PROFESSOR LESETJA J LEGOABE (Orcid ID : 0000-0003-2440-4993)

Article type : Research Article

Evaluation of 2-benzylidene-1-tetralone derivatives as antagonists of A_1 and A_{2A} adenosine receptors

Lesetja J. Legoabe[1,*], Mietha M. Van der Walt[1] and Gisella Terre'Blanche[1,2]

1. Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

2. Pharmaceutical Chemistry, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Antagonists of the adenosine receptors (A_1 and A_{2A}) are thought to be beneficial in neurological disorders, such as Alzheimer's and Parkinson's disease. The aim of this study was to explore 2-benzylidene-1-tetralone derivatives as antagonists of A_1 and/or A_{2A} adenosine receptors. In general, the test compounds were found to be selective for the A_1 adenosine receptor, with only three test compounds possessing affinity for both the A_1 and A_{2A} adenosine receptor. The 2-benzylidene-1-tetralones bearing a hydroxyl substituent at either position C5, C6 or C7 of ring A displayed favorable adenosine A_1 receptor binding, while C5 hydroxy substitution led to favorable A_{2A} adenosine receptor affinity. Interestingly, *para*-hydroxy substitution on ring B in combination with ring A bearing a hydroxy at position C6 or C7 provided the 2-benzylidene-1-tetralones with both A_1 and A_{2A} adenosine receptor affinity. Compounds **4** and **8** displayed the highest A_1 and A_{2A} adenosine receptor affinity with values below 7μM. Both these compounds behaved as A_1 adenosine receptor antagonists in the performed GTP shift assays. In conclusion, the 2-benzylidene-1-tetralone derivatives can be considered as lead compounds to design a new class of dual acting adenosine A_1/A_{2A} receptor antagonists that may have potential in treating both dementia and locomotor deficits in Parkinson's disease.

KEYWORDS

2-Benzylidene-1-tetralone derivatives, A_1 adenosine receptor antagonists, A_{2A} adenosine receptor antagonists

Correspondence

Lesetja J Legoabe, Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa, Tel +27 18 299 2182, Fax +27 18 299 4243, email lesetja.legoabe@nwu.ac.za.

Parkinson's disease (PD) is a common but complex neurodegenerative disease characterized by a loss of dopaminergic neurons in the substantia nigra *pars compacta*[1] and abnormal aggregates of α-synuclein protein called Lewy bodies and Lewy neurites.[2] The decrease of dopamine in the basal ganglia leads to classical motor symptoms associated with PD, which include bradykinesia, rigidity, resting tremor and postural instability.[3] Although these four cardinal signs generally define PD, it is important not to dismiss the non-motor symptoms associated with PD.[4] Non-motor symptoms range from dribbling saliva, constipation, depression, sleeping disorders, apathy, hallucinations, and dementia.[5]

Current treatment of PD is symptomatic and consists of drugs that restore dopamine concentrations and/or effects.[6] L-3,4-dihydroxyphenylalanine (L-dopa), dopamine's immediate precursor, is considered the most effective drug for the treatment of the motor symptoms of PD.[7] Though L-dopa provides the greatest symptomatic relief, its adverse effects include motor fluctuations ("wearing-off phenomenon" and "on-off phenomenon"), non-motor fluctuations, dyskinesia, and drug-induced psychosis.[8] Other drugs used for the treatment of PD are dopamine agonists, catechol-*O*-methyltransferase inhibitors, monoamine oxidase inhibitors, amantadine, and anti-cholinergic drugs.[9] These drugs are focused on either replacing the concentrations and/or effects of dopamine in the brain and only address motor symptoms and not non-motor symptoms.[10] Therefore an ideal drug would address motor symptoms and non-motor symptoms, be disease modifying, and neuroprotective. Adenosine receptor (AR) antagonists may address the aforementioned problems.[11]

Adenosine is a neuro- and homeostatic modulator with wide ranging effects throughout the human body.[12, 13] Currently four AR subtypes, namely A_1, A_{2A}, A_{2B} and A_3, are known[14] and chemical compounds acting on them such as adenosine analogues and conjugates exhibited therapeutic potential. [15, 16] The A_1 ARs are highly expressed throughout the brain, while A_{2A} AR expression is limited to the striatum, nucleus accumbens and the olfactory tubercle.[17] The A_1 and A_{2A} ARs have been identified as drug targets for the treatment of PD and other neurological conditions.[18, 19] Adenosine plays a role opposite to dopamine in the brain.[20] Agonists and antagonists of ARs produce behavioural effects similar to antagonists and agonists of dopamine receptors, respectively.[11] Therefore, the antagonism of A_{2A} ARs in striatopallidal neurons reduces postsynaptic

effects of dopamine depletion, and sequentially reduces motor symptoms of PD.[21] Furthermore, A_{2A} AR antagonists might protect dopaminergic neurons from deterioration and thus be neuroprotective.[22] The combination of A_{2A} AR antagonists and L-dopa could reduce the risk of developing dyskinesia associated with long term L-dopa treatment.[23] In addition cognitive impairment associated with PD may potentially improve through A_1 AR antagonism.[19] Evidence for the improvement of cognitive impairment associated with PD through the antagonism of the A_1 AR came from a study where a dual A_1/A_{2A} receptor antagonist, ASP-5854 (Figure 1), reversed scopolamine-induced memory deficits in rats, whereas a specific A_{2A} AR antagonist, KW-6002 (Figure 1) did not.[19]

It is plausible that a dual-target A_1/A_{2A} AR antagonist may have significance in the treatment of PD as it reduces motor symptoms, is neuroprotective through the antagonism of A_{2A} ARs, and improves cognitive impairment through the antagonism of A_1 ARs.[19] Furthermore a dual-target A_1/A_{2A} AR antagonist may have a synergistic motor activating effect; antagonism of the A_1 AR facilitates presynaptic dopamine release and antagonism of the A_{2A} AR facilitates postsynaptic dopamine release.[24] For example, the motor activating effect of caffeine (Figure 1) may be due to the synergistic activity of antagonising both the A_1 and A_{2A} ARs.[25]

Affinity for both the A_1 and A_{2A} ARs were found for selected aurone derivatives.[26] An aurone (2-benzylidene-1-benzofuran-3-one) is a heterocyclic chemical compound (fused 6- and 5-membered rings) and a type of flavonoid.[27] Hispidol , an aurone derivative, was found to be a selective A_1 AR antagonist with K_i values of 0.352 μM and 52.7 μM for the A_1 and A_{2A} ARs, respectively.[26] Maritimetin, another aurone derivative, was found to possess affinity for both the A_1 (K_i=3.47 μM) and A_{2A} (K_i=9.35 μM) ARs.[26] It seems that single hydroxy substitution (hispidol) in ring A (position 6) and ring B (*para* position) is well tolerated for both A_1 and A_{2A} AR affinity. In addition, di-substitution of hydroxy on ring A (position 6 and 7) and ring B (*para* and *meta* position) lead to a 10-fold decrease in A_1 AR affinity but surprisingly a 5-fold increase in A_{2A} AR affinity (hispidol vs maritimetin) was obtained. However, in the case of Auresidin, where the di-hydroxy-substitution on ring A is in position 4 and 6 and on ring B in the *para* and *meta* (3' and 4') position, it is found that A_1 AR affinity (K_i=13.2 μM) is retained, but A_{2A} AR affinity is diminished (K_i > 100 μM). Sulfuretin bearing a single hydroxy substitutent on ring A (position 6) and di-hydroxy-substitution on ring B (3' and 4') retained both A_1 (K_i=4.44 μM) and A_{2A} (K_i=28.1 μM) AR affinity, thereby making the assumption that hydroxy substitution at position 4 of ring A is detrimental for A_{2A} AR affinity (Figure 2).

In addition, the isoflavones (heterocyclic fused 6- and 6-membered rings) were also evaluated for their affinity at A_1 ARs.[26] Overall, hydroxy substitution at position 5 and 7 of ring A and *para* position of ring B (4') were well tolerated for A_1 AR affinity. Methoxy substitution in the *para* (4') position of ring B (Biochanin) slightly decreased A_1 AR affinity, while methoxy substitution at position 7 (Prunetin) of ring A diminished A_1 AR affinity (Figure 2).

2-Benzylidene-1-tetralones are bicyclic benzofused ring systems and are structurally related to aurones and isoflavones (Figure 2). In addition, 2-benzylidene-1-tetralone derivatives are also known for their antitumor,[28] antifungal[29] and anti-microbial properties.[30] The 2-benzylidene-1-tetralone has not previously been evaluated as a possible scaffold for developing AR antagonists. Based on the aforementioned literature findings that the aurone derivatives possess affinity for the A_1 and A_{2A} ARs, as well as the observation that isoflavones exert A_1 AR binding, we conducted a pilot study to investigate the potential of several structurally related 2-benzylidene-1-tetralones to possess affinity for the A_1 and A_{2A} ARs. Thus, in order to find new insights into the structural requirements for AR binding by 2-benzylidene-1-tetralone based analogues, various modifications to both ring A and B was explored.

1. METHODS AND MATERIALS

1.1 Materials and general procedures

Unless otherwise noted, all starting materials for the synthesis were obtained from Sigma-Aldrich and were used without further purification. 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. NMR measurements were carried out in $CDCl_3$ or DMSO-d_6 as NMR solvent. All chemical shifts are reported in parts per million (ð) downfield from the signal of $Si(CH_3)_4$. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). The melting points (mp) were determined with a Buchi B-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using silica gel 60 (Merck). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. The affinities of 2-benzylidene-1-tetralones for rat adenosine A_1 and A_{2A} receptor subtypes were determined with radioligand competition experiments as described previously.[31] All commercially available reagents were obtained from various manufacturers (Sigma–Aldrich, Ascent Scientific and Merck). The radioligands [^{3}H]NECA (specific activity 25 Ci/mmol) and [^{3}H]DPCPX (specific activity 120 Ci/mmol) were obtained from Amersham Biosciences and PerkinElmer, respectively. Filter-count was purchased from PerkinElmer, while Whatman GF/B

25 mm diameter filters was obtained from Merck. Counting of radio activities were performed using a Packard Tri-CARB 2810 TR liquid scintillation counter.

1.2 General method for preparation of 2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one analogues (1–22)

The target compounds were prepared by employing an acid-catalysed Claisen-Schmidt condensation method.[32] This chemical transformation involves the nucleophilic addition of a ketone enolate to an aldehyde to form a β-hydroxyaldehyde or β-hydroxyketone, before dehydration to give a conjugated enone. [33] Briefly, the appropriate substituted tetralone derivatives (2 mmol) were reacted with appropriate benzaldehydes (2 mmol) in a mixture of methanol (15 mL) and hydrochloric acid (32%; 22.5 mL) and were reflux for 1–6 h. The reaction progress was monitored using silica gel TLC. Upon completion of the reaction, ice-cold water (20 mL) was added and the resulting precipitate was collected by filtration, dried and purified by recrystallization from a suitable solvent (mainly ethanol) (**S**cheme 1).

All the final products in this study possessed *E*-configuration. Although in principle chalcones can exist as *E*- or *Z*-isomers; previous studies found that the *E*-isomer is the most thermodynamically stable form.[34] It was further demonstrated that the *Z*-configuration is highly unfavourable due to strong steric interaction between the aryl and carbonyl groups. [35]

1.2.1 (2*E*)-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (1)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 67.4%; clear yellow crystals; mp: 106.0–107.3 ^{o}C (ethanol). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.12 (d, *J* = 7.7 Hz, 1H), 7.86 (s, 1H), 7.50 – 7.31 (m, 7H), 7.23 (d, *J* = 7.2 Hz, 1H), 3.12 (t, *J* = 6.4 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.88, 143.20, 136.63, 135.80, 135.42, 133.43, 133.25, 129.85, 128.51, 128.41, 128.19, 128.15, 126.99, 28.85, 27.16. APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O$: 234.292 found: 235.1121 $[M+1]^{+}$.

1.2.2 (2*E*)-2-benzylidene-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 71.5%: mp 151.2–151.9 ^{o}C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 – 7.84 (m, 2H), 7.41 (dt, *J* = 15.2, 7.4 Hz, 5H), 7.35 (d, *J* = 7.0 Hz, 1H), 7.16 – 7.06 (m, 2H), 3.09 (dd, *J* = 9.1, 3.7 Hz, 2H), 2.86 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.82,

155.40, 137.55, 135.71, 135.67, 135.36, 134.03, 129.94, 129.67, 128.68, 128.42, 121.82, 113.99, 27.96, 27.42; APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O_2$: 250.29186, found: 251.1078 $[M+1]^+$.

1.2.3 (2*E*)-2-benzylidene-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (3)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 76 %; purple crystal. mp 199.8–200.8 °C (ethanol). ^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.44 (s, 1H), 7.85 (d, J=8.6 Hz, 1H), 7.62 (s, 1H), 7.48 (d, J=7.4 Hz, 2H), 7.44 (t, J=7.6 Hz, 2H), 7.37 (t, J=7.3 Hz, 1H), 6.77 (dd, J=8.6, 2.3 Hz, 1H), 6.67 (s, 1H), 3.01 (t, J=5.8 Hz, 2H), 2.82 (t, J=6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 185.25, 162.29, 146.06, 135.88, 135.47, 134.48, 130.30, 129.77, 128.54, 128.51, 125.08, 114.77, 113.94, 28.24, 26.75; APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O_2$: 250.292, found: 251.1047 $[M+1]^+$.

1.2.4 (2*E*)-2-benzylidene-5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (4)

The title compound is a product of 5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 88%: mp 174.2–175.3 °C (ethanol). ^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 9.85 (s, 1H), 7.65 (s, 1H), 7.51 (d, *J*=7.4 Hz, 2H), 7.45 (t, *J*=7.3 Hz, 3H), 7.38 (t, *J*=7.3 Hz, 1H), 7.20 (t, *J*=7.9 Hz, 1H), 7.07 (d, *J*=8.0 Hz, 1H), 3.03 (t, *J*=5.8 Hz, 2H), 2.81 (t, *J*=6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 187.07, 154.35, 135.61, 135.30, 135.19, 134.02, 130.11, 129.88, 128.68, 128.58, 127.06, 119.27, 117.97, 26.10, 21.13; APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O_2$: 250.29186, found: 251.1049 $[M+1]^+$

1.2.5 (2*E*)-2-benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (5)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 51.2%; pale yellow crystals; mp: 121.5–122.7 °C (dichloromethane). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.61 (d, *J* = 2.8 Hz, 1H), 7.41 (dt, *J* = 15.2, 7.4 Hz, 4H), 7.33 (t, *J* = 7.0 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 7.06 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.09 (td, *J* = 6.7, 1.6 Hz, 2H), 2.90 – 2.84 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.80, 158.62, 136.68, 135.92, 135.85, 135.43, 134.23, 129.84, 129.42, 128.49, 128.40, 121.50, 110.26, 55.52, 28.02, 27.34; APCI-HRMS *m/z*: calculated for $C_{18}H_{16}O_2$: 264.318, found: 265.1212 $[M+1]^+$.

1.2.6 (2*E*)-2-benzylidene-6,7-dimethoxy-3,4-dihydronaphthalen-1(2*H*)-one (6)

The title compound is a product of 6,7-dimethoxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 91%: mp 135.1–135.8 °C. ^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 7.64 (s,

1H), 7.47 (dd, *J*=34.3, 8.3 Hz, 5H), 7.38 (t, *J*=7.3 Hz, 1H), 6.92 (s, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.04 (t, *J*=5.8 Hz, 2H), 2.86 (t, *J*=6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 185.33, 153.47, 147.86, 138.35, 135.55, 135.39, 134.65, 129.76, 128.52, 125.63, 110.66, 108.95, 55.79, 55.46, 27.63, 26.95; APCI-HRMS *m/z*: calculated for $C_{19}H_{18}O_3$: 294.34442, found: 295.1352 $[M+1]^+$.

1.2.7 (2*E*)-6-amino-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (7)

The title compound is a product of 6-amino-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 51.3%; brown shinny crystals; mp: 230.0–233.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 7.81 (d, J = 8.5 Hz, 1H), 7.60 (s, 1H), 7.50 – 7.39 (m, 4H), 7.36 (d, J = 7.2 Hz, 1H), 6.80 (dd, J = 8.5, 1.7 Hz, 1H), 6.67 (s, 1H), 4.92 (s, br, 3H), 2.99 (t, J = 5.8 Hz, 2H), 2.79 (t, J = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 184.81, 148.33, 145.49, 136.10, 135.60, 134.24, 129.92, 129.80, 128.59, 128.50, 124.98, 115.69, 114.58, 28.32, 26.74; APCI-HRMS *m/z*: calculated for $C_{17}H_{15}NO$: 249.3071, found: 250.1226 $[M+1]^+$.

1.2.8 (2*E*)-7-hydroxy-2-(4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-on (8)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-hydroxybenzaldehyde in a yield of 39.2%; brown crystals, mp: 249.5–249.7 °C (ethyl acetate).^{1}H NMR (600 MHz, DMSO) δ 9.92 (s, 1H), 9.60 (s, 1H), 7.61 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 2.6 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.96 (dd, *J* = 8.2, 2.7 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.02 (t, *J* = 5.9 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.61, 158.35, 156.17, 136.17, 133.96, 133.82, 132.49, 132.08, 129.57, 126.16, 121.04, 115.51, 112.58, 27.96, 27.01; APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O_3$: 266.291, found: 267.1027 $[M+1]^+$.

1.2.9 (2*E*)-6-hydroxy-2-(4'-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (9)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-hydroxybenzaldehyde in a yield of 72.3%: mp 279.4–280.9 °C (ethanol).^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.37 (s, 1H), 9.89 (s, 1H), 7.82 (d, *J*=8.6 Hz, 1H), 7.56 (s, 1H), 7.36 (d, *J*=8.5 Hz, 2H), 6.83 (d, *J*=8.6 Hz, 2H), 6.75 (dd, *J*=8.6, 2.3 Hz, 1H), 6.65 (s, 1H), 3.01 (t, *J*=6.0 Hz, 2H), 2.81 (t, *J*=6.5 Hz, 2H);^{13}C NMR (151 MHz, DMSO) δ 185.25, 162.05, 158.13, 145.78, 135.15, 132.81, 131.90, 130.16, 126.33, 125.34, 115.49, 114.63, 113.87, 28.20, 26.79; APCI-HRMS *m/z*: calculated for $C_{17}H_{14}O_3$: 266.29126, found: 267.0988 $[M+1]^+$.

1.2.10 (2*E*)-6-hydroxy-2-(3'-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (10)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-methoxybenzaldehyde in a yield of 90%: mp 287.5–289.5 °C (ethanol).^{1}H NMR(DMSO-d_6, 600 MHz):

δ (ppm) 10.45 (s, 1H), 7.84 (d, *J*=8.6 Hz, 1H), 7.59 (s, 1H), 7.35 (t, *J*=7.9 Hz, 1H), 7.07 – 7.00 (m, 2H), 6.94 (dd, *J*=8.2, 2.2 Hz, 1H), 6.77 (dd, *J*=8.6, 2.3 Hz, 1H), 6.66 (d, *J*=2.1 Hz, 1H), 3.77 (s, 3H), 3.01 (t, *J*=5.8 Hz, 2H), 2.82 (t, *J*=6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 185.27, 162.32, 159.26, 146.13, 136.85, 136.15, 134.47, 130.33, 129.62, 125.09, 121.98, 115.13, 114.80, 114.25, 113.97, 55.17, 28.26, 26.85; APCI-HRMS *m/z*: calculated for $C_{18}H_{16}O_3$: 280.31784, found: 281.1147 $[M+1]^+$.

1.2.11 (2*E*)-2-(3',4'-dichlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (11)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3,4-dichlorobenzaldehyde in a yield of 83%: mp 183.3–184.1 °C (ethanol).^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 9.69 (s, 1H), 7.76 (s, 1H), 7.68 (d, *J*=8.3 Hz, 1H), 7.59 (s, 1H), 7.48 (d, *J*=8.3 Hz, 1H), 7.33 (d, *J*=2.6 Hz, 1H), 7.18 (d, *J*=8.3 Hz, 1H), 6.99 (dd, *J*=8.2, 2.7 Hz, 1H), 2.98 (t, *J*=5.8 Hz, 2H), 2.81 (t, *J*=6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.48, 156.28, 137.42, 136.11, 134.23, 133.47, 132.79, 131.52, 131.34, 131.11, 130.66, 129.88, 129.83, 121.67, 112.58, 26.98, 26.95; APCI-HRMS *m/z*: calculated for $C_{17}H_{12}Cl_2O_2$: 319.18198, found: 319.0296 $[M]^+$

1.2.12 (2*E*)-2-(3',4'-dichlorobenzylidene)-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (12)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one and 3,4-dichlorobenzaldehyde in a yield of 88%: mp 214.2–215.5 °C (ethanol). ^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.49 (s, 1H), 7.84 (d, *J*=8.6 Hz, 1H), 7.74 (s, 1H), 7.68 (d, *J*=8.3 Hz, 1H), 7.55 (s, 1H), 7.47 (d, *J*=8.3 Hz, 1H), 6.77 (dd, *J*=8.6, 2.2 Hz, 1H), 6.66 (s, 1H), 2.97 (t, *J*=5.8 Hz, 2H), 2.83 (t, *J*=6.4 Hz, 2H), 2.49; ^{13}C NMR (151 MHz, DMSO) δ 184.90, 162.48, 146.17, 137.72, 136.31, 131.85, 131.43, 131.30, 130.90, 130.63, 130.40, 129.83, 124.89, 114.90, 114.00, 28.09, 26.70; APCI-HRMS *m/z*: calculated for $C_{17}H_{12}Cl_2O_2$: 319.18198, found: 319.0306 $[M]^+$.

1.2.13 (2*E*)-2-(3',4'-dichlorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (13)

The title compound is a product of 5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3,4-dichlorobenzaldehyde in a yield of 78%: mp 398.6–398.7 °C. ^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 9.89 (s, 1H), 7.77 (s, 1H), 7.69 (d, *J*=8.3 Hz, 1H), 7.57 (s, 1H), 7.49 (d, *J*=8.3 Hz, 1H), 7.44 (d, *J*=7.2 Hz, 1H), 7.20 (t, *J*=7.9 Hz, 1H), 7.07 (d, *J*=7.9 Hz, 1H), 2.99 (t, *J*=5.9 Hz, 2H), 2.81 (t, *J*=6.4 Hz, 2H);^{13}C NMR (151 MHz, DMSO) δ 186.82, 154.41, 137.43, 136.12, 133.81, 132.53, 131.54, 131.35, 131.09, 130.68, 130.20, 129.89, 127.16, 119.46, 118.02, 26.06, 21.06; APCI-HRMS *m/z*: calculated for $C_{17}H_{12}Cl_2O_2$: 319.18198, found: 319.0301 $[M]^+$

1.2.14 (2*E*)-2-(4'-fluorobenzylidene)-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (14)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-fluorobenzaldehyde in a yield of 38.1%: mp 186.3–186.6 ^{o}C (ethanol).^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.46 (s, 1H), 7.84 (d, *J*=8.6 Hz, 1H), 7.60 (s, 1H), 7.54 (dd, *J*=8.4, 5.7 Hz, 2H), 7.27 (t, *J*=8.8 Hz, 2H), 6.77 (dd, *J*=8.6, 2.3 Hz, 1H), 6.66 (d, *J*=2.1 Hz, 1H), 2.99 (t, *J*=5.9 Hz, 2H), 2.82 (t, *J*=6.5 Hz, 2H).^{13}C NMR (151 MHz, DMSO) δ 185.19, 162.75, 162.33, 161.11, 146.06, 135.75, 133.45, 132.10, 132.04, 131.98, 131.96, 130.33, 125.07, 115.62, 115.48, 114.81, 113.96, 28.18, 26.67; APCI-HRMS *m/z*: calculated for $C_{17}H_{13}FO_2$: 268.2823232, found: 269.0954 $[M+1]^+$.

1.2.15 (2*E*)-2-(4'-chlorobenzylidene)-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (15)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-chlorobenzaldehyde in yield of 52.6 %; mp: 209.1–210.2 ^{o}C .^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.46 (s, 1H), 7.84 (d, *J*=8.6 Hz, 1H), 7.59 (s, 1H), 7.54 – 7.47 (m, 4H), 6.66 (d, *J*=2.1 Hz, 1H), 2.98 (t, *J*=6.4 Hz, 2H), 2.82 (t, *J*=6.5 Hz, 2H);^{13}C NMR (151 MHz, DMSO) δ 185.05, 162.37, 146.07, 136.54, 134.35, 133.12, 133.06, 131.56, 130.33, 128.56, 124.97, 114.81, 113.95, 28.14, 26.70; APCI-HRMS *m/z*: calculated for $C_{17}H_{13}ClO_2$: 284.73692, found: 285.0694 $[M+1]^+$.

1.2.16 (2*E*)-2-(4'-bromobenzylidene)-6-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (16)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-bromobenzaldehyde in yield of 71.6 %: mp 202.0–203.4 ^{o}C (ethanol).^{1}H NMR(DMSO-d_6, 600 MHz): δ (ppm) 10.47 (s, 1H), 7.84 (d, *J*=8.6 Hz, 1H), 7.62 (d, *J*=8.4 Hz, 2H), 7.56 (s, 1H), 7.43 (d, *J*=8.4 Hz, 2H), 6.79 – 6.74 (m, 1H), 6.66 (d, *J*=2.1 Hz, 1H), 2.97 (t, *J*=5.8 Hz, 2H), 2.82 (t, *J*=6.4 Hz, 2H);^{13}C NMR (151 MHz, DMSO) δ 185.10, 162.39, 146.11, 136.62, 134.72, 133.21, 131.82, 131.51, 130.36, 125.00, 121.81, 114.84, 113.98, 28.16, 26.73; APCI-HRMS *m/z*: calculated for $C_{17}H_{13}BrO_2$: 329.18792, found: 329.0176 $[M]^+$.

1.2.17 3'-[(*E*)-(6-hydroxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (17)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-formylbenzonitrile in yield of 85 %: mp 51.3–51.4 ^{o}C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 10.49 (s, 1H), 7.95 (s, 1H), 7.88 – 7.77 (m, 3H), 7.63 (dd, *J* = 19.0, 11.2 Hz, 2H), 6.77 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.67 (d, *J* = 2.0 Hz, 1H), 2.99 (t, *J* = 5.9 Hz, 2H), 2.84 (t, *J* = 6.4 Hz, 2H).;^{13}C NMR (151 MHz, DMSO) δ 184.95, 162.51, 146.21, 137.86, 136.78, 134.31, 133.02, 132.18, 131.86, 130.41, 129.75, 124.87, 118.60, 114.90, 114.00, 111.76, 28.10, 26.64; APCI-HRMS *m/z*: calculated for $C_{18}H_{13}NO_2$: 275.30132, found: 276.0992 $[M+1]^+$.

1.2.18 4'-[(*E*)-(6-hydroxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (18)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-formylbenzonitrile in yield of 56.0 %: mp 231.4–233.3 °C (ethanol).^{1}H NMR (600 MHz, DMSO) δ 10.50 (s, 5H), 7.87 (dd, *J* = 19.8, 8.4 Hz, 14H), 7.69 – 7.58 (m, 15H), 6.77 (dd, *J* = 8.6, 2.3 Hz, 5H), 6.67 (d, *J* = 2.1 Hz, 5H), 2.98 (t, *J* = 5.8 Hz, 11H), 2.83 (t, *J* = 6.4 Hz, 11H); ^{13}C NMR (151 MHz, DMSO) δ 184.93, 162.55, 146.23, 140.41, 138.51, 132.54, 132.35, 130.48, 130.46, 124.87, 118.77, 114.94, 114.02, 110.61, 28.11, 26.77; APCI-HRMS *m/z*: calculated for $C_{18}H_{13}NO_2$: 275.30132, found: 276.1025 $[M+1]^+$.

1.2.19 (2*E*)-6-hydroxy-2-(3'-nitrobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (19)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-nitrobenzaldehyde in yield of 69.4 %: mp 51.3–51.4 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 10.47 (s, 1H), 8.26 (d, *J* = 8.7 Hz, 4H), 7.86 (d, *J* = 8.6 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 4H), 7.66 (s, 2H), 6.78 (dd, *J* = 8.6, 2.3 Hz, 2H), 6.67 (d, *J* = 2.1 Hz, 2H), 3.00 (t, *J* = 5.7 Hz, 4H), 2.85 (t, *J* = 6.4 Hz, 4H);^{13}C NMR (151 MHz, DMSO) δ 184.87, 162.62, 146.74, 146.27, 142.44, 139.08, 132.05, 130.87, 130.49, 124.82, 123.60, 114.98, 114.04, 28.10, 26.82; APCI-HRMS *m/z*: calculated for $C_{17}H_{13}NO_4$: 295.28942, found: 296.0900 $[M+1]^+$.

1.2.20 (2*E*)-6-hydroxy-2-(4'-nitrobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (20)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-nitrobenzaldehyde in yield of 55.7 %: mp 51.5–51.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 10.50 (s, 1H), 8.26 (s, 1H), 8.20 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.67 (s, 1H), 6.77 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.67 (d, *J* = 2.1 Hz, 1H), 3.01 (t, *J* = 5.8 Hz, 2H), 2.84 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 184.91, 162.54, 147.92, 146.18, 138.21, 137.20, 136.08, 132.00, 130.43, 130.07, 124.86, 123.98, 122.97, 114.93, 114.02, 28.11, 26.65; APCI-HRMS *m/z*: calculated for $C_{17}H_{13}NO_4$: 295.28942, found: 296.0908 $[M+1]^+$.

1.2.21 (2*E*)-2-[4'-(dimethylamino)benzylidene]-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (21)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-(dimethylamino)benzaldehyde in yield of 62.8 %: mp 219.5–240.7 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 7.81 (d, *J* = 8.5 Hz, 1H), 7.58 (s, 1H), 7.44 (d, *J* = 8.6 Hz, 2H), 6.96 (s, 2H), 6.76 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.66 (d, *J* = 2.1 Hz, 1H), 3.80 (s, 1H), 3.02 (t, *J* = 6.1 Hz, 2H), 2.99 (s, 6H), 2.80 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 185.10, 162.03, 145.66, 134.78, 131.65, 130.09, 125.41, 114.62, 113.85, 40.74, 28.18, 26.86; APCI-HRMS *m/z*: calculated for $C_{19}H_{19}NO_2$: 293.35966, found: 294.1480 $[M+1]^+$.

1.2.22 (2*E*)-2-[(5'-chlorothiophen-2'-yl)methylidene]-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (22)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 5-chlorothiophene-2-carbaldehyde in yield of 64.0 %: mp 234.2–235.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 10.46 (s, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.72 (s, 1H), 7.45 (d, *J* = 4.0 Hz, 1H), 7.24 (d, *J* = 4.0 Hz, 1H), 6.76 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.68 (d, *J* = 2.1 Hz, 1H), 2.99 (dd, *J* = 9.4, 3.8 Hz, 2H), 2.90 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 184.31, 162.29, 145.85, 137.56, 133.36, 132.68, 131.95, 130.28, 127.71, 127.00, 125.08, 114.81, 113.96, 27.47, 26.72; APCI-HRMS *m/z*: calculated for $C_{15}H_{11}ClO_2S$: 290.76464, found: 291.0266 $[M+1]^+$.

1.3 Radioligand binding assays

The A_1 and A_{2A} AR binding affinity of the test compounds (**1–22**) were determined with radioligand competition experiments described previously.[31] The A_1 AR binding affinity was performed with rat whole brain membranes in the presence of the radioligand [^{3}H]-8-cylcopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX).[31, 36] In turn, the A_{2A} AR binding affinity was measured at rat striatal membranes with 5'-N-[^{3}H]-ethylcarboxamideadenosine ([^{3}H]NECA) as radioligand.[31, 37] In addition, N_6-cyclopentyladenosine (CPA) was added to the A_{2A} AR binding studies to minimize the binding of the radioligand [^{3}H]NECA to A_1 ARs.[31, 37] Nonspecific binding was defined by the addition of 100 µM CPA.[31] The adenosine A_1 agonist CPA and adenosine A_{2A} antagonist ZM-241385 was chosen to validate the radioligand binding assays of the current study.

1.4 Functional characterization of compounds 4 and 8

In order to determine if compounds **4** and **8** function as agonists or as antagonists, the competition curves of the latter compounds were evaluated via a GTP shift assay reported previously. [29] The GTP shift assays were carried out with rat whole brain membranes in the absence and presence of 0.1 mM GTP. Nonspecific binding was defined by the addition of 10 µM DPCPX (unlabelled). [31] The adenosine A_1 agonist CPA and adenosine A_1 antagonist DPCPX was chosen as reference compounds of the current study.

1.5 Data analysis

The competition curves were obtained by using the Prism software package (GraphPad Software Inc.). The results of the competition experiments were expressed as dissociation constant values (K_i)

of triplicate determinations and are expressed as mean ± standard error of mean (SEM). The K_i values were calculated by using the K_d values of 0.36 nM for [^{3}H]DPCPX at rat whole brain membranes,[31, 36] while 15.3 nM for [^{3}H]NECA at rat striata membrane[31, 37] were used. Furthermore, GTP shifts are 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.[38] A compound with a calculated GTP shift of approximately 1 is considered an antagonist; in turn the presence of GTP affects the competition curves of an agonist and shifts the curve to the right.[31, 39]

2. RESULTS

2.1 Radioligand binding assays

The results of the radioligand binding experiments for the reference (CPA and ZM-241385) and test compounds (**4** to **8**) are summarized in Table 1. Furthermore, the experimental results of CPA and ZM-241385 were found in accordance with literature values (Table 1).

2.2 Functional characterization of compounds 4 and 8

The affinities of the reference (CPA and DPCPX) and test compounds (**4** and **8**) were determined in the absence and presence of 0.1 mM GTP and are reported with the calculated GTP shifts in Table 2. The calculated GTP shift results for CPA and DPCPX (Table 2) was found to correspond with literature values, where CPA act as an agonist and DPCPX as an antagonist (Figure 3).

3. DISCUSSION

3.1 Radioligand binding assays – A_1 and A_{2A} AR affinity

Structural modifications on ring A: First of all, in analogy with the aforementioned aurones, the impact of hydroxy substitution at either the C5-, C6- or C7-position of ring A was evaluated by comparing the dissociation constant (K_i) values of these compounds (**2**–**4**) to their unsubstituted counterpart compound **1** (A_1K_i = 22.78 µM). Similarly to the aurones, the position of the hydroxy group on ring A was found to be important to modulate both the A_1 and A_{2A} AR affinity. For instance, the hydroxy substitution at C7 (**2**; A_1K_i = 11.75 µM) increased the A_1 AR binding affinity approximately two-fold compared to compound **1**. In turn, hydroxy substitution at C6 (**3**; A_1K_i = 25.42 µM) resulted in a similar K_i value as compound **1**. Of note, compound **4** (C5-OH) exhibited the second highest A_1 AR affinity (A_1K_i = 5.93 µM) and highest A_{2A} AR binding ($A_{2A}K_i$ = 2.90 µM) among compounds **1**, **2** and **3**. Thus, substitution with a hydroxy group at position C5 of ring A may modulate the AR affinity of 2-benzylidene-1-tetralones to such an extent that both A_1 and A_{2A} AR affinity are obtained. Thus, A_1 AR affinity may be governed by hydroxy substitution at position C5, C6

and C7 of ring A, with the order of increased binding affinity documented as follow: **4** (C5-OH) > **2** (C7-OH) > **3** (C6-OH). Furthermore, in agreement with the abovementioned literature findings of the isoflavone derivatives, the investigated 2-benzylidene-1-tetralones seems to favour hydroxy substitution of ring A over substitution with a methoxy group. For example, compound **2** (C7-OH) exhibited a four-fold enhanced affinity for A_1 ARs compared to compound **5** that bear a methoxy group at C7 of ring A. In addition, compound **6** was synthesized containing a di-methoxy substitution at position C6 and C7 of ring A. However, this structural modification resulted in a diminished affinity for the A_1 AR. Compound **7** (C6-NH_2) exhibited an approximate three-fold reduced A_1 affinity compared to the unsubstituted compound **1** and C6-hydroxy substituted compound **3**. Consequently, optimization of ring A, of the 2-benzylidene-1-tetralones, towards A_1 AR affinity seems to favour hydroxy substitution (at C5, C6 or C7) over methoxy or amino substitution. With the exception of compound **4**, the structural modifications to ring A (without changes to ring B) did not result in A_{2A} AR affinity (at a maximum tested concentration of 100 µM) for compounds **1** to **7**.

Structural modifications on ring B: Notably, C4'-hydroxy substitution of ring B led to an increase of the A_1 AR binding and significantly yielded gained A_{2A} AR affinity. For example, both the C6- and C7-hydroxy substituted derivatives (**8** and **9**) exerted binding affinity for the A_1 and A_{2A} ARs. Compound **8** possessed K_i values of 5.39 µM and 6.60 µM for the A_1 and A_{2A} ARs, respectively. Furthermore, compound **8** displayed an approximate three-fold higher affinity towards the A_1 and A_{2A} ARs compared to compound **9** (A_1K_i = 16.15 µM; $A_{2A}K_i$ = 19.14 µM). Interestingly, both of the latter compounds (**8** and **9**) exhibited a non-selective activity towards the A_1 and A_{2A} ARs. Similar to the above findings, the compound bearing C7-hydroxy substitution (**8**) is preferred over the compound possessing a C6-hydroxy substituent (**9**). Introduction of a 3'-methoxy group (compound **3** vs compound **10**) did not increase A_1 AR affinity but A_1 AR activity was retained (**10** A_1K_i = 28.59 µM vs **3** A_1K_i = 25.42 µM). Furthermore, affinity at the A_{2A} AR subtype remained elusive. The influence of halogen substitution on ring B was also investigated. Di-substitution with chlorine at positions 3' and 4' of ring B led to compounds **11** (A_1K_i = 37.10 µM), **12** (A_1K_i = 39.29 µM) and **13** (A_1K_i = 23.29 µM) displaying A_1 AR affinity. Although a decrease in A_1 AR affinity was observed compared to their corresponding counterparts compounds **2** (C7-OH), **3** (C6-OH) and **4** (C5-OH), compound **13**, possessing a C5-hydroxy substituent on ring A, exhibited the highest A_1 AR affinity among compounds **11** (C7-OH), **12** (C6-OH) and **13** (C5-OH), thus showing the same trend as their corresponding homologues (e.g. **13** > **11** > **12** vs **4** > **2** > **3**) where compound **4** possess a C5-hydroxy substitute on ring A. Furthermore, compounds **11**, **12** and **13** were deemed inactive towards the A_{2A} AR. As part of the pilot study, various substitutions on ring B of compound **3** (C6-OH) was additionally explored to gain further insight into compound **3**'s structural requirements. For

instance, the presence of C4'-fluorine (**14**; A_1K_i = 24.85 μM) substitution on ring B displayed a similar binding affinity towards the A_1 AR for compound **14** compared to compound **3** (unsubstituted ring B), while C4'-bromine (**16**; A_1K_i = 34.57 μM) substitution on ring B resulted in a slight decrease in A_1 AR affinity in comparison with compound **3**. Both of these compounds (**14** and **16**) showed no affinity towards the A_{2A} AR subtype ($A_{2A}K_i$ > 100 μM). Surprisingly C4'-chlorine substitution (**15**) on ring B resulted in a loss of A_1 AR affinity in comparison to compounds **3**, **14** and **16**. Further substitution of compound **3** with electron withdrawing groups (CN, NO_2) at either position C3' or C4' (**17**–**20**) of ring B did not govern A_1 or A_{2A} AR binding. By replacing the chlorine substituted phenyl ring of compound **15** with a chlorine substituted thiophene ring (**22**), A_1 AR affinity (A_1K_i = 42.87 μM) was gained as compound **15** displayed no affinity towards the A_1 ARs. Compound **22** also showed no affinity towards the A_{2A} ARs ($A_{2A}K_i$ > 100 μM). It could be assumed that by replacing phenyl ring B with another ring system (e.g. thiophene) that there would be no significant impact on the A_1 AR activity and future research with other ring systems are necessary to gain more insight. In general, the test compounds (**11**–**16** and **22**) possessing halogen substitution on ring B exerted a more favourable effect on the A_1 AR, over the A_{2A} AR subtype, with the only exception being compound **15** (C4'-Cl) that exhibited no affinity towards either one of the AR subtypes. Overall, both A_1 and A_{2A} AR binding affinity is favoured by ring B bearing a C4'-hydroxy group (**8** and **9**), whereas other substituents on ring B led to a decreased or loss in A_1 AR affinity with A_{2A} AR binding remaining elusive.

3.2 Functional characterization of compounds 4 and 8

In order to determine if the test compounds (**1**–**22**) act as agonists or antagonists, GTP shift experiments were carried out. For this purpose compounds **4** and **8** were chosen as they exhibited the highest A_1 AR binding affinity among the investigated compounds. Generally, a rightward shift of the binding curve in the presence of GTP (due to an uncoupling of the A_1 AR from its G_i protein) is expected for an A_1 AR agonist.[31, 39] In the case of an A_1 AR antagonist no significant shift is anticipated in the presence of GTP. [31, 39] The results suggest that compounds **4** and **8** act as A_1 AR antagonists as no significant rightward shift of the binding curves were observed in the presence of GTP (Figure 3).

4. CONCLUSION

In conclusion, the pilot study indicated the importance of structural modifications at both rings A and B of the 2-benzylidene-1-tetralone scaffold for gaining and even losing A_1 and/or A_{2A} AR affinity. Overall, the test compounds (**1**–**22**) were found to be selective for the A_1 AR, with only three test compounds (**4**, **8** and **9**) possessing affinity for the A_{2A} AR subtype. In general, substitution with a

hydroxy group on ring A and/or B play a key role in modulating the binding affinity and selectivity at A_1 and A_{2A} ARs. Specifically, C5-hydroxy substitution on ring A was preferred over the C6- and C7-positions (i.e. **4** vs **2**&**3**; **13** vs **11**&**12**) for A_1 affinity, while C4'-hydroxy substitution (**8** and **9**) on ring B favored both A_1 and A_{2A} AR affinity. Compounds **4** and **8** are two promising drug candidates that both act as A_1 AR antagonists *in vitro* with K_i values below 7 μM. Compound **8** was found to possess the highest A_1 AR affinity (A_1K_i = 5.39 μM) and the second highest A_{2A} AR affinity ($A_{2A}K_i$ = 6.60 μM) among the test compounds (**1–22**) and acted non-selectively. Furthermore, compound **4** was reported with affinity for both the A_1 (A_1K_i =5.93 μM) and A_{2A} ($A_{2A}K_i$ =2.90 μM) ARs and displayed selectivity towards the A_{2A} AR. Therefore, compounds **4** and **8** are ideal drug candidates for future *in vivo* investigation of the 2-benzylidene-1-tetralone class of compounds as adenosine receptor antagonists in neurodegenerative disorders.

ACKNOWLEDGEMENTS

We are grateful to Dr. J. Jordaan and Mr. A. Joubert of the SASOL Centre for Chemistry, North-West University, for recording the NMR and MS spectra, respectively. We are also thankful to Prof. J. du Preez for HPLC analysis. Financial support for this work was provided by the North-West University, the National Research Foundation (Grant No: 96135) and the Medical Research Council, South Africa.

CONFLICT OF INTEREST

The authors declared that they have no competing financial interest.

Figure legends section

FIGURE 1 The structures of caffeine, KW-6002 and ASP-5854.

FIGURE 2 The structures of various aurones and isoflavones previously documented to possess affinity for the adenosine receptor and the general structure of the target 2-benzylidene-1-tetralone scaffold being explored in the current study.

SCHEME 1 General synthetic route for preparation of target substituted (2*E*)-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one derivatives

FIGURE 3 The binding curves of CPA (reference compound) and compounds **4** and **8**, indicating their A_1 AR agonist/antagonistic action as determined via GTP shift assays (with and without 0.1 mM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. (**A**) GTP shift of 1 calculated for the A_1 AR

antagonist compound **4**, (**B**) GTP shift of 1 calculated for the A_1 AR antagonist compound **8** and (**C**) GTP shift of 6 calculated for the A_1 AR agonist CPA.

TABLE 1 The dissociation constant values (K_i values) for the binding of the 2-benzylidene-1-tetralones to rat adenosine A_1 and A_{2A} receptors.

TABLE 2 The adenosine A_1 affinities (in the absence and presence of GTP) and GTP shifts of selected test compounds (**4** and **8**) and reference compounds.

REFERENCES

[1] H. EhringerO. Hornykiewicz, Parkinsonism Relat Disord. 1998, 4 (2), 53-57.

[2] M. G. Spillantini, M. L. Schmidt, V. M. Lee, J. Q. Trojanowski, R. JakesM. Goedert, Nature. 1997, 388 (6645), 839-840.

[3] W. R. GibbA. J. Lees, J. Neurol. Neurosurg. Psychiatry. 1988, 51 (6), 745-752.

[4] J. W. Langston, Ann Neurol. 2006, 59 (4), 591-596.

[5] K. R. Chaudhuri, L. YatesP. Martinez-Martin, Curr Neurol Neurosci Rep. 2005, 5 (4), 275-283.

[6] L. V. KaliaA. E. Lang, Lancet. 2015, 386 (9996), 896-912.

[7] D. B. Calne, N Engl J Med. 1993, 329 (14), 1021-1027.

[8] G. C. Cotzias, P. S. PapavasiliouR. Gellene, N Engl J Med. 1969, 280 (7), 337-345.

[9] S. Fahn, J Neurol. 1998, 245 (11 Suppl 3), P15-24.

[10] S. S. WuS. J. Frucht, CNS Drugs. 2005, 19 (9), 723-743.

[11] S. Ferre, K. Fuxe, G. von Euler, B. JohanssonB. B. Fredholm, Neuroscience. 1992, 51 (3), 501-512.

[12] A. C. Newby, Trends Biochem. Sci., 9 (2), 42-44.

[13] R. A. Cunha, Neurochem Int. 2001, 38 (2), 107-125.

[14] T. M. PalmerG. L. Stiles, Neuropharmacology. 1995, 34 (7), 683-694.

[15] M. SamselK. Dzierzbicka, Pharmacol Rep. 2011, 63 (3), 601-617.

[16] M. Samsel, K. DzierzbickaP. Trzonkowski, Postepy Hig Med Dosw (Online). 2013, 67, 1189-1203.

[17] J. H. Stehle, S. A. Rivkees, J. J. Lee, D. R. Weaver, J. D. DeedsS. M. Reppert, Mol Endocrinol. 1992, 6 (3), 384-393.

[18] P. J. Richardson, H. KaseP. G. Jenner, Trends Pharmacol Sci. 1997, 18 (9), 338-344.

[19] T. Mihara, K. Mihara, J. Yarimizu, Y. Mitani, R. Matsuda, H. Yamamoto, S. Aoki, A. Akahane, A. IwashitaN. Matsuoka, J. Pharmacol. Exp. Ther. . 2007, 323 (2), 708-719.

[20] S. Ferré, P. Popoli, L. Gimenez-Llort, R. Rimondini, C. Müller, I. Strömberg, S. O. ÖgrenK. Fuxe, Parkinsonism Relat. Disord. 2001, 7 (3), 235-241.

[21] M. A. Schwarzschild, L. Agnati, K. Fuxe, J. F. ChenM. Morelli, Trends in neurosciences. 2006, 29 (11), 647-654.

[22] J. F. Chen, K. Xu, J. P. Petzer, R. Staal, Y. H. Xu, M. Beilstein, P. K. Sonsalla, K. Castagnoli, N. Castagnoli, Jr.M. A. Schwarzschild, J Neurosci. 2001, 21 (10), Rc143.

[23] T. Kanda, M. J. Jackson, L. A. Smith, R. K. Pearce, J. Nakamura, H. Kase, Y. KuwanaP. Jenner, Exp Neurol. 2000, 162 (2), 321-327.

[24] B. C. ShookP. F. Jackson, ACS Chem. Neurosci. 2011, 2 (10), 555-567.

[25] K. A. Jacobson, C. Gallo-Rodriguez, N. Melman, B. Fischer, M. Maillard, A. van Bergen, P. J. van GalenY. Karton, J Med Chem. 1993, 36 (10), 1333-1342.

[26] K. A. Jacobson, S. Moro, J. A. Manthey, P. L. WestX. D. Ji, Adv Exp Med Biol. 2002, 505, 163-171.

[27] T. Nakayama, J Biosci Bioeng. 2002, 94 (6), 487-491.

[28] H. Shih, L. Deng, C. J. Carrera, S. Adachi, H. B. CottamD. A. Carson, Bioorganic & medicinal chemistry letters. 2000, 10 (5), 487-490.

[29] T. Al Nakib, V. Bezjak, M. J. MeeganR. Chandy, European journal of medicinal chemistry. 1990, 25 (5), 455-462.

[30] S. Arora, A. Pareek, N. AgrawalB. P. Nagori, International Journal of Research in Pharmacy and Chemistry. 2013, 3 (4), 797-802.

[31] M. M. Van der Walt, G. Terre'Blanche, Bioorg Med Chem. 2015, 23 (20), 6641-6649.

[32] M. S. Nel, A. Petzer, J. P. PetzerL. J. Legoabe, Bioorg. Med. Chem. Lett. 2016, 26 (19), 4599-4605.

[33] T. Mukaiyama, in *Organic Reactions* (John Wiley & Sons, Inc., 2004).

[34] M. Larsen, H. Kromann, A. KharazmiS. F. Nielsen, Bioorg Med Chem Lett. 2005, 15 (21), 4858-4861.

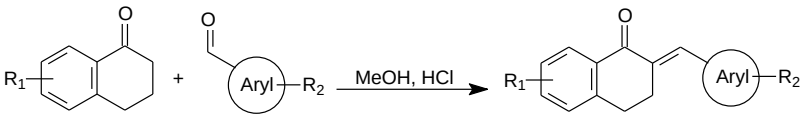
[35] B. Hallgas, Z. Dobos, E. Ősz, F. Hollósy, R. E. Schwab, E. Z. Szabó, D. Erős, M. Idei, G. KériT. Lóránd, Journal of Chromatography B. 2005, 819 (2), 283-291.

[36] R. Bruns, J. Fergus, E. Badger, J. Bristol, L. Santay, J. Hartman, S. HaysC. Huang, Naunyn-Schmiedeberg's Arch. Pharmacol. . 1987, 335 (1), 59-63.

[37] R. F. Bruns, G. H. LuT. A. Pugsley, Mol. Pharmacol. 1986, 29 (4), 331-346.

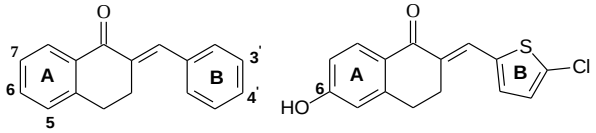
[38] H.-W. H. Van der Wenden EM, Roelen HCPF, Von Frijtag Drabbe Künzel JK, Pirovano IM, Mathôt RAA, Danhof M, Van Aerschot A, Lidaks MJ, IJzerman AP, Soudijn W., Eur J Pharmacol-Mol Pharmacol Sect. 1995, 290, 189-199.

[39] M. Gütschow, M. Schlenk, J. Gäb, M. Paskaleva, M. W. Alnouri, S. Scolari, J. IqbalC. E. Müller, J.Med. Chem. 2012, 55 (7), 3331-3341.



SCHEME 1 General synthetic route for preparation of target substituted (2*E*)-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one derivatives

TABLE 1 The dissociation constant values (K_i values) for the binding of the 2-benzylidene-1-tetralones to rat adenosine A_1 and A_{2A} receptors.



	Ring A			Ring B		K_i ± SEM (μM)[a]		SI[c]
Compd	7	6	5	3'	4'	A_1[b] vs [^{3}H]DPCPX	A_{2A}[b] vs [^{3}H]NECA	(A_{2A}/A_1)
Structural modifications of ring A								
1	-H	-H	-H	-H	-H	22.78 ± 1.28	> 100	-
2	-OH	-H	-H	-H	-H	11.75 ± 0.615	> 100	-
3	-H	-OH	-H	-H	-H	25.42 ± 0.94	> 100	-
4	-H	-H	-OH	-H	-H	5.93 ± 0.45	2.90 ± 0.66	0.5
5	-OCH_3	-H	-H	-H	-H	48.16 ± 5.18	> 100	-
6	-OCH_3	-OCH_3	-H	-H	-H	> 100	> 100	-
7	-H	-NH_2	-H	-H	-H	65.99 ± 4.85	> 100	-
Structural modifications of ring B								
8	-OH	-H	-H	-H	-OH	5.39 ± 0.29	6.60 ± 0.75	1
9	-H	-OH	-H	-H	-OH	16.15 ± 1.23	19.14 ± 7.00	1
10	-H	-OH	-H	- OCH_3	-H	28.59 ± 2.59	> 100	-
11	-OH	-H	-H	-Cl	-Cl	37.10 ± 3.60	> 100	-
12	-H	-OH	-H	-Cl	-Cl	39.29 ± 2.26	> 100	-
13	-H	-H	-OH	-Cl	-Cl	23.29 ± 0.74	> 100	-
14	-H	-OH	-H	-H	-F	24.85± 1.65	> 100	-
15	-H	-OH	-H	-H	-Cl	> 100	> 100	-
16	-H	-OH	-H	-H	-Br	34.57 ± 4.52	> 100	-
17	-H	-OH	-H	-CN	-H	>100	> 100	-
18	-H	-OH	-H	-H	-CN	>100	> 100	-
19	-H	-OH	-H	-NO_2	-H	>100	> 100	-
20	-H	-OH	-H	-H	-NO_2	>100	> 100	-
21	-H	-OH	-H	-H	-$N(CH_3)_2$	>100	> 100	-
22	-H	-OH	-H	-	-	42.87 ± 4.22	> 100	-
Reference compounds								
CPA (A_1 agonist)						0.010 ± 0.002 (0.015)[d]	-	-
ZM-241385 (A_{2A} antagonist)						-	0.002 ± 0.001 (0.002)[e]	-

[a] All K_i values determined in triplicate and expressed as mean ± SEM.

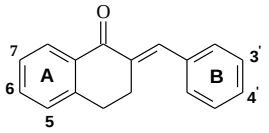
[b] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes).

[c] Selectivity index (SI) for the A_1 receptor isoform calculated as the ratio of K_i (A_{2A})/K_i (A_1).

[d] Literature value obtained from reference.[31]

[e] Literature value obtained from reference.[40]

TABLE 2 The adenosine A_1 affinities (in the absence and presence of GTP) and GTP shifts of selected test compounds (**4** and **8**) and reference compounds.



	Ring A			Ring B		K_i ± SEM[a]		
Compd	**7**	**6**	**5**	**3'**	**4'**	**A_1 vs [^{3}H]DPCPX**	**A_1 + GTP[b] vs [^{3}H]DPCPX**	**GTP Shift[c]**
4	-H	-H	-OH	-H	-H	5.93 ± 0.45 µM	6.92 ± 0.81 µM	1
8	-OH	-H	-H	-H	-OH	5.39 ± 0.29 µM	6.91 ± 1.19 µM	1
Reference compounds								
CPA (A_1 agonist)						10.9 ± 2.34 nM	62.17 ± 3.2 nM	6 (6.48)[d]
DPCPX (A_{2A} antagonist)						0.5 ± 0.10 nM	0.5 ± 0.05 nM	1 (1)[d]

[a] All K_i values determined in triplicate and expressed as mean ± SEM.
[b] GTP shift assay, where the 0.1 mM GTP was added to the A_1 AR radioligand binding assay.
[c] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP.
[d] Literature values obtained from reference.[31]

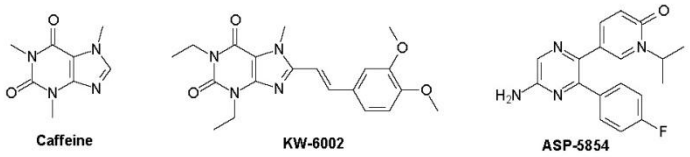
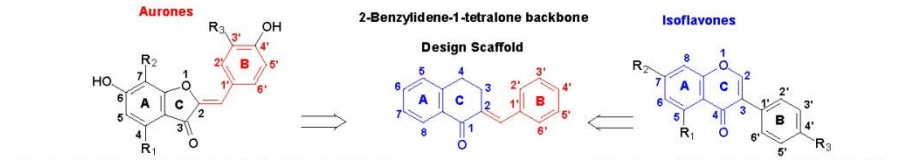


FIGURE 1 The structures of caffeine, KW-6002 and ASP-5854.



Sulfuretin: R_1=H; R_2=H; R_3=OH; $\mathbf{A_1}K_i$=4.44 μM; $\mathbf{A_{2A}}K_i$=28.1 μM

Aureusidin: R_1=OH; R_2=H; R_3=OH; $\mathbf{A_1}K_i$=13.2 μM; $\mathbf{A_{2A}}K_i$=>100 μM

Hispidol: R_1=H; R_2=H; R_3=OH; $\mathbf{A_1}K_i$=0.352 μM; $\mathbf{A_{2A}}K_i$=52.7 μM

Maritimetin: R_1=H; R_2=OH; R_3=OH; $\mathbf{A_1}K_i$=3.47 μM; $\mathbf{A_{2A}}K_i$=9.35 μM

Daidzein: R_1=H; R_2=OH; R_3=OH; $\mathbf{A_1}K_i$=23.4 μM

Genistein: R_1=OH; R_2=OH; R_3=OH; $\mathbf{A_1}K_i$=12.6 μM

Biochanin: R_1=OH; R_2=OH; R_3=OCH_3; $\mathbf{A_1}K_i$=34.5 μM

Prunetin: R_1=OH; R_2=OCH_3; R_3=OH; $\mathbf{A_1}K_i$=>100 μM

FIGURE 2 The structures of various aurones and isoflavones previously documented to possess affinity for the adenosine receptor and the general structure of the target 2-benzylidene-1-tetralone scaffold being explored in the current study.