# 2–Benzylidene–1–Indanone Analogues as Dual Adenosine $A_1/A_{2a}$ Receptor Antagonists for the Potential Treatment of Neurological Conditions

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**ABSTRACT**

Previous studies explored 2-benzylidine-1-tetralone derivatives as innovative adenosine $A_1$ and $A_{2A}$ receptor antagonists for alternative non-dopaminergic treatment of Parkinson's disease. This study's aim is to investigate structurally related 2-benzylidene-1-indanones with substitutions on ring A and B as novel, potent and selective adenosine $A_1$ and $A_{2A}$ receptor blockers. 2-Benzylidene-1-indanone derivatives were synthesised via acid catalysed aldol condensation reactions and evaluated via radioligand binding assays to ascertain structure activity relationships to govern $A_1$ and $A_{2A}$ AR affinity. The results indicated that hydroxy substitution at C4 of ring A and meta (3'), or para (4') substitution on ring B of the 2-benzylidene-1-indanone scaffold (**2c**) is preferred over substitution at C5 (**2d**) or C6 (**2e**) of ring A for adenosine $A_1$ receptor activity and selectivity in the micromolar range. Furthermore, substitution at the meta (3') position of ring B with chlorine lead to the highly potent and selective adenosine $A_{2A}$ receptor antagonist, compound **2 h**. Compound **2c** and the **2q** behaved as adenosine $A_1$ receptor antagonists in the performed GTP shift assays. In view of these findings, compounds **2c**, **2 h**, **2q** and **2p** are potent and selective adenosine $A_1$ and $A_{2A}$ receptor antagonists for the potential treatment of neurological conditions.

## Introduction

Existing treatment for Parkinson's disease (PD) is controversial; on the one hand the gold standard of PD treatment L-3,4-dihydroxyphenylalanine (Levo-dopa/L-dopa) effectively relieves motor symptoms, yet, on the other hand, its adverse effects comprise of motor and non-motor complications [1]. Additionally, it does not address non-motor symptoms or neurodegeneration [2]. Justly, a novel drug that addresses all said problems is needed, seeing as non-dopaminergic treatment may possibly improve PD patients' quality of life.

Adenosine receptor (AR) antagonists may be the solution to the PD–conundrum; as an epidemiological study has established an association between the consumption of coffee or caffeine and a reduced risk of developing PD. Caffeine is a xanthine derivative and non–selective $A_1$ and $A_{2A}$ AR antagonist [3]. An $A_1$ AR antagonist could alleviate cognitive deficits in PD, a non-motor symptom of the disease [4]. This is substantiated by the $A_1$ AR's distribution and expression in the prefrontal cortex and hippocampus [5], which are areas linked to cognition [6]. Moreover, blockade of $A_1$ AR's increase acetylcholine — a neurotransmitter associated with learning and memory [7]. Another non–motor symptom of PD, namely depression, may be addressed by $A_{2A}$ AR antagonists; evidenced by a decrease in immobility time during the forced swim test and tail suspension test in rodents when KW-6002

(selective $A_{2A}$ AR antagonist) was administered [8, 9]. $A_{2A}$ AR antagonists are also relevant to motor control — which is affected in PD [1]. Firstly, these receptors are abundantly expressed in the striatum [5], a brain area associated with motor control [6]. Secondly, adenosine $A_{2A}$ and dopamine $D_2$ receptors are co-expressed on the striatopallidal (inhibitory) pathway, inhibition of $A_{2A}$ AR's compensate for hypolocomotion as a result of dwindling stimulation of striatopallidal dopamine $D_2$ receptors by dopamine [1]. Adverse effects of L–dopa (most effective drug for treatment of motor symptoms), such as dyskinesias, may be lessened by concomitant administration of an $A_{2A}$ AR antagonist to L-dopa [10]. Blockade of both $A_1$ and $A_{2A}$ AR's synergistically improve motor control by increasing presynaptic dopamine release via $A_1$ AR inhibition and postsynaptic dopamine response via $A_{2A}$ AR inhibition [11]. Additionally, blockade of the $A_{2A}$ AR may possibly facilitate neuroprotection in neurodegenerative disorders, like PD [12].

Recently, 2-benzylidene-1-tetralone analogues were explored as $A_1$ and/or $A_{2A}$ AR antagonists [13, 14]. The 2-benzylidene-1-tetralone derivative (**1a**), possessed both $A_1$ and $A_{2A}$ AR affinity ($A_1K_i$ = 5.93 µM; and $A_{2A}K_i$ + 2.90 µM) with a selectivity index of 2 towards the $A_{2A}$ AR. Compound **1a** has a basic benzylidene tetralone backbone (fused 6- and 6-membered rings, namely ring A and ring C), where ring C bears a C2-phenyl substituted side-chain (ring B). It was found that C5–hydroxy substitution on ring A is optimal for $A_1$ and $A_{2A}$ AR binding and that the 2-benzylidene side-chain, ring B, also governs AR binding. Similar to these bicyclic benzofused ring systems are the heterocyclic aurones — fused 6- and 5-membered rings — which also possess $A_1$ and/or $A_{2A}$ AR affinity. Hispidol (**1b**) and maritimetin (**1c**) are examples of aurones with AR affinity [15].

In analogy to the structure of compound **1a** and the aurones (**1b-c**) (▶ **Fig. 1**), the present study investigates the structurally related 2-benzylidene-1-indanone scaffold as potent $A_1$ and $A_{2A}$ AR antagonists.

The 2-benzylidene-1-indanone scaffold will be modified to include changes to ring A and ring B (▶ **Fig. 1**) and, subsequently, evaluated to ascertain which structure activity relationships govern $A_1$ and $A_{2A}$ AR affinity.

## Materials and Methods

### Chemistry

Unless otherwise noted, all starting materials and solvents were procured from Sigma–Aldrich and used without further purification. Proton ($^1$H) and carbon ($^{13}$C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively, with deuterated dimethylsulfoxide (DMSO–d6) as solvent. Chemical shifts are reported in parts per million (δ) in relation to the signal of tetramethylsilane ($Si(CH_3)_4$). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF–Q II mass spectrometer in atmospheric pressure chemical ionisation (APCI) mode. High performance liquid chromatography (HPLC) analyses were determined on an Agilent 1100 HPLC system. Melting points (mp) were measured with a Buchi B545 melting point apparatus and are uncorrected.

### Synthesis of 4-hydroxy-2,3-dihydro-1H–inden-1-one (2a)

$AlCl_3$ (148 mmol) and NaCl (86 mmol) were mixed and mechanically stirred at 120–150 °C. At 150 °C, 3,4-dihydrocoumarin (27 mmol) was slowly added to the $AlCl_3$ and NaCl mixture. Subsequently, the temperature was raised to 200 °C and the reaction mixture mechanically stirred under reflux for 1h30 min. Ice (103 g) and HCl (32 %; 53 mL) were added to the reaction mixture and mechanically stirred at room temperature for 2 h. The resulting grey solid was washed with water, filtered and dried to yield **2a** as a grey powder, used without further purification: Yield 85 %; mp 231.4–232.5 °C; $^1$H NMR (600 MHz, DMSO) δ 10.00 (s, 1 H), 7.23 (t, J = 7.6 Hz, 1 H), 7.08 (d, J = 7.4 Hz, 1 H), 7.05 (d, J = 7.3 Hz, 1 H), 2.92 (t, J = 5.7 Hz, 2 H), 2.59 (t, J = 5.7 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 206.68, 155.17, 141.90, 138.43, 128.66, 119.82, 113.34, 35.81, 22.33. APCI–HRMS m/z calculated for $C_9H_9O_2$ ($MH^+$): 149.0597, found: 149.0597. Purity (HPLC): 97.2 %.

### General procedure for synthesis of 2-benzylidene-1-indanone analogues (2b–q)

Firstly, 4-hydroxy-2,3-dihydro-1H-inden-1-one (**2a**) (2.025 mmol), 5-hydroxy-2,3-dihydro-1H-inden-1-one (2.025 mmol) or 6-hydroxy-2,3-dihydro-1H-inden-1-one (2.025 mmol) was added to an empty flat bottomed flask and, secondly, the appropriate benzaldehyde (2.025 mmol) (as stated in **b**–**q**), thereafter MeOH (4 mL) was added to the contents of the flat bottomed flask, followed by HCl (32 %; 190.9 mmol, 6 mL). The subsequent suspension was mechanically stirred at 120 °C under reflux for 24 h. Thereafter, the reaction mixture was cooled to room temperature, ice (20 g) was added and the resulting precipitate was filtered, dried and recrystallized from a suitable solvent to yield compounds **2b**–**q**.

#### (E)-2-benzylidene-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 b)

The title compound (light brown powder) was prepared in a yield of 24 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and benzaldehyde: mp 327.2–327.5 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.11 (s, 1 H), 7.79 (d, J = 7.5 Hz, 2 H), 7.52 (dd, J = 9.7, 5.1 Hz, 3 H), 7.46 (t, J = 7.3 Hz, 1 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.25 (d, J = 7.4 Hz, 1 H), 7.10 (d, J = 7.7 Hz, 1 H), 3.94 (d, J = 1.0 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.60, 154.83, 138.87, 136.37, 135.11, 134.93, 132.80, 130.75, 129.81, 129.08, 129.04, 120.44, 114.17, 29.08. APCI–HRMS m/z calculated for $C_{16}H_{13}O_2$ ($MH^+$): 237.0910, found: 237.0909. Purity (HPLC): 100 %.

#### (E)-2-(3,4-dihydroxybenzylidene)-4-hydroxy-2,3-dihydro-2,3-dihydro-1H-inden-1-one (2 c)

The title (green powder) compound was prepared in a yield of 55 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 3,4-dihydroxybenzaldehyde: 25.8–25.9 °C; $^1$H NMR (600 MHz, DMSO) δ 10.10 (s, 1 H), 9.67 (s, 1 H), 9.33 (s, 1 H), 7.36 (s, 1 H), 7.33–7.24 (m, 2 H), 7.22 (d, J = 7.4 Hz, 1 H), 7.14–7.05 (m, 2 H), 6.86 (d, J = 8.2 Hz, 1 H), 3.83 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.47, 154.75, 148.05, 145.65, 139.35, 136.03, 133.76, 131.34, 128.94, 126.46, 124.49, 119.98, 117.24, 116.06, 113.97, 29.17. APCI–HRMS m/z calculated for $C_{16}H_{13}O_4$ ($MH^+$): 269.0808, found 269.0810. Purity (HPLC): 98.7 %.

#### (E)-2-(3,4-dihydroxybenzylidene)-5-hydroxy-2,3-dihydro-1H-inden-1-one (2 d)

The title compound (beige powder) was prepared in a yield of 48 % from 5-hydroxy-2,3-dihydro-1H-inden-1-one and 3,4-dihydroxybenzaldehyde: 280.8–287.2 °C; $^1$H NMR (600 MHz, DMSO) δ 10.55

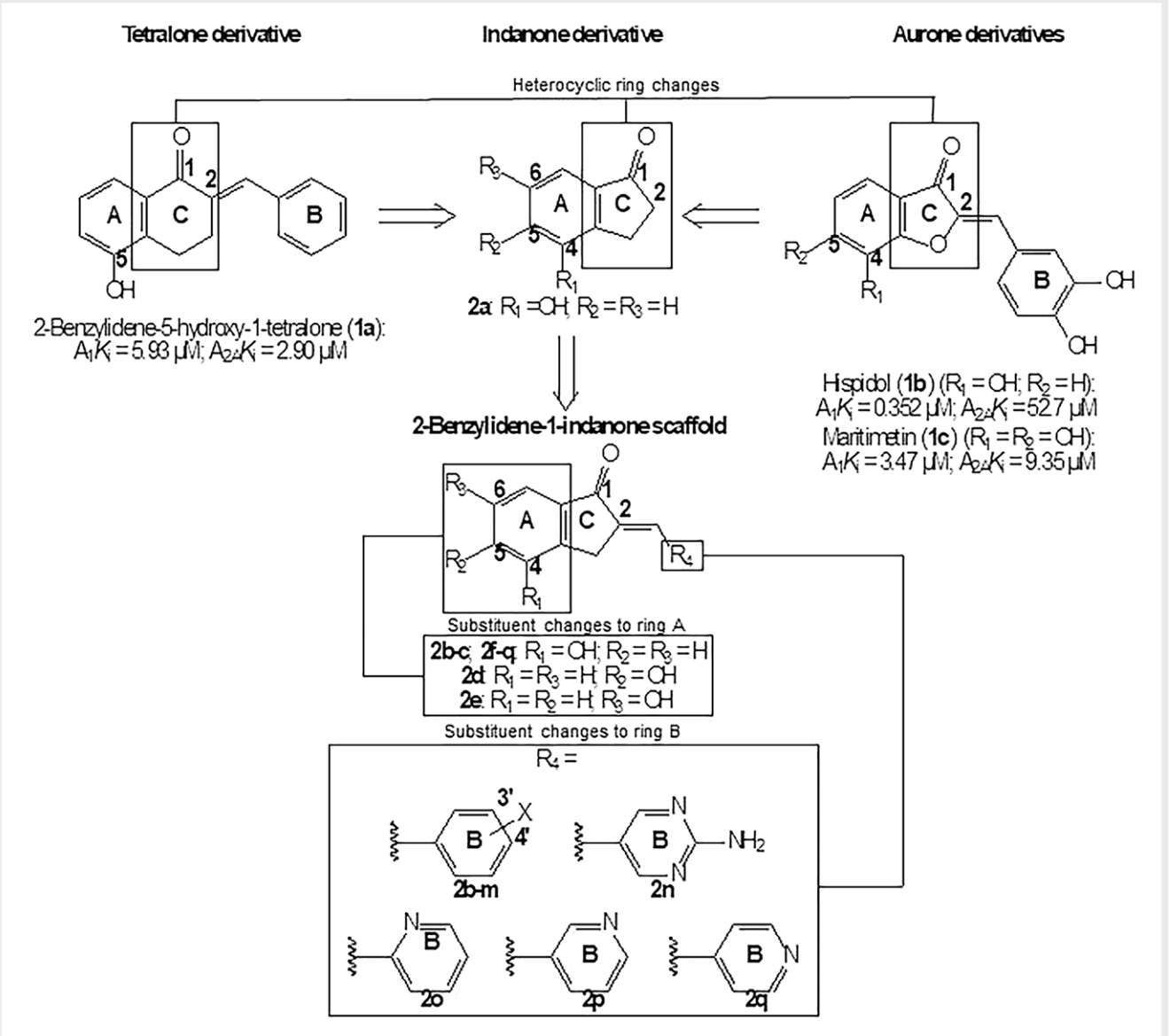
(s, 1 H), 9.59 (s, 1 H), 9.22 (s, 1 H), 7.61 (d, J = 8.3 Hz, 1 H), 7.25 (t, J = 1.7 Hz, 1 H), 7.18 (d, J = 2.0 Hz, 1 H), 7.07 (dd, J = 8.3, 2.0 Hz, 1 H), 6.96 (d, J = 1.8 Hz, 1 H), 6.87–6.82 (m, 2 H), 3.92 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 191.43, 163.55, 152.67, 147.58, 145.55, 132.17, 131.89, 129.64, 126.66, 125.53, 123.76, 117.33, 115.99, 111.92, 39.52, 31.90. APCI–HRMS m/z calculated for $C_{16}H_{13}O_4$ ($MH^+$): 269.0808, found 269.0769. Purity (HPLC): 92 %.

### (E)-2-(3,4-dihydroxybenzylidene)-6-hydroxy-2,3-dihydro-1H-inden-1-one (2 e)

The title compound (dark brown powder) was prepared in a yield of 11 % from 6-hydroxy-2,3-dihydro-1H-inden-1-one and 3,4-dihydroxybenzaldehyde: 26.1–26.2 °C; $^1$H NMR (600 MHz, DMSO) δ 9.81 (s, 1 H), 9.67 (s, 1 H), 9.27 (s, 1 H), 7.46 (d, J = 8.2 Hz, 1 H), 7.33 (s, 1 H), 7.20 (d, J = 1.9 Hz, 1 H), 7.14–7.04 (m, 3 H), 6.85 (d, J = 8.2 Hz, 1 H), 3.89 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.20, 157.11, 147.97, 145.61, 140.43, 138.92, 133.40, 127.34, 126.50, 124.21, 122.92, 117.44, 116.05, 108.09, 39.52, 31.29. APCI–HRMS m/z calculated for $C_{16}H_{13}O_4$ ($MH^+$): 269.0808, found 268.0730. Purity (HPLC): 96.3 %.

### (E)-2-(3-fluorobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 f)

The title compound (light brown crystals) was prepared in a yield of 58 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 3-fluorobenzaldehyde: mp 30.0–30.1 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.12 (d, J = 1.2 Hz, 1 H), 7.62 (t, J = 10.2 Hz, 2 H), 7.59–7.49 (m, 2 H), 7.34–7.23 (m, 3 H), 7.10 (dd, J = 7.7, 0.7 Hz, 1 H), 3.95 (d, J = 1.4 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.55 (s), 163.16 (s), 161.54 (s), 154.86 (s), 138.69 (s), 137.34 (d, J = 8.0 Hz), 136.44 (d, J = 10.0 Hz), 131.40 (d, J = 2.4 Hz), 130.95 (d, J = 8.4 Hz), 129.17 (s), 126.95 (d, J = 2.5 Hz), 120.63 (s), 116.83 (d, J = 21.9 Hz), 116.54 (d,




▶ **Fig. 1** Structural and heterocyclic ring changes to compound 1a, hispidol and maritimetin to determine features essential for dual A1/A2A antagonistic activity.

J = 21.2 Hz), 114.24 (s), 28.96 (s). APCI–HRMS m/z calculated for $C_{16}H_{12}FO_2$ ($MH^+$): 255.0816, found: 255.0816. Purity (HPLC): 100 %.

### (E)-2-(4-fluorobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 g)

The title compound (light brown crystals) was prepared in a yield of 44 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 4-fluorobenzaldehyde: mp 51.5–51.6 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.12 (s, 1 H), 7.86 (dd, J = 8.4, 5.7 Hz, 2 H), 7.52 (s, 1 H), 7.33 (dt, J = 15.2, 8.2 Hz, 3 H), 7.25 (d, J = 7.4 Hz, 1 H), 7.10 (d, J = 7.7 Hz, 1 H), 3.91 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.55, 163.56, 161.91, 154.82, 138.83, 136.33, 134.78 (d, J = 2.2 Hz), 133.06 (d, J = 8.6 Hz), 131.64, 131.59 (d, J = 3.1 Hz), 129.09, 120.42, 116.15, 116.01, 114.15, 28.90. APCI–HRMS m/z calculated for $C_{16}H_{12}FO_2$ ($MH^+$): 255.0816, found: 255.0816. Purity (HPLC): 98.1 %.

### (E)-2-(3-chlorobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 h)

The title compound (beige powder) was prepared in a yield of 75 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 3-chlorobenzaldehyde: mp 386.7–386.8 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.14 (s, 1 H), 7.85 (s, 1 H), 7.77 (d, J = 7.4 Hz, 1 H), 7.58–7.48 (m, 3 H), 7.32 (t, J = 7.6 Hz, 1 H), 7.26 (d, J = 6.9 Hz, 1 H), 7.11 (dd, J = 7.8, 0.8 Hz, 1 H), 3.95 (d, J = 1.5 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.46, 154.82, 138.65, 137.10, 136.60, 136.38, 133.68, 131.15, 130.79, 129.95, 129.41, 129.24, 129.15, 120.63, 114.23, 39.52, 28.91. APCI–HRMS m/z calculated for $C_{16}H_{12}ClO_2$ ($MH^+$): 271.0520, found: 271.0520. Purity (HPLC): 100 %.

### (E)-2-(4-chlorobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 i)

The title compound (gold crystals) was prepared in a yield of 61 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 4-chlorobenzaldehyde: mp 30.9–31.0 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.14 (d, J = 10.6 Hz, 1 H), 7.81 (d, J = 8.4 Hz, 2 H), 7.57 (d, J = 8.4 Hz, 2 H), 7.50 (s, 1 H), 7.30 (t, J = 7.6 Hz, 1 H), 7.24 (d, J = 7.4 Hz, 1 H), 7.10 (d, J = 7.8 Hz, 1 H), 3.91 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.53, 154.86, 138.77, 136.33, 135.78, 134.42, 133.85, 132.38, 131.42, 129.14, 129.07, 120.53, 114.19, 28.98. APCI–HRMS m/z calculated for $C_{16}H_{12}ClO_2$ ($MH^+$): 271.0520, found: 271.0520. Purity (HPLC): 100 %.

### (E)-2-(3-bromobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1–one (2 j)

The title compound (beige powder) was prepared in a yield of 68 % from 4-hydroxy-2,3–dihydro-1H-inden-1-one and 3-bromobenzaldehyde: mp 30.8–30.9 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.15 (d, J = 4.8 Hz, 1 H), 7.97 (s, 1 H), 7.80 (d, J = 7.8 Hz, 1 H), 7.64 (dd, J = 7.9, 1.1 Hz, 1 H), 7.53–7.44 (m, 2 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.25 (d, J = 7.3 Hz, 1 H), 7.13–7.08 (m, 1 H), 3.93 (d, J = 1.3 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.48, 154.86, 138.68, 137.40, 136.59, 136.40, 132.86, 132.33, 131.13, 131.06, 129.60, 129.17, 122.31, 120.66, 114.24, 28.91. APCI–HRMS m/z calculated for $C_{16}H_{12}BrO_2$ ($MH^+$): 315.0015, found: 315.0015. Purity (HPLC): 100 %.

### (E)-2-(4-bromobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2 k)

The title compound (gold crystals) was prepared in a yield of 49 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 4-bromobenzaldehyde: mp 30.9–31.0 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.14 (d, J = 13.7 Hz, 1 H), 7.72 (qd, J = 8.4, 2.6 Hz, 4 H), 7.49 (d, J = 1.8 Hz, 1 H), 7.31 (td, J = 7.7, 1.5 Hz, 1 H), 7.25 (d, J = 7.5 Hz, 1 H), 7.10 (d, J = 7.8 Hz, 1 H), 3.91 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.53, 154.85, 138.76, 136.33, 135.90, 134.17, 132.58, 132.01, 131.51, 129.15, 123.31, 120.54, 114.19, 28.99. APCI–HRMS m/z calculated for $C_{16}H_{12}BrO_2$ ($MH^+$): 315.0015, found: 315.0015. Purity (HPLC): 100 %.

### (E)-4–hydroxy-2-(4-(trifluoromethyl)benzylidene)-2,3-dihydro-1H-inden-1–one (2 l)

The title compound (green crystals) was prepared in a yield of 39 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 4-(trifluoromethyl)benzaldehyde: mp 317.3–397.4 °C (EtOH); $^1$H NMR (600 MHz, DMSO) δ 10.18 (d, J = 4.9 Hz, 1 H), 7.99 (d, J = 8.1 Hz, 2 H), 7.85 (d, J = 8.2 Hz, 2 H), 7.57 (s, 1 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.26 (d, J = 7.4 Hz, 1 H), 7.11 (d, J = 7.7 Hz, 1 H), 3.96 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.50, 154.90, 138.94, 138.61, 137.66, 136.50, 131.19, 130.92, 129.36, 129.22, 129.15, 125.75 (dd, J = 7.3, 3.5 Hz), 125.00, 123.20, 120.69, 114.27, 28.96. APCI–HRMS m/z calculated for $C_{17}H_{12}F_3O_2$ ($MH^+$): 305.0784, found: 305.0807. Purity (HPLC): 98.4 %.

### (E)-4-((4-hydroxy-1-oxo-1H-inden-2(3 H)-ylidene)methyl) benzonitrile (2 m)

The title compound (green crystals) was prepared in a yield of 53 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 4-formylbenzonitrile: mp 304.5–307.4 °C (MeOH); $^1$H NMR (600 MHz, DMSO) δ 10.17 (s, 1 H), 7.95 (s, 4 H), 7.54 (d, J = 1.8 Hz, 1 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.25 (d, J = 7.4 Hz, 1 H), 7.11 (d, J = 7.6 Hz, 1 H), 3.95 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.40, 154.86, 139.48, 138.51, 138.25, 136.43, 132.69, 131.13, 130.70, 129.21, 120.74, 118.66, 114.26, 111.52, 29.00. APCI–HRMS m/z calculated for $C_{17}H_{12}NO_2$ ($MH^+$): 262.0863, found: 262.0857. Purity (HPLC): 96.6 %.

### (E)-2((2-aminopyrimidine-5-yl)methylene)-4-hydroxy-2,3-dihydro-1H–inden-1-one (2 n)

The title compound (beige powder) was prepared in a yield of 10 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 2-aminopyrimidine-5-carbaldehyde: mp 398.5–398.8 °C (MeOH); $^1$H NMR (600 MHz, DMSO) δ 10.05 (s, 1 H), 8.67 (s, 2 H), 7.38–7.26 (m, 4 H), 7.22 (d, J = 7.3 Hz, 1 H), 7.08 (d, J = 7.7 Hz, 1 H), 3.87 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.09, 162.95, 160.38, 154.74, 139.21, 135.83, 131.74, 129.00, 128.27, 120.20, 117.94, 114.07, 39.52, 29.19. APCI–HRMS m/z calculated for $C_{14}H_{12}N_3O_2$ ($MH^+$):254.0924, found: 254.0924. Purity (HPLC): 76 %.

### (E)-4-hydroxy-2-(pyridin-2-ylmethylene)-2,3-dihydro-1H-inden-1-one (2 o)

The title compound (green powder) was prepared in a yield of 71 % from 4-hydroxy-2,3-dihydro–1H-inden-1-one and picolinaldehyde: mp 30.8–30.9 °C ($H_2O$); $^1$H NMR (600 MHz, DMSO) δ 10.28 (s, 1 H), 8.85 (dd, J = 4.9, 0.8 Hz, 1 H), 8.16 (t, J = 7.4 Hz, 1 H), 8.04 (d,

J = 7.9 Hz, 1 H), 7.66–7.56 (m, 2 H), 7.35–7.24 (m, 2 H), 7.18–7.13 (m, 1 H), 4.09 (d, J = 1.6 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.64, 154.98, 151.92, 147.81, 139.84, 138.52, 137.21, 129.17, 128.07, 127.49, 124.62, 120.95, 115.73, 114.31, 29.80. APCI–HRMS m/z calculated for $C_{15}H_{12}NO_2$ ($MH^+$): 238.0863, found: 238.0879. Purity (HPLC): 100 %.

### (E)-4-hydroxy-2-(pyridin-3-ylmethylene)-2,3-dihydro-1H-inden-1-one (2 p)

The title compound (green powder) was prepared in a yield of 50 % from 4-hydroxy-2,3-dihydro–1H-inden-1-one and nicotinaldehyde: mp 30.9–31.0 °C (MeOH); $^1$H NMR (600 MHz, DMSO) δ 10.32 (s, 1 H), 9.18 (d, J = 1.7 Hz, 1 H), 8.83 (dd, J = 5.3, 1.2 Hz, 1 H), 8.70 (d, J = 8.2 Hz, 1 H), 7.98 (dd, J = 8.1, 5.4 Hz, 1 H), 7.63 (t, J = 2.0 Hz, 1 H), 7.33 (t, J = 7.6 Hz, 1 H), 7.28 (d, J = 7.0 Hz, 1 H), 7.17 (dd, J = 7.8, 0.8 Hz, 1 H), 4.04 (d, J = 1.6 Hz, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.16, 154.94, 146.01, 144.27, 142.73, 139.56, 138.33, 136.52, 133.15, 129.30, 127.14, 126.22, 120.94, 114.34, 28.76. APCI–HRMS m/z calculated for $C_{15}H_{12}NO_2$ ($MH^+$): 238.0863, found: 238.0876. Purity (HPLC): 100 %.

### (E)-4-hydroxy-2-(pyridin-4-ylmethylene)-2,3-dihydro-1H-inden-1-one (2 q)

The title (green powder) compound was prepared in a yield of 52 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and isonicotinaldehyde: mp 30.9–31.0 °C (MeOH); $^1$H NMR (600 MHz, DMSO) δ 10.29 (d, J = 0.7 Hz, 1 H), 8.86 (d, J = 5.6 Hz, 2 H), 8.07 (d, J = 4.6 Hz, 2 H), 7.58 (s, 1 H), 7.38–7.25 (m, 2 H), 7.17 (d, J = 7.6 Hz, 1 H), 4.05 (s, 2 H); $^{13}$C NMR (151 MHz, DMSO) δ 193.20, 154.91, 146.02, 138.50, 138.20, 136.59, 135.16, 129.38, 128.42, 125.79, 121.10, 118.17, 114.41, 28.92. APCI–HRMS m/z calculated for $C_{15}H_{12}NO_2$ ($MH^+$): 238.0863, found: 238.0879. Purity (HPLC): 100 %.

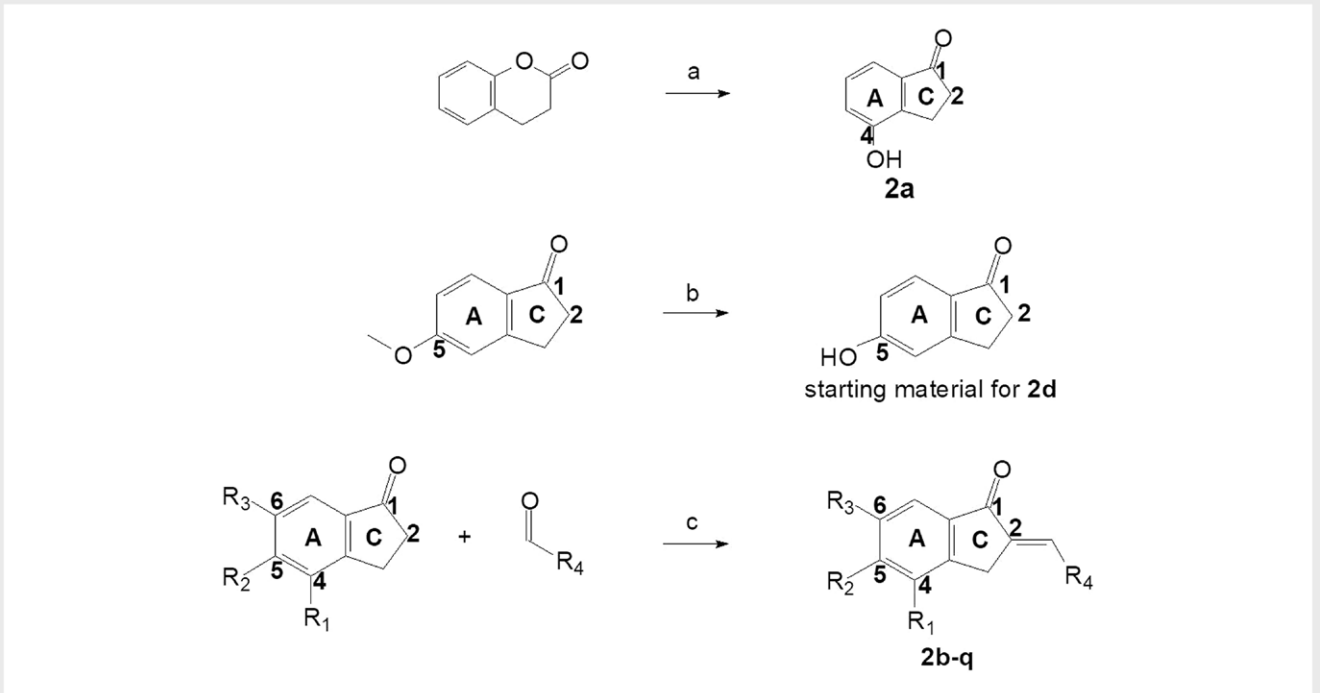
## Biology

All commercially available reagents were obtained from various manufacturers: radioligands [$^3$H]NECA (specific activity 27.1 Ci/mmol) procured from PerkinElmer and [$^3$H]DPCPX (specific activity 120 Ci/mmol) from Amersham Biosciences, filter-count from PerkinElmer and Whatman GF/B 25 mm diameter filters from Merck. Radio activity was calculated by a Packard Tri-CARB 2810 TR liquid scintillation counter.

## Radioligand binding assays

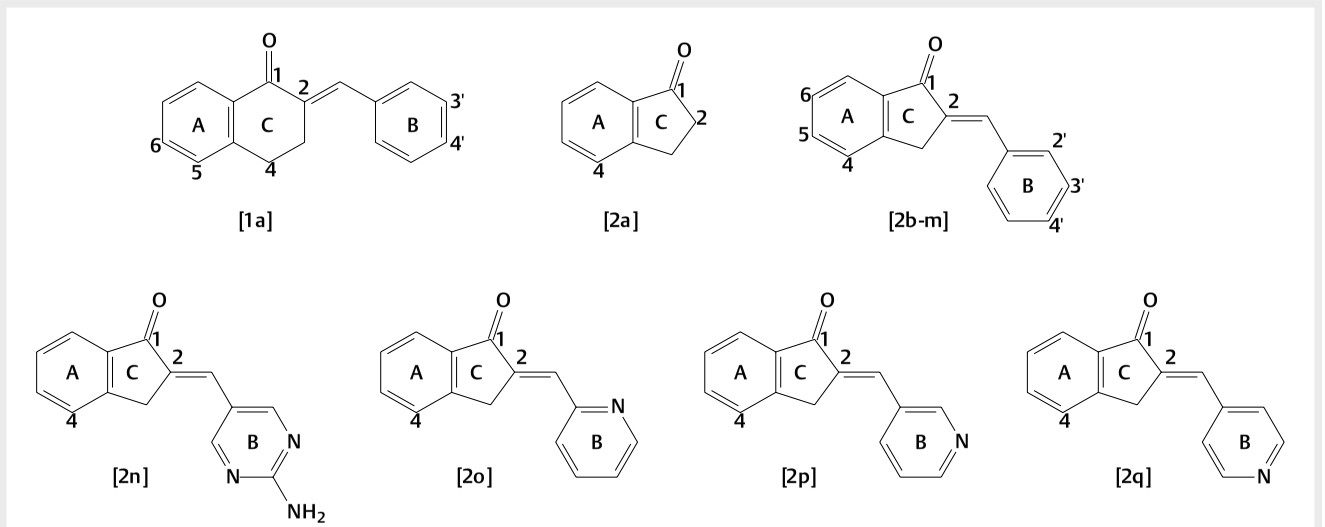
The collection of tissue samples for the $A_1$ and $A_{2A}$ AR binding studies were approved by the Research Ethics Committee of the North-West University (application number NWU-0035–10-A5). The rat whole brains (expressing $A_1$ AR's) and rat striata (expressing $A_{2A}$ AR's ) were prepared according to the protocol described in literature [16, 17].

The competition experiments were carried out in the presence of the radioligands [$^3$H]-8-cylcopentyl-1,3-dipropylxanthine ([$^3$H]DPCPX; 0.1 nM; $K_d$ = 0.36 nM) and 5'-N-[$^3$H]-ethylcarboxamideadenosine ([$^3$H]NECA; 4 nM; $K_d$ = 15.3 nM) for the $A_1$ and $A_{2A}$ AR radioligand binding assays, respectively [16, 17]. In addition, the $A_{2A}$ AR binding studies were determined in the presence of $N^6$-cyclopentyladenosine (CPA) to minimize the binding of [$^3$H]NECA to $A_1$ AR's. Non-specific binding was defined by the addition of a final concentration of 100 µM CPA. The sigmoidal-dose response curves, via Graphpad Software Inc. package, were obtained by plotting the specific binding vs. the logarithm of the test compound's concentrations. Subsequently, the $K_i$ values were obtained by using the $IC_{50}$ values that were determined from sigmoidal–dose response curves. All incubations were carried out in triplicate and




▶ **Fig. 2** Synthesis of 2a, starting material for 2d and 2b–q.Reagents and conditions: **a**) AlCl3, NaCl, 120–150 °C, 3,4-dihydrocoumarin, 200 °C (1 h 30 min), ice, HCl, rt (2 h); **b**) AlCl3, toluene, 120 °C (1 h); **c**) MeOH, HCl (32 %), 120 °C (24 h).

▶ **Table 1** The dissociation constant values ($K_i$ values) for the binding of the 2-benzylidene-1-indanones at rat $A_1$ and $A_{2A}$ AR's.




| | Ring A | | | Ring B | | $K_i$ ± SEM (μM)[a] ( % displacement)[b] | | SI[d] | $K_i$ ± SEM (μM)[e] | GTP Shift [f] |
|---|---|---|---|---|---|---|---|---|---|---|
| # | 4 | 5 | 6 | 3' | 4' | $A_1$[c] vs [$^3$H]DPCPX | $A_{2A}$[c] vs [$^3$H]NECA | ($A_1/A_{2A}$) | $A_1$[c] + GTP vs [$^3$H]DPCPX | |
| **Tetralones** | | | | | | | | | | |
| **1a** | H | OH | H | H | H | 5.93 ± 0.45[a,g] | 2.90 ± 0.66[a,g] | 13[d] | 6.92 ± 0.81[a,g] | 1[f] |
| **Indanones** | | | | | | | | | | |
| **2a** | OH | H | H | – | | > 100 (58 %)[b] | > 100 (68 %)[b] | – | – | – |
| **2b** | OH | H | H | H | H | > 100 (29 %)[b] | 1.55 ± 0.28[a] | – | – | – |
| **2c** | OH | H | H | OH | OH | 0.435 ± 0.050[a] | 0.903 ± 0.081[a] | 0.5[d] | 0.339 ± 0.071[e] | 0.9[f] |
| **2d** | H | OH | H | OH | OH | 5.31 ± 0.50[a] | > 100 (95 %)[b] | – | – | – |
| **2e** | H | H | OH | OH | OH | 4.01 ± 0.30[a] | 2.12 ± 0.38[a] | 1.9[d] | – | – |
| **2f** | OH | H | H | F | H | > 100 (85 %)[b] | > 100 (26 %)[b] | – | – | – |
| **2g** | OH | H | H | H | F | > 100 (26 %)[b] | > 100 (24 %)[b] | – | – | – |
| **2h** | OH | H | H | Cl | H | > 100 (75 %)[b] | 0.512 ± 0.051[a] | – | – | – |
| **2i** | OH | H | H | H | Cl | > 100 (40 %)[b] | 2.73 ± 0.28[a] | – | – | – |
| **2j** | OH | H | H | Br | H | > 100 (69 %)[b] | 1.04 ± 0.18[a] | – | – | – |
| **2k** | OH | H | H | H | Br | > 100 (51 %)[b] | > 100 (46 %)[b] | – | – | – |
| **2l** | OH | H | H | H | $CF_3$ | > 100 (88 %)[b] | > 100 (56 %)[b] | – | – | – |

▲ **Table 1** Continued.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **2m** | OH | H | H | H | CN | >100 (49 %)[b] | >100 (66 %)[b] | – | – |
| **2n** | OH | H | H | – | – | >100 (57 %)[b] | >100 (77 %)[b] | – | – |
| **2o** | OH | H | H | – | – | 4.71 ± 0.58[a] | 1.79 ± 0.13[a] | 2.6[d] | – |
| **2p** | OH | H | H | – | – | 6.58 ± 0.68[a] | 1.61 ± 0.17[a] | 4.1[d] | – |
| **2q** | OH | H | H | – | – | 1.69 ± 0.13[a] | 3.37 ± 0.90[a] | 0.5[d] | 1.83 ± 0.09[e] |
| **Reference compounds** | | | | | | | | | |
| **CPA ($A_1$agonist)** | | | | | | 0.0068 ± 0.0001[a] (0.0079)[h]; (0.015)[i] | 0.163 ± 0.001[a] (0.331)[i] | 24[d] (22)[i] | 0.099 ± 0.015[a] (0.099)[i] | 15[f] (14)[i] |
| **DPCPX ($A_1$antagonist)** | | | | | | 0.0004 ± 0.0002[a] (0.0005)[i]; (0.0003)[j] | 0.545 ± 0.204[a] (0.530)[i]; (0.340)[j] | 1363[d] (958)[i]; (1130)[j] | 0.0004 ± 0.0002[a] (0.0004)[j] | 1.0[f] |

[a]All $K_i$ values determined in triplicate and expressed as mean ± SEM; [b]Percentage displacement of the radioligand at a maximum tested concentration (100 µM); [c]Rat receptors were used ($A_1$: rat whole brain membranes; $A_{2A}$: rat striatal membranes); [d]Selectivity index (SI) for the $A_{2A}$ receptor isoform calculated as the ratio of $K_i$ ($A_1$)/$K_i$ ($A_{2A}$); [e]GTP shift assay, where the 100 µM GTP was added to the $A_1$ AR radioligand binding assay; [f]GTP shifts calculated by dividing the $K_i$ in the presence of GTP by the $K_i$ in the absence of GTP; [g]Literature value obtained from reference [13]; [h]Literature value obtained from reference [19]; [i]Literature value obtained from reference [16]; [j]Literature value obtained from reference [20].

the dissociation constants ($K_i$ values) are expressed as the mean ± standard error of mean (SEM). CPA and DPCPX (unlabelled) were used as reference compounds and their assay results confirmed validity of the radioligand binding assays.

### GTP shift assays

In addition, compounds **2c** and **2q** were explored via a GTP shift assay to determine the agonistic or antagonistic functionality of the investigated 2-benzylidene-1-indanones towards the $A_1$ AR. The GTP shift assay was performed as described previously with rat whole brain membranes and [$^3$H]DPCPX (0.1 nM; $K_d$ = 0.36 nM) in the absence and presence of a final concentration of 100 µM GTP [16, 18]. Non–specific binding was defined by the addition of 10 µM DPCPX (unlabeled). If a calculated GTP shift of approximately 1 is obtained, that compound is considered to function as an antagonist. On the other hand, the presence of GTP affects the competition curve of an agonist and shifts the curve to the right, as previously demonstrated by the $A_1$ AR agonist CPA [16, 18]. The sigmoidal-dose response curves were obtained via the Graphpad Software Inc. package and the $K_i$ values determined as described above. The GTP shift was calculated by dividing the $K_i$ value of a compound reported in the presence of GTP by the $K_i$ value obtained in the absence of GTP [16, 18].

## Results and Discussion

### Chemistry

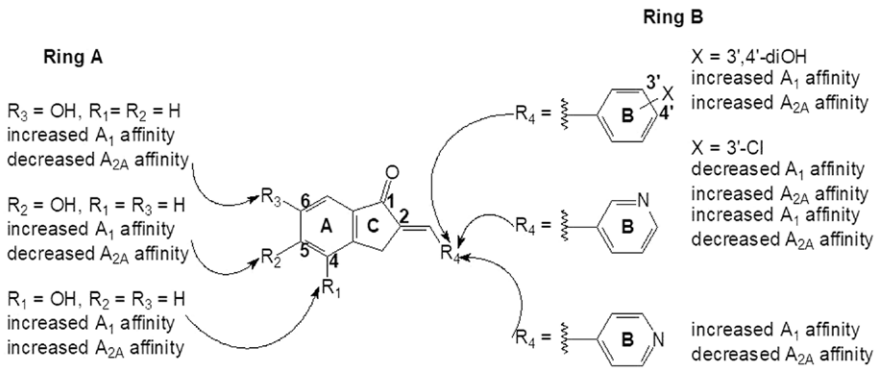
(▶ **Fig. 2**) The key starting material 4-hydroxy-2,3-dihydro-1H-inden-1-one (**2a**) was synthesised in a good yield by a rearrangement reaction. The crude product was used without further purification to synthesise compounds **2b**–**c** and **2f**–**q**. The starting material for compound **2d**, was synthesised via demethylation of 5-methoxy-2,3-dihydro-1H-inden-1-one using $AlCl_3$ to obtain 5-hydroxy-2,3-dihydro-1H-inden-1-one. Conversely, starting material for compound **2e**, namely 6-hydroxy-2,3-dihydro-1H-inden-1-one was commercially available and procured from Sigma–Aldrich. The target 2-benzylidene-1-indanones were synthesised through an acid catalysed aldol condensation reaction. The novel compounds **2b**–**q** were purified by recrystallization from a suitable solvent (yields of 10–85 %) and, in each instance, the structures and purity of these compounds were verified by $^1$H-NMR, $^{13}$C-NMR, mass spectrometry and HPLC analysis.

The novel synthesized compounds (**2b**–**q**) possess E-configuration, similar to the 2-benzylidene-1-tetralone analogues synthesized by Legoabe and co–workers [13] and Janse van Rensburg and co–workers [14].

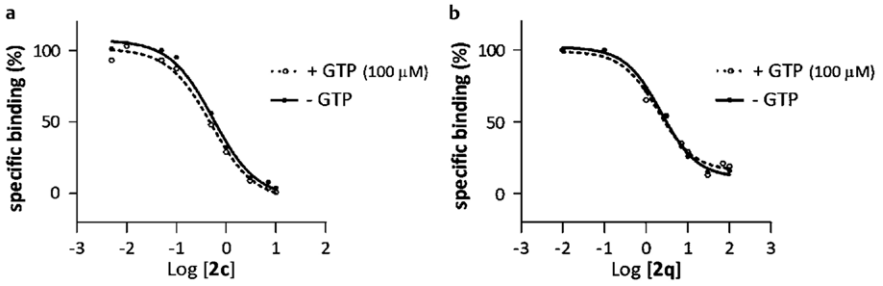
### Biology

The degree and type of binding affinity of the 2–benzylidene–1–indanone analogues (**2a**–**q**) at rat $A_1$ and $A_{2A}$ AR's were determined by radioligand binding assays and GTP shift assays, respectively, and results are summarized in ▶ **Table 1**. Two reference compounds, namely CPA and DPCPX, were included in the study and the results are in accordance with literature values.

Previous studies identified the 5-hydroxy substituted 2-benzylidene-1-tetralone derivative, **1a**, as a lead compound to design novel and potent $A_1$ and $A_{2A}$ AR antagonists [13]. The 4-hydroxy




▶ **Fig. 3** A broad overview of ring **a** and **b** substitutions on 2-benzylidene-1-indanone core's influence on A1 and A2A AR affinity.




▶ **Fig. 4** The binding curves of compounds 2c and 2q are examples of A1 AR antagonistic action determined via GTP shift assays (with and without 100 µM GTP) in rat whole brain membranes expressing A1 ARs with [3 H]DPCPX as radioligand. **a** GTP shift of 0.9 calculated for compound 2c, **b** GTP shift of 1.0 calculated for compound 2q.

substituted compound **2a**, the parent scaffold of this study, is unsubstituted at position 2 and lacked $A_1$ and/or $A_{2A}$ AR activity.

## Structural modifications to ring A

In analogy to previous studies of the 2-benzylidene-1-tetralones which determined optimal ring A substitution [13, 14], the impact of OH-substitution at position 4, 5 or 6 of ring A and meta (3') and para (4') substitution on ring B of the 2-benzylidene-1-indanones were evaluated by comparing the dissociation constant values ($K_i$ values) of these compounds (**2c**–**2e**) to each other. Similar to the 2-benzylidene-1-tetralones, the position of the OH-group on ring A, together with meta (3') and para (4') substitution on ring B, of the 2-benzylidene-1-indanones modulates $A_1$ and $A_{2A}$ AR binding affinity and C4-OH substitution (**2c**; $A_1K_i$ = 0.435 µM and $A_{2A}K_i$ = 0.903 µM) on ring A is preferred over C6- (**2e**; $A_1K_i$ = 4.01 µM and $A_{2A}K_i$ = 2.12 µM) and C5-OH substitution (**2d**; $A_1K_i$ = 5.31 µM and $A_{2A}K_i$ = > 100 µM).

## Structural modifications to ring B

Comparison of compound **2a** to **2b** showed that the 2-benzylidene side chain increases both $A_1$ and $A_{2A}$ AR affinity — conveying the necessity of C2 substitution on ring C. The $A_{2A}K_i$ value of compound **2b** (1.55 µM) suggests that phenyl ring B is valuable to $A_{2A}$ AR affinity.

In correlation to previous studies, $A_1$ and $A_{2A}$ AR binding affinity favour OH-group substitution on meta (3') and para (4') positions of ring B. For example, compound **2c** possessed a 1.7 fold increase in $A_{2A}$ AR affinity compared to its unsubstituted counterpart **2b** ($A_1K_i$ = > 100 µM and $A_{2A}K_i$ = 1.55 µM).

Further investigation of halide substituents on C3' or C4' of ring B (retaining C4-OH ring A) provided results similar to research by Legoabe and co-workers [13], as well as Janse van Rensburg and colleagues [14]. Generally, halogen substitution at either the meta (3') or para (4') position of ring B proved detrimental to both $A_1$ and $A_{2A}$ AR binding affinity ($K_i$ values = > 100 µM) when compared to compound **2b**. While, halogen substitution with Cl at the meta (3') or para (4') position (**2 h** & **2i**) and Br at the meta (3') position (**2j**) is detrimental to $A_1$ AR affinity ($K_i$ values = > 100 µM), it was beneficial to $A_{2A}$ AR affinity. Additionally, comparison of **2 h** and **2i** indicated that C3'-Cl substitution (**2 h**; $A_{2A}K_i$ = 0.512 µM) is preferred over C4'-Cl substitution (**2i**; $A_{2A}K_i$ = 2.73 µM), as **2 h** shows a 5.3 fold increase in $A_{2A}$ AR affinity.

As with previous studies [13, 14], other ring systems were also explored by replacing phenyl ring B with either a pyridine ring or 2-aminopyrimidine ring. Pyridine ring substitution proved advantageous to both $A_1$ and $A_{2A}$ AR binding affinity; with compounds **2o**–**2q** exhibiting, in decreasing order of affinity, $A_1$**2q**; N4' $K_i$ = 1.69 µM > **2o**; N2' $K_i$ = 4.71 µM > **2p** N3' $K_i$ = 6.58 µM and $A_{2A}$**2p**; N3' $K_i$ = 1.61 µM > **2o**; N2' $K_i$ = 1.79 µM > **2q**; N4' $K_i$ = 3.37 µM AR affinity. It 7seems that $A_1$ AR binding favours N4'-substitution, whereas $A_{2A}$ AR binding prefers N3'-substitution. Compound **2n**, contain-

ing a 2-aminopyrimidine ring, was devoid of $A_1$ and $A_{2A}$ AR binding affinity ($A_1$&$A_{2A}K_i$ value = > 100 µM).

### Structural modifications to ring C

Evaluation of compound **2b** in relation to compound **1a** showed that reduction of ring C from a 6 membered ring (tetralone) to a 5-membered ring (indanone) decreased $A_1$ AR affinity and increased $A_{2A}$ AR binding affinity approximately 2 fold (**2b**, $A_{2A}K_i$ = 1.55 µM vs **1a**; $A_{2A}K_i$ = 2.90 µM).

Of the 2-benzylidene-1-indanones, 3',4'-diOH substituted compound **2c** shows the best $A_1$ AR affinity and the second best $A_{2A}$ AR affinity, while 2-'Cl substituted compound **2 h** possessed the highest $A_{2A}$ AR affinity and no $A_1$ AR affinity. Other compounds exhibiting relatively good $A_1$ and/or $A_{2A}$ AR affinity are: 6-OH substituted **2e** and pyridine ring substituted compounds **2o**–**q** (▶ **Fig. 3**).

The GTP shift assay results suggest that compounds **2c** and **2q** act as $A_1$ AR antagonists – as no significant rightward shift of the binding curves were observed in the presence of GTP (▶ **Fig. 4**).

## Conclusions

In summary, this study involved the synthesis, characterization and evaluation of novel 2-benzylidene-1-indanone analogues to understand the importance of structural modifications to ring A, B and C of the 2-benzylidene-1-tetralone scaffold in gaining or even losing $A_1$ and/or $A_{2A}$ AR affinity. Upon analysis, it was found that C4-OH substitution on ring A and 3'- and 4'-OH substitution on ring B (**2c**) is complimentary to $A_1$ and $A_{2A}$ AR affinity, affording this non-selective compound $K_i$ values below 1 µM for both the $A_1$ and $A_{2A}$ AR. C3'-Cl substitution on ring B (retaining C4-OH ring A) provided compound **2 h** with the highest $A_{2A}$ AR affinity ($K_i$ = 0.512 µM) and selectivity. Replacing phenyl ring B with a pyridine ring increased $A_1$ AR affinity and slightly decreased $A_{2A}$ AR affinity (**2o**–**q**), in comparison to **2b** — yet compounds **2o**–**q** still possess relatively good $A_1$ and $A_{2A}$ AR affinity. Additionally, it seems that $A_1$ AR binding favours N4'-substitution (**2q**), whereas $A_{2A}$ AR binding favours N3'–substitution (**2p**). In general, conversion from fused 6– and 6–membered rings (2-benzylidene-1-tetralones) to fused 6- and 5-membered rings (2-benzylidene-1-indanones) in combination with ring B substitutions improved $A_1$ and $A_{2A}$ AR affinity. In view of these findings, compounds **2c**, **2 h**, **2q** and **2p** are worthy candidates to further explore as potent and selective $A_1$ and $A_{2A}$ AR antagonists for the potential treatment of neurological conditions, achieved by optimization of the 2-benzylidene-1-indanone scaffold.

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## Conflict of interest

The authors have no conflict of interest to declare.

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