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 compensates 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 ineffective drug for treatment of motor symptoms, such as dyskinesias, and A2AK i

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 = 5.93 μM; and A2A(K) = 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 benzfused ring systems are the heterocyclic aurones — fused 6- and 5-membered rings, namely ring A and ring C), where ring C bears a C5–hydroxy substitution on ring A is optimal for A1 and A2AR binding and that the 2-benzylidene side-chain, ring B, also governs AR binding. Similar to these bicyclic benzfused ring systems are the heterocyclic

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–d6) as solvent. Chemical shifts are reported in parts per million (δ) in relation to the tetramethylsilane (Si(CH3)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-dihydropyrind-1-one-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 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;

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, 141.90, 138.43, 128.66, 119.82, 113.34, 35.81, 22.33. APCI–HRMS m/z calculated for C16H10O3 (MH+): 269.0808, found: 269.0801. Purity (HPLC): 98.7%.

The title compound (beige powder) was prepared in a yield of 48 % from 5-hydroxy-2,3-dihydropyrind-1-one-1-one and 3,4-dihydroxybenzaldehyde: 280.8–287.2 °C; 1H NMR (600 MHz, DMSO) δ 10.55
(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); $^{13}$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, 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 = 8.2 Hz, 1H), 3.89 (s, 2H); $^{13}$C 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 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, 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); $^{13}$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.
(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); 1H NMR (600 MHz, DMSO) δ 10.12 (s, 1 H), 7.86 (dd, J = 8.4, 5.7 Hz, 2H), 7.52 (s, 1H), 7.33 (dt, J = 15.2, 8.2 Hz, 2H), 7.25 (d, J = 7.4 Hz, 1H), 7.10 (d, J = 7.7 Hz, 1H), 3.91 (s, 2H); 13C NMR (151 MHz, DMSO) δ 193.53, 154.85, 138.76, 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, 129.42, 116.15, 116.01, 114.15, 28.90. APCI–HRMS m/z calculated for C16H12FO2 (MH+): 255.0816, found: 255.0816. Purity (HPLC): 98.1%.

(Janse van Rensburg et al., 2018)
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); 13C 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); 1H 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); 13C 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); 1H 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); 13C NMR (151 MHz, DMSO) δ 193.20, 154.91, 146.02, 145.79, 141.10, 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 [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 A1 and A2A 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 A1 AR’s) and rat striata (expressing A2A 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; Kd = 0.36 nM) and 5’-N-[3H]-ethylcarboxamideadenosine ([3H]NECA; 4 nM; Kd = 15.3 nM) for the A1 and A2A AR radioligand binding assays, respectively [16, 17]. In addition, the A2A AR binding studies were determined in the presence of N6-cyclopentyladenosine (CPA) to minimize the binding of [3H]NECA to A1 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.

<table>
<thead>
<tr>
<th>Ring A</th>
<th>Ring B</th>
<th>$K_i$ ± SEM (µM)$^a$ (% displacement)$^b$</th>
<th>$S_i$$^d$</th>
<th>$K_i$ ± SEM (µM)$^e$</th>
<th>GTP Shift $^f$</th>
</tr>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2a</td>
<td>OH</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 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 H OH OH</td>
<td>0.435 ± 0.05$^a$</td>
<td>0.903 ± 0.081$^a$</td>
<td>0.5$^a$</td>
</tr>
<tr>
<td>2d</td>
<td>H</td>
<td>OH 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 OH OH OH</td>
<td>4.01 ± 0.30$^a$</td>
<td>2.12 ± 0.38$^a$</td>
<td>1.9$^a$</td>
</tr>
<tr>
<td>2f</td>
<td>OH</td>
<td>H H F H</td>
<td>$&gt;100$ (85%)$^b$</td>
<td>$&gt;100$ (26%)$^b$</td>
<td>–</td>
</tr>
<tr>
<td>2g</td>
<td>OH</td>
<td>H H H F</td>
<td>$&gt;100$ (26%)$^b$</td>
<td>$&gt;100$ (24%)$^b$</td>
<td>–</td>
</tr>
<tr>
<td>2h</td>
<td>OH</td>
<td>H H Cl H</td>
<td>$&gt;100$ (75%)$^b$</td>
<td>0.512 ± 0.05$^a$</td>
<td>–</td>
</tr>
<tr>
<td>2i</td>
<td>OH</td>
<td>H H Cl H</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 H Br H</td>
<td>$&gt;100$ (69%)$^b$</td>
<td>1.04 ± 0.18$^a$</td>
<td>–</td>
</tr>
<tr>
<td>2k</td>
<td>OH</td>
<td>H H H Br</td>
<td>$&gt;100$ (51%)$^b$</td>
<td>$&gt;100$ (46%)$^b$</td>
<td>–</td>
</tr>
<tr>
<td>2l</td>
<td>OH</td>
<td>H H H CF$_3$</td>
<td>$&gt;100$ (88%)$^b$</td>
<td>$&gt;100$ (56%)$^b$</td>
<td>–</td>
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</tbody>
</table>

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### Table 1 Continued.

<table>
<thead>
<tr>
<th>Compound</th>
<th>OH</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>CN</th>
<th>Dissociation Constant Values (Kᵢ) Values</th>
<th>Reference Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>2m</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (49%)&lt;sup&gt;\textbf{a}&lt;/sup&gt;</td>
<td>CPA (A&lt;sub&gt;1&lt;/sub&gt; agonist)</td>
</tr>
<tr>
<td>2n</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (57%)&lt;sup&gt;\textbf{a}&lt;/sup&gt;</td>
<td>CPA (A&lt;sub&gt;1&lt;/sub&gt; agonist)</td>
</tr>
<tr>
<td>2o</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (57%)&lt;sup&gt;\textbf{a}&lt;/sup&gt;</td>
<td>DPCPX (A&lt;sub&gt;1&lt;/sub&gt; antagonist)</td>
</tr>
<tr>
<td>2p</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (57%)&lt;sup&gt;\textbf{a}&lt;/sup&gt;</td>
<td>DPCPX (A&lt;sub&gt;1&lt;/sub&gt; antagonist)</td>
</tr>
<tr>
<td>2q</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>–</td>
<td>–</td>
<td>&gt;100 (57%)&lt;sup&gt;\textbf{a}&lt;/sup&gt;</td>
<td>DPCPX (A&lt;sub&gt;1&lt;/sub&gt; antagonist)</td>
</tr>
</tbody>
</table>

Reference compounds

- **CPA (A<sub>1</sub> agonist)**: 0.0068 ± 0.0001<sup>\textbf{a}</sup> (0.0079<sup>\textbf{a}</sup>; 0.015)
- **DPCPX (A<sub>1</sub> antagonist)**: 0.0004 ± 0.0002<sup>\textbf{a}</sup> (0.0005<sup>\textbf{a}</sup>; 0.0003)

<sup>\textbf{a}</sup> Kᵢ values determined in triplicate and expressed as mean ± SEM; <sup>\textbf{b}</sup> Percentage displacement of the radioligand at a maximum tested concentration (100 µM); <sup>\textbf{c}</sup> Rat receptors were used (A<sub>1</sub>: rat whole brain membranes; A<sub>2A</sub>: rat striatal membranes); <sup>\textbf{d}</sup> Selectivity index (SI) for the A<sub>2A</sub> receptor isoform calculated as the ratio of Kᵢ (A<sub>1</sub>)/Kᵢ (A<sub>2A</sub>); <sup>\textbf{e}</sup> GTP shift assay, where the 100 µM GTP was added to the A<sub>1</sub> AR radioligand binding assay; <sup>\textbf{f}</sup> GTP shifts calculated by dividing the Kᵢ in the presence of GTP by the Kᵢ in the absence of GTP; <sup>\textbf{g}</sup> Literature value obtained from reference [16]; <sup>\textbf{h}</sup> Literature value obtained from reference [13]; <sup>\textbf{i}</sup> Literature value obtained from reference [14]; <sup>\textbf{j}</sup> Literature value obtained from reference [15].
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 (Ki 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; A1Ki = 0.435 µM and A2AKi = 0.903 µM) on ring A is preferred over C6-(2e; A1Ki = 4.01 µM and A2AKi = 2.12 µM) and C5-OH substitution (2d; A1Ki = 5.31 µM and A2AKi = > 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 A2AKi 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 (A1Ki = > 100 µM and A2AKi = 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 (Ki 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 (Ki values = > 100 µM), it was beneficial to A2A AR affinity. Additionally, comparison of 2h and 2i indicated that C3'-Cl substitution (2h; A2AKi = 0.512 µM) is preferred over C4'-Cl substitution (2i; A2AKi = 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, A1 2q: N4' Ki = 1.69 µM > 2o; N2' Ki = 4.71 µM > 2p N3' Ki = 6.58 µM and A2A 2p: N3' Ki = 1.51 µM > 2o; N2' Ki = 1.79 µM > 2q; N4' Ki = 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 (indanone) 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_i = 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
