **4a** (A_1K_i (r) = 4.343 µM) and **4e** (A_1 & $A_{2A}K_i$ (r) > 100 µM) as opposed to **4b** (A_1K_i (r) = 6.843 µM; $A_{2A}K_i$ (r) = 7.054 µM). A prior study came to the same conclusion (**1o–q**: A_1 & $A_{2A}K_i$ (r) > 100 µM). Therefore, regardless of ring B substitution (either -H (**1o**), -OH (**1p–q**) or -OCH_3 (**4c–e**)), C5 OCH_3-group substitution on ring A of the 2-benzylidene-1-tetralone derivatives is detrimental to A_1 and A_{2A} AR affinity.

Interestingly, the structurally related 2-benzylidene-1-indanone derivatives generally preferred OCH_3- to OH-group substitution on ring A and OH- to OCH_3-group substitution on ring B – contradictory to the 2-benzylidene-1-tetralone derivatives [23, 24, 26].

Comparison of *meta* (C3') OCH_3-group substituted **4a** to *meta* (C3') OH-group substituted **1l** showed a more than 2.5-fold decrease in A_1 AR affinity (**4a**: A_1K_i (r) = 4.343 µM versus **1l**: A_1K_i (r) = 1.62 µM), whereas A_{2A} AR affinity was completely lost (**4a**: A_1K_i (r) > 100 µM). Compound **4b** possessed dual A_1 and A_{2A} AR affinity below 10 µM, with a SI of approximately 1. Interestingly, the benzylidene tetralones **4a** and **4b** contrasted with the benzylidene indanones **2c** and **2e**, respectively, revealing that (unlike the benzylidene indanones **2c–h**) *meta* (C3') and *para* (C4') OCH_3-group substitution on ring B of the 2-benzylidene-1-tetralone scaffold is not disadvantageous to A_1 and A_{2A} AR affinity.

The influence of ring C on A_1 and A_{2A} AR affinity is inconclusive. Comparison of benzylidene tetralone **4a** to benzylidene indanone **2c,** as well as **4b–2e**, **4c–2f**, **4d–2g** and **4e–2h**, respectively, led to mixed results. On the one hand, compounds **4a** and **4b** (6-membered ring C) possess A_1 and/or A_{2A} AR affinity and compounds **2c** and **2e** (5-membered ring C) are without A_1 and/or A_{2A} AR affinity, leading to the assumption that a 6-membered ring C (tetralone) is favoured over a 5-membered ring C (indanone). On the other hand, neither **4c–e** (tetralone) nor **2f–h** (indanone) possess A_1 and/or A_{2A} AR affinity. Moreover, the 2-benzylidene-1-tetralone derivative **1p** had only micromolar affinity whereas its counterpart, the 2-benzylidene-1-indanone derivative **1h**, had nanomolar affinity.

The type of binding affinity that test compounds **2a, 3b** and **4a** exhibited at the rat A_1 AR was determined via a GTP shift assay, as described previously [31, 33, 34]. GTP shifts were calculated by dividing the K_i values of compounds reported in the presence of GTP by the K_i values obtained in the absence of GTP and the results are summarized in ▶ **Table 4**. Test compounds **2a, 3b** and **4a** were selected as they possess the highest A_1 AR affinity in the three respective series (▶ **Table 1**, ▶ **2**, ▶ **3**, ▶ **4**). The results suggest that compounds **2a, 3b** and **4a** act as A_1 AR antagonists, as the binding curves in the presence of GTP are almost unaffected and the calculated GTP shifts are approximately 1 (▶ **Table 4** and ▶ **Fig. 7**) [43, 44]. On account of the structural similarity of the test compounds (**2a–h**, **3a–f** & **4a–e**), it may be supposed that these 2-benzylidene-1-indanone and –tetralone derivatives are all A_1 AR antagonists.

The A_1 and A_{2A} AR radioligand binding assays, as well as GTP shift assays were validated with CPA (A_1 agonist), DPCPX (A_1 antagonist) and istradefylline (**1a**) (A_{2A} antagonist) as reference compounds and results are in accordance with literature values. These results, expressed as K_i values (μM), are summarized in ▶ **Table 5**.

In silico evaluation

The physical-chemical properties (▶ **Table 6**), as well as drug-likeness and medicinal chemistry friendliness (▶ **Table 7**) of compounds **2a–b**, **3b–c** and **4a–b** (which showed A_1 and/or A_{2A} AR affinity) indicate that compounds **2a–b**, **3b–c** and **4a–b** are drug-like according to rule-based filters [45–49], despite their low A_1 and/or A_{2A} AR affinity. However, only compounds **2a–b** are lead-like (i. e. a molecular entity suitable for optimization), as compounds **3b–c** and **4a–b** do not satisfy the criteria set out (**3b–c**: MW > 350 & **4a–b**: XLOGP3 > 3.5) [50]. A lead compound is subject to chemical modifications that will, in all likelihood, increase size (MW) and lipophilicity (XLOGP3); therefore, a lead-like compound is required to be smaller and less hydrophobic than a drug-like compound [51].

Conclusions

The obtained results allowed for the conclusion that affinity and/or selectivity of the 2-benzylidene-1-indanone and -tetralone derivatives toward A_1 and/or A_{2A} ARs may be modulated by the nature of the substituents attached at position C4 of the 1-indanone core and C5 of the 1-tetralone core, as well as the *meta* (C3') and/or *para* (C4') position(s) on ring B. However, the present series of 2-benzylidene-1-indanone derivatives (**2a–h**) did not produce a single compound with higher A_1 and A_{2A} AR affinity than compounds **1h** and **1j**. It is, nevertheless, worth mentioning that all compounds that possessed affinity for the A_1 and/or A_{2A} AR had K_i values below 10 μM. Additionally, the combination of moieties from compounds **1c** as well as **1h** and **1j** to yield morpholine substituted 2-benzylidene-1-indanone derivatives (**3b–c** & **3e–f**) did not lead to hybrid compounds with affinity in the nanomolar-range. The morpholine substituted 2-benzylidene-1-indanone derivatives (**3c** & **3f**), however, answered a question previously raised regarding the C4-OCH_3 substituted 2-benzylidene-1-indanone derivatives, namely that the catechol functional group (as seen in **1j**) is not the liability it was thought to be. In fact, the OCH_3-group at position C4 on ring A of the 2-benzylidene-1-indanone derivative **1j** also contributed to A_1 and A_{2A} AR affinity – and not only the catechol functional group on ring B. In the future the concerns raised about the α,β-unsaturated ketone system (perceived as a potential Michael acceptor) present in the 2-benzylidene-1-indanone and -tetralone scaffold may be eased by converting the lead-like compounds (**2a–b**) into either isoxazole or pyrazole derivatives (thus eliminating the said reactive functional group, while retaining the necessary substituents on ring A and phenyl ring B).

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Conflict of Interest

The authors have no conflict of interest to declare.

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