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 A1 and A2A AR antagonist [3]. An A1 AR antagonist could alleviate cognitive deficits in PD, a non-motor symptom of the disease [4]. This is substantiated by the A1 AR’s distribution and expression in the prefrontal cortex and hippocampus [5], which are areas linked to cognition [6]. Moreover, blockade of A1 AR’s increase acetylcholine — a neurotransmitter associated with learning and memory [7]. Another non–motor symptom of PD, namely depression, may be addressed by A2A AR antagonists; evidenced by a decrease in immobility time during the forced swim test and tail suspension test in rodents when KW-6002...
(selective A2A AR antagonist) was administered [8, 9]. A2A 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 A2A and dopamine D2 receptors are co-expressed on the striatopallidal (inhibitory) pathway, inhibition of A2A AR’s compensation for hypolocomotion as a result of dwindling striatopallidal dopamine D2 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 A2A AR antagonist to L-dopa [10]. Blockade of both A1 and A2A AR’s synergistically improve motor control by increasing presynaptic dopamine release via A1 AR inhibition and postsynaptic dopamine response via A2A AR inhibition [11]. Additionally, blockade of the A2A AR may possibly facilitate neuroprotection in neurodegenerative disorders, like PD [12].

Recently, 2-benzylidene-1-tetralone analogues were explored as A1 and/or A2A AR antagonists [13, 14]. The 2-benzylidene-1-tetralone derivative (1a), possessed both A1 and A2A AR affinity (A1K i = 5.93 μM; and A2A(K δ) 2.90 μM) with a selectivity index of 2 towards the A2A 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 A1 and A2A 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 (6-membered rings, namely ring A and ring C), where ring C bears hydroxy substitution on ring A is optimal for A1 and A2A AR binding [16].

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 A1 and A2A 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 A1 and A2A 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 (1H) and carbon (13C) 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) as solvent. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Si(CH3)4) as internal standard. High resolution mass spectra (HRMS) were recorded on a Bruker microOTOF–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)

AlCl3 (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 AlCl3 and NaCl mixture. Subsequently, the temperature was raised to 200 °C and the reaction mixture mechanically stirred under reflux for 1 h 30 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; 1H 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, 2H), 2.59 (t; J = 5.7 Hz, 2H); 13C NMR (151 MHz, DMSO) δ 206.68, 155.17, 149.05, 138.43, 128.66, 119.82, 113.34, 35.81, 22.33. APCI–HRMS m/z calculated for C16H13O2 (MH+): 237.0910, found: 237.0909. 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 (2a) (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 (2b)

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); 1H NMR (600 MHz, DMSO) δ 10.11 (s, 1 H), 7.79 (d; J = 7.5 Hz, 2H), 7.52 (dd; J = 9.7, 5.1 Hz, 3H), 7.46 (t; J = 7.3 Hz, 1H), 7.31 (t; J = 7.6 Hz, 1H), 7.25 (d; J = 7.4 Hz, 1H), 7.10 (d; J = 7.7 Hz, 1H), 3.94 (d; J = 1.0 Hz, 2H); 13C NMR (151 MHz, DMSO) δ 193.40, 154.83, 153.97, 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 C16H13O2 (MH+): 237.0910, found: 237.0909. Purity (HPLC): 100 %.

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

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: mp 280.8–287.2 °C; 1H NMR (600 MHz, DMSO) δ 10.55

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(s, 1H), 9.59 (s, 1H), 9.22 (s, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.25 (t, J = 1.7 Hz, 1H), 7.18 (d, J = 2.0 Hz, 1H), 7.07 (dd, J = 8.3, 2.0 Hz, 1H), 6.96 (d, J = 1.8 Hz, 1H), 6.87–6.82 (m, 2H), 3.92 (s, 2H); 13C 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 C16H13O4 (MH+): 269.0808, found 269.0769. Purity (HPLC): 92%.

(E)-2-(3,4-dihydroxybenzylidene)-6-hydroxy-2,3-dihydro-1H-inden-1-one (2e)
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; 1H NMR (600 MHz, DMSO) δ 9.81 (s, 1H), 9.67 (s, 1H), 9.27 (s, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.33 (s, 1H), 7.20 (d, J = 1.9 Hz, 1H), 7.14–7.04 (m, 3H), 6.85 (d, J = 8.3 Hz, 1H), 3.89 (s, 2H); 13C NMR (151 MHz, DMSO) δ 193.20, 157.11, 147.97, 145.61, 140.43, 138.92, 133.40, 126.50, 124.21, 122.92, 117.44, 116.05, 108.09, 39.52, 31.90. APCI–HRMS m/z calculated for C16H13O4 (MH+): 269.0808, found 268.0730. Purity (HPLC): 96.3%.

(E)-2-(3-fluorobenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (2f)
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); 1H NMR (600 MHz, DMSO) δ 10.12 (d, J = 1.2 Hz, 1H), 7.62 (t, J = 10.2 Hz, 2H), 7.59–7.49 (m, 2H), 7.34–7.23 (m, 3H), 7.10 (dd, J = 7.7, 0.7 Hz, 1H), 3.95 (d, J = 1.4 Hz, 2H); 13C NMR (151 MHz, DMSO) δ 193.55 (s), 163.16 (s), 161.54 (s), 138.69 (s), 137.4 (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, J = 8.4 Hz), 115.99 (s), 111.92 (s), 39.52 (s, 2H); 13C NMR (151 MHz, DMSO) δ 193.20, 157.11, 147.97, 145.61, 140.43, 138.92, 133.40, 126.50, 124.21, 122.92, 117.44, 116.05, 108.09, 39.52, 31.90. APCI–HRMS m/z calculated for C16H13O4 (MH+): 269.0808, found 268.0730. Purity (HPLC): 96.3%.

Fig. 1 Structural and heterocyclic ring changes to compound 1a, hispidol and maritimetin to determine features essential for dual A1/A2A antagonistic activity.
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); [1]3C 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, 128.51, 114.19, 28.99. APCI–HRMS m/z calculated for C16H19BrO2 (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); [1]3C 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 C17H17F4O2 (MH+): 305.0784, found: 305.0807. Purity (HPLC): 98.4%.

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

The title compound (gold 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); [1]3C 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 C17H17NO2 (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-carboxaldehyde: 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); [1]3C 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 C19H19N2O2 (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 (H2O); [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, 146.02, 138.50, 138.20, 136.59, 135.16, 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%.

(Biology)
All commercially available reagents were obtained from various manufacturers: radioligands [3H]NECA (specific activity 27.1 Ci/mmol) procured from PerkinElmer and [3H]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 [3H]-8-cyclopentyl-1,3-dipropylxanthine ([3H]DPCPX; 0.1 nM; K$_d$ = 0.36 nM) and 5’-N-[3H]-ethylcarboxamideadenosine ([3H]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 [3H]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

\[ \text{Fig. 2} \quad \text{Synthesis of 2a, starting material for 2d and 2b–q. Reagents and conditions: a) AlCl$_3$, NaCl, 120–150 °C, 3,4-dihydrocoumarin, 200 °C (1 h 30 min), ice, HCl, rt (2 h); b) AlCl$_3$, toluene, 120 °C (1 h); c) MeOH, HCl (32 %), 120 °C (24 h).} \]
Table 1  The dissociation constant values ($K_i$ values) for the binding of the 2-benzylidene-1-indanones at rat A1 and A2A AR's.

<table>
<thead>
<tr>
<th></th>
<th>Ring A</th>
<th>Ring B</th>
<th>$K_i$ ± SEM (µM)$^a$ (% displacement)$^b$</th>
<th>SI$^d$</th>
<th>$K_i$ ± SEM (µM)$^e$</th>
<th>GTP Shift $^f$</th>
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<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>5</td>
<td>6 3' 4'</td>
<td>A1c vs [3H]DPCPX</td>
<td>A2Ac vs [3H]NECA</td>
<td>(A1c/A2Ac)</td>
</tr>
<tr>
<td>1a</td>
<td>H</td>
<td>OH</td>
<td>H     H     H</td>
<td>5.93 ± 0.45$^a$</td>
<td>2.90 ± 0.66$^a$</td>
<td>6.92 ± 0.81$^a$</td>
</tr>
<tr>
<td>2a</td>
<td>OH</td>
<td>H</td>
<td>H     H     H</td>
<td>&gt;100 (58%)$^b$</td>
<td>&gt;100 (68%)$^b$</td>
<td>–</td>
</tr>
<tr>
<td>2b</td>
<td>OH</td>
<td>H</td>
<td>H     H     H</td>
<td>&gt;100 (29%)$^b$</td>
<td>1.55 ± 0.28$^a$</td>
<td>–</td>
</tr>
<tr>
<td>2c</td>
<td>OH</td>
<td>H</td>
<td>H     OH    OH</td>
<td>0.435 ± 0.050$^a$</td>
<td>0.903 ± 0.081$^a$</td>
<td>0.5$^d$</td>
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<tr>
<td>2d</td>
<td>H</td>
<td>OH</td>
<td>OH    OH    OH</td>
<td>5.31 ± 0.50$^a$</td>
<td>&gt;100 (95%)$^b$</td>
<td>–</td>
</tr>
<tr>
<td>2e</td>
<td>H</td>
<td>H</td>
<td>OH    OH    OH</td>
<td>4.01 ± 0.30$^a$</td>
<td>2.12 ± 0.38$^a$</td>
<td>1.9$^d$</td>
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<tr>
<td>2f</td>
<td>OH</td>
<td>H</td>
<td>H     H     F</td>
<td>&gt;100 (85%)$^b$</td>
<td>&gt;100 (26%)$^b$</td>
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<tr>
<td>2g</td>
<td>OH</td>
<td>H</td>
<td>H     H     H</td>
<td>&gt;100 (26%)$^b$</td>
<td>&gt;100 (24%)$^b$</td>
<td>–</td>
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<tr>
<td>2h</td>
<td>OH</td>
<td>H</td>
<td>H     H     Cl</td>
<td>&gt;100 (75%)$^b$</td>
<td>0.512 ± 0.05$^a$</td>
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<tr>
<td>2i</td>
<td>OH</td>
<td>H</td>
<td>H     H     Cl</td>
<td>&gt;100 (40%)$^b$</td>
<td>2.73 ± 0.28$^a$</td>
<td>–</td>
</tr>
<tr>
<td>2j</td>
<td>OH</td>
<td>H</td>
<td>H     Br    H</td>
<td>&gt;100 (69%)$^b$</td>
<td>1.04 ± 0.18$^a$</td>
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</tr>
<tr>
<td>2k</td>
<td>OH</td>
<td>H</td>
<td>H     H     Br</td>
<td>&gt;100 (51%)$^b$</td>
<td>&gt;100 (46%)$^b$</td>
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<tr>
<td>2l</td>
<td>OH</td>
<td>H</td>
<td>H     H     CF₃</td>
<td>&gt;100 (88%)$^b$</td>
<td>&gt;100 (56%)$^b$</td>
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The dissociation constant values (Table 1) for the binding of the 2-benzylidene-1-indanones at rat A 1 and A2A AR's.

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<th>Compound</th>
<th>OH</th>
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<th>H</th>
<th>H</th>
<th>CN</th>
<th>Kᵢ</th>
<th>Kᵢ</th>
<th>Kᵢ</th>
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<tr>
<td>2m</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (49%)</td>
<td>&gt;100 (66%)</td>
<td>–</td>
</tr>
<tr>
<td>2n</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (57%)</td>
<td>&gt;100 (77%)</td>
<td>–</td>
</tr>
<tr>
<td>2o</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>4.7±0.5 b</td>
<td>1.7±0.13 a</td>
<td>2.6 b</td>
</tr>
<tr>
<td>2p</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>6.5±0.68 a</td>
<td>1.6±0.17 a</td>
<td>4.1 b</td>
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<td>2q</td>
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<td>1.69±0.13 a</td>
<td>3.37±0.90 a</td>
<td>0.5 d</td>
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**Reference compounds**

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<thead>
<tr>
<th>CPA (A₁ agonist)</th>
<th>Kᵢ</th>
<th>Kᵢ</th>
<th>Kᵢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0068±0.0001 a</td>
<td>(0.0079); (0.015)</td>
<td>0.163±0.001 a</td>
<td>(0.331)</td>
</tr>
<tr>
<td>DPCPX (A₁ antagonist)</td>
<td>Kᵢ</td>
<td>Kᵢ</td>
<td>Kᵢ</td>
</tr>
<tr>
<td>0.0004±0.0002 a</td>
<td>(0.0005); (0.0003)</td>
<td>0.545±0.204 a</td>
<td>(0.530); (0.340)</td>
</tr>
</tbody>
</table>

**Results and Discussion**

The novel synthesized compounds 2b–q were purified by recrystallization from a suitable solvent (yields of 10–85%). The novel synthesized compounds 2b–q possess 6 configurations. The novel synthesized compounds 2b–q were afterwards purified by chromatography on silica gel to give the pure products. The novel synthesized compounds 2b–q were then subjected to GC/MS analysis.

**CYP3A4 inhibition assays**

The inhibition of CYP3A4 activity by the novel synthesized compounds 2b–q was then evaluated in vitro in a microsomal enzyme assay. The inhibitory activity of the novel synthesized compounds 2b–q was compared with that of the reference compound CPA. The IC₅₀ values were determined and expressed as mean ± SEM (n = 3). CPA (0.0004 ± 0.0002) was used as reference 

**GTP shift assays**

The GTP shift assay was performed as described previously with rat whole brain membranes and [³²P]DPCPX (0.1 nM; Kᵢ d = 0.36 nM) in the absence and presence of a final concentration of 100 μM GTP [16, 18]. The GTP shift was calculated as the ratio of Kᵢ value obtained in the presence of GTP by the Kᵢ value of a compound reported in the presence and absence of GTP [16, 18].

**Non-specific binding**

Non-specific binding was defined by the addition of 10 μM DPCPX (unlabeled). If a calculated GTP shift of approximately 1 is obtained, then the compound is considered to function as an antagonist. On the other hand, the presence of the GTP shift increases the curve to the right, as previously demonstrated (unpublished). The GTP shift was calculated as described above. The GTP shift was calculated from the equation (value of Kᵢ in the presence of GTP / value of Kᵢ in the absence of GTP) [16, 18].
substituted compound 2a, the parent scaffold of this study, is unsubstituted at position 2 and lacked A1 and/or A2A 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 A1 and A2A AR binding affinity and C4-OH substitution (2c; A1K_i = 0.435 μM and A2AK_i = 0.903 μM) on ring A is preferred over C6- (2e; A1K_i = 4.01 μM and A2AK_i = 2.12 μM) and C5-OH substitution (2d; A1K_i = 5.31 μM and A2AK_i = > 100 μM).

Structural modifications to ring B
Comparison of compound 2a to 2b showed that the 2-benzylidene side chain increases both A1 and A2A AR affinity — conveying the necessity of C2 substitution on ring C. The A2AK_i value of compound 2b (1.55 μM) suggests that phenyl ring B is valuable to A2A AR affinity.

In correlation to previous studies, A1 and A2A 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 A2A AR affinity compared to its unsubstituted counterpart 2b (A1K_i > 100 μM and A2AK_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 A1 and A2A 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 (2h & 2i) and Br at the meta (3’) position (2j) is detrimental to A1 AR affinity (K_i values = > 100 μM), it was beneficial to A2A AR affinity. Additionally, comparison of 2h and 2i indicated that C3’-Cl substitution (2h; A2AK_i = 0.512 μM) is preferred over C4’-Cl substitution (2i; A2AK_i = 2.73 μM) as 2h shows a 5.3 fold increase in A2A 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 A1 and A2A AR binding affinity; with compounds 2o–2q exhibiting, in decreasing order of affinity, A1q: N4’ K_i = 1.69 μM > 2o; N2’ K_i = 4.71 μM > 2p N3’ K_i = 6.58 μM and A2A2p: N3’ K_i = 1.61 μM > 2o; N2’ K_i = 1.79 μM > 2q; N4’ K_i = 3.37 μM AR affinity. It seems that A1 AR binding favours N4’-substitution, whereas A2A AR binding prefers N3’-substitution. Compound 2n, contain-
ing a 2-aminopyrimidine ring, was devoid of A1 and A2A AR binding affinity (A1 & A2A Ki 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 (indenone) decreased A1 AR affinity and increased A2A AR binding affinity approximately 2 fold (2b, A2A Ki = 1.55 µM vs 1a; A2A Ki = 2.90 µM).

Of the 2-benzylidene-1-indanones, 3',4'-diOH substituted compound 2c shows the best A1 AR affinity and the second best A2A AR affinity, while 2'-Cl substituted compound 2h possessed the highest A2A AR affinity and no A1 AR affinity. Other compounds exhibiting relatively good A1 and/or A2A 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 A1 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 A1 and/or A2A 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 A1 and A2A AR affinity, affording this non-selective compound K values below 1 µM for both the A1 and A2A AR. C3’-Cl substitution on ring B (retaining C4-OH ring A) provided compound 2h with the highest A2A AR affinity (K 2h = 0.512 µM) and selectivity. Replacing phenyl ring B with a pyridine ring increased A1 AR affinity and slightly decreased A2A AR affinity (2o–q), in comparison to 2b – yet compounds 2o–q still possess relatively good A1 and A2A AR affinity. Additionally, it seems that A1 AR binding favours N4'-substitution (2q), whereas A2A 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 A1 and A2A AR affinity. In view of these findings, compounds 2c, 2h, 2q and 2p are worthy candidates to further explore as potent and selective A1 and A2A 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.

References
