2-Benzylidene-1-Indanone Analogues as Dual Adenosine A₁/A_{2A} Receptor Antagonists for the Potential Treatment of Neurological Conditions

Authors

Helena Dorathea Janse van Rensburg¹, Lesetja J. Legoabe², Gisella Terre'Blanche^{1, 2}, Mietha M. Van der Walt²

Affiliations

- 1 Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom, South Africa
- 2 Centre of Excellence for Pharmaceutical Sciences, School of Pharmacy, North-West University, Potchefstroom, South Africa

Key words

parkinson's disease, alzheimer's disease, 4-hydroxy-2-benzylidene-1-indanones, adenosine antagonists

received 11.10.2018

accepted 26.11.2018

Bibliography

DOI <https://doi.org/10.1055/a-0808-3993>

Published online: 7.1.2019

Drug Res 2019; 69: 382–391

© Georg Thieme Verlag KG Stuttgart · New York

ISSN 2194-9379

Correspondence

L. J. Legoabe

Centre of Excellence for Pharmaceutical Sciences

School of Pharmacy

North-West University

Private Bag X6001

2520 Potchefstroom

South Africa

Tel.: + 27/18/299 2182, Fax: + 27/18/299 4243

Lesetja.Legoabe@nwu.ac.za

ABSTRACT

Previous studies explored 2-benzylidene-1-tetralone derivatives as innovative adenosine A₁ and A_{2A} receptor antagonists for alternative non-dopaminergic treatment of Parkinson's disease. This study's aim is to investigate structurally related 2-benzylidene-1-indanones with substitutions on ring A and B as novel, potent and selective adenosine A₁ and A_{2A} receptor blockers. 2-Benzylidene-1-indanone derivatives were synthesised via acid catalysed aldol condensation reactions and evaluated via radioligand binding assays to ascertain structure activity relationships to govern A₁ and A_{2A} AR affinity. The results indicated that hydroxy substitution at C4 of ring A and meta (3'), or para (4') substitution on ring B of the 2-benzylidene-1-indanone scaffold (**2c**) is preferred over substitution at C5 (**2d**) or C6 (**2e**) of ring A for adenosine A₁ receptor activity and selectivity in the micromolar range. Furthermore, substitution at the meta (3') position of ring B with chlorine lead to the highly potent and selective adenosine A_{2A} receptor antagonist, compound **2h**. Compound **2c** and the **2q** behaved as adenosine A₁ receptor antagonists in the performed GTP shift assays. In view of these findings, compounds **2c**, **2h**, **2q** and **2p** are potent and selective adenosine A₁ and A_{2A} receptor antagonists for the potential treatment of neurological conditions.

Introduction

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

Adenosine receptor (AR) antagonists may be the solution to the PD-conundrum; as an epidemiological study has established an asso-

ciation between the consumption of coffee or caffeine and a reduced risk of developing PD. Caffeine is a xanthine derivative and non-selective A₁ and A_{2A} 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 A_{2A} AR antagonists; evidenced by a decrease in immobility time during the forced swim test and tail suspension test in rodents when KW-6002

(selective A_{2A} AR antagonist) was administered [8, 9]. A_{2A} AR antagonists are also relevant to motor control — which is affected in PD [1]. Firstly, these receptors are abundantly expressed in the striatum [5], a brain area associated with motor control [6]. Secondly, adenosine A_{2A} and dopamine D₂ receptors are co-expressed on the striatopallidal (inhibitory) pathway, inhibition of A_{2A} AR's compensate for hypolocomotion as a result of dwindling stimulation of striatopallidal dopamine 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 A_{2A} AR antagonist to L-dopa [10]. Blockade of both A₁ and A_{2A} AR's synergistically improve motor control by increasing presynaptic dopamine release via A₁ AR inhibition and postsynaptic dopamine response via A_{2A} AR inhibition [11]. Additionally, blockade of the A_{2A} AR may possibly facilitate neuroprotection in neurodegenerative disorders, like PD [12].

Recently, 2-benzylidene-1-tetralone analogues were explored as A₁ and/or A_{2A} AR antagonists [13, 14]. The 2-benzylidene-1-tetralone derivative (**1a**), possessed both A₁ and A_{2A} AR affinity (A₁K_i = 5.93 μM; and A_{2A}K_i = 2.90 μM) with a selectivity index of 2 towards the A_{2A} AR. Compound **1a** has a basic benzylidene tetralone backbone (fused 6- and 6-membered rings, namely ring A and ring C), where ring C bears a C2-phenyl substituted side-chain (ring B). It was found that C5-hydroxy substitution on ring A is optimal for A₁ and A_{2A} AR binding and that the 2-benzylidene side-chain, ring B, also governs AR binding. Similar to these bicyclic benzofused ring systems are the heterocyclic aurones — fused 6- and 5-membered rings — which also possess A₁ and/or A_{2A} AR affinity. Hispidol (**1b**) and maritimetin (**1c**) are examples of aurones with AR affinity [15].

In analogy to the structure of compound **1a** and the aurones (**1b-c**) (► **Fig. 1**), the present study investigates the structurally related 2-benzylidene-1-indanone scaffold as potent A₁ and A_{2A} AR antagonists.

The 2-benzylidene-1-indanone scaffold will be modified to include changes to ring A and ring B (► **Fig. 1**) and, subsequently, evaluated to ascertain which structure activity relationships govern A₁ and A_{2A} AR affinity.

Materials and Methods

Chemistry

Unless otherwise noted, all starting materials and solvents were procured from Sigma–Aldrich and used without further purification. Proton (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-*d*₆) as solvent. Chemical shifts are reported in parts per million (δ) in relation to the signal of 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 Büchi 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, 2 H), 2.59 (t, *J* = 5.7 Hz, 2 H); 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 C₉H₉O₂ (MH⁺): 149.0597, found: 149.0597. Purity (HPLC): 97.2 %.

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

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

(E)-2-benzylidene-4-hydroxy-2,3-dihydro-1H-inden-1-one (**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, 2 H), 7.52 (dd, *J* = 9.7, 5.1 Hz, 3 H), 7.46 (t, *J* = 7.3 Hz, 1 H), 7.31 (t, *J* = 7.6 Hz, 1 H), 7.25 (d, *J* = 7.4 Hz, 1 H), 7.10 (d, *J* = 7.7 Hz, 1 H), 3.94 (d, *J* = 1.0 Hz, 2 H); ¹³C NMR (151 MHz, DMSO) δ 193.60, 154.83, 138.87, 136.37, 135.11, 134.93, 132.80, 130.75, 129.81, 129.08, 129.04, 120.44, 114.17, 29.08. APCI–HRMS *m/z* calculated for C₁₆H₁₃O₂ (MH⁺): 237.0910, found: 237.0909. Purity (HPLC): 100 %.

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

The title (green powder) compound was prepared in a yield of 55 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and 3,4-dihydroxybenzaldehyde: 25.8–25.9 °C; ¹H NMR (600 MHz, DMSO) δ 10.10 (s, 1 H), 9.67 (s, 1 H), 9.33 (s, 1 H), 7.36 (s, 1 H), 7.33–7.24 (m, 2 H), 7.22 (d, *J* = 7.4 Hz, 1 H), 7.14–7.05 (m, 2 H), 6.86 (d, *J* = 8.2 Hz, 1 H), 3.83 (s, 2 H); ¹³C NMR (151 MHz, DMSO) δ 193.47, 154.75, 148.05, 145.65, 139.35, 136.03, 133.76, 131.34, 128.94, 126.46, 124.49, 119.98, 117.24, 116.06, 113.97, 29.17. APCI–HRMS *m/z* calculated for C₁₆H₁₃O₄ (MH⁺): 269.0808, found 269.0810. Purity (HPLC): 98.7 %.

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

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

(s, 1 H), 9.59 (s, 1 H), 9.22 (s, 1 H), 7.61 (d, $J=8.3$ Hz, 1 H), 7.25 (t, $J=1.7$ Hz, 1 H), 7.18 (d, $J=2.0$ Hz, 1 H), 7.07 (dd, $J=8.3, 2.0$ Hz, 1 H), 6.96 (d, $J=1.8$ Hz, 1 H), 6.87–6.82 (m, 2 H), 3.92 (s, 2 H); ^{13}C NMR (151 MHz, DMSO) δ 191.43, 163.55, 152.67, 147.58, 145.55, 132.17, 131.89, 129.64, 126.66, 125.53, 123.76, 117.33, 115.99, 111.92, 39.52, 31.90. APCI–HRMS m/z calculated for $\text{C}_{16}\text{H}_{13}\text{O}_4$ (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; ^1H NMR (600 MHz, DMSO) δ 9.81 (s, 1 H), 9.67 (s, 1 H), 9.27 (s, 1 H), 7.46 (d, $J=8.2$ Hz, 1 H), 7.33 (s, 1 H), 7.20 (d, $J=1.9$ Hz, 1 H), 7.14–7.04 (m, 3 H), 6.85 (d, $J=8.2$ Hz, 1 H), 3.89 (s, 2 H); ^{13}C NMR (151 MHz, DMSO) δ 193.20,

157.11, 147.97, 145.61, 140.43, 138.92, 133.40, 127.34, 126.50, 124.21, 122.92, 117.44, 116.05, 108.09, 39.52, 31.29. APCI–HRMS m/z calculated for $\text{C}_{16}\text{H}_{13}\text{O}_4$ (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); ^1H NMR (600 MHz, DMSO) δ 10.12 (d, $J=1.2$ Hz, 1 H), 7.62 (t, $J=10.2$ Hz, 2 H), 7.59–7.49 (m, 2 H), 7.34–7.23 (m, 3 H), 7.10 (dd, $J=7.7, 0.7$ Hz, 1 H), 3.95 (d, $J=1.4$ Hz, 2 H); ^{13}C NMR (151 MHz, DMSO) δ 193.55 (s), 163.16 (s), 161.54 (s), 154.86 (s), 138.69 (s), 137.34 (d, $J=8.0$ Hz), 136.44 (d, $J=10.0$ Hz), 131.40 (d, $J=2.4$ Hz), 130.95 (d, $J=8.4$ Hz), 129.17 (s), 126.95 (d, $J=2.5$ Hz), 120.63 (s), 116.83 (d, $J=21.9$ Hz), 116.54 (d,

► **Fig. 1** Structural and heterocyclic ring changes to compound 1a, hispidol and maritimetin to determine features essential for dual A_1/A_{2A} antagonistic activity.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The title compound (green powder) was prepared in a yield of 71 % from 4-hydroxy-2,3-dihydro-1H-inden-1-one and picolinaldehyde: mp 30.8–30.9 °C (H_2O); 1H 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 $\text{C}_{15}\text{H}_{12}\text{NO}_2$ (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); ^{13}C NMR (151 MHz, DMSO) δ 193.16, 154.94, 146.01, 144.27, 142.73, 139.56, 138.33, 136.52, 133.15, 129.30, 127.14, 126.22, 120.94, 114.34, 28.76. APCI–HRMS m/z calculated for $\text{C}_{15}\text{H}_{12}\text{NO}_2$ (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); ^{13}C NMR (151 MHz, DMSO) δ 193.20, 154.91, 146.02, 138.50, 138.20, 136.59, 135.16, 129.38, 128.42, 125.79, 121.10, 118.17,

114.41, 28.92. APCI–HRMS m/z calculated for $\text{C}_{15}\text{H}_{12}\text{NO}_2$ (MH^+): 238.0863, found: 238.0879. Purity (HPLC): 100 %.

Biology

All commercially available reagents were obtained from various manufacturers: radioligands $[^3\text{H}]\text{NECA}$ (specific activity 27.1 Ci/mmol) procured from PerkinElmer and $[^3\text{H}]\text{DPCPX}$ (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 $\text{A}_{2\text{A}}$ 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 $\text{A}_{2\text{A}}$ AR's) were prepared according to the protocol described in literature [16, 17].

The competition experiments were carried out in the presence of the radioligands $[^3\text{H}]\text{-8-cyclopentyl-1,3-dipropylxanthine}$ ($[^3\text{H}]\text{DPCPX}$; 0.1 nM; $K_d = 0.36$ nM) and 5'-N- $[^3\text{H}]\text{-ethylcarboxamideadenosine}$ ($[^3\text{H}]\text{NECA}$; 4 nM; $K_d = 15.3$ nM) for the A_1 and $\text{A}_{2\text{A}}$ AR radioligand binding assays, respectively [16, 17]. In addition, the $\text{A}_{2\text{A}}$ AR binding studies were determined in the presence of N⁶-cyclopentyladenosine (CPA) to minimize the binding of $[^3\text{H}]\text{NECA}$ to A_1 AR's. Non-specific binding was defined by the addition of a final concentration of 100 μM CPA. The sigmoidal-dose response curves, via Graphpad Software Inc. package, were obtained by plotting the specific binding vs. the logarithm of the test compound's concentrations. Subsequently, the K_i values were obtained by using the IC_{50} values that were determined from sigmoidal-dose response curves. All incubations were carried out in triplicate and

► **Fig. 2** Synthesis of 2a, starting material for 2d and 2b–q. Reagents and conditions: **a**) 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).

Chemical structures of compounds 1a, 2a, 2b-m, 2p, 2q, 2n, and 2o are shown. Each structure consists of a fluorene-9-one core (rings A and C) with a substituent B at the 2-position. The numbering for rings A and C is 1, 2, 3, 4, 5, 6. The substituent B is numbered 1', 2', 3', 4'.

- 1a: 2-(4-phenyl)-9-fluorenone
- 2a: 2-(2-phenyl)-9-fluorenone
- 2b-m: 2-(4-phenyl)-9-fluorenone
- 2p: 2-(2-pyridyl)-9-fluorenone
- 2q: 2-(4-pyridyl)-9-fluorenone
- 2n: 2-(2-amino-5-pyridyl)-9-fluorenone
- 2o: 2-(2-pyridyl)-9-fluorenone

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

► Table 1 Continued.

	OH	H	H	H	H	CN	> 100 (49%) ^b	> 100 (66%) ^b	-	-	-
2m											-
2n	OH	H	H	H	H	-	> 100 (57%) ^b	> 100 (77%) ^b	-	-	-
2o	OH	H	H	H	H	-	4.71 ± 0.58 ^a	1.79 ± 0.13 ^a	2.6 ^d	-	-
2p	OH	H	H	H	H	-	6.58 ± 0.68 ^a	1.61 ± 0.17 ^a	4.1 ^d	-	-
2q	OH	H	H	H	H	-	1.69 ± 0.13 ^a	3.37 ± 0.90 ^a	0.5 ^d	1.83 ± 0.09 ^e	1 ^f
Reference compounds											
	CPA (A₁-agonist)						0.0068 ± 0.0001 ^a (0.0079) ^b ; (0.015) ⁱ	0.163 ± 0.001 ^a (0.331) ^j	24 ^d (22) ^j	0.099 ± 0.015 ^a (0.099) ^j	15 ^f (14) ^j
	DPCPX (A₁-antagonist)						0.0004 ± 0.0002 ^a (0.0005) ^j ; (0.0003) ^j	0.545 ± 0.204 ^a (0.530) ^j ; (0.340) ^j	1363 ^d (958) ^j ; (1130) ^j	0.0004 ± 0.0002 ^a (0.0004) ^j	1.0 ^f

^aAll K_i values determined in triplicate and expressed as mean ± SEM; ^bPercentage displacement of the radioligand at a maximum tested concentration (100 μM); ^cRat receptors were used (A₁: rat whole brain membranes; A_{2A}: rat striatal membranes); ^dSelectivity index (SI) for the A_{2A} receptor isoform calculated as the ratio of K_i (A₁)/K_i (A_{2A}); ^eGTP shift assay, where the 100 μM GTP was added to the A₁ AR radioligand binding assay; ^fGTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP; ^gLiterature value obtained from reference [13]; ^hLiterature value obtained from reference [19]; ⁱLiterature value obtained from reference [16]; ^jLiterature value obtained from reference [20].

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

GTP shift assays

In addition, compounds **2c** and **2q** were explored via a GTP shift assay to determine the agonistic or antagonistic functionality of the investigated 2-benzylidene-1-indanones towards the A₁ AR. The GTP shift assay was performed as described previously with rat whole brain membranes and [³H]DPCPX (0.1 nM; K_d = 0.36 nM) in the absence and presence of a final concentration of 100 μM GTP [16, 18]. Non-specific binding was defined by the addition of 10 μM DPCPX (unlabelled). If a calculated GTP shift of approximately 1 is obtained, that compound is considered to function as an antagonist. On the other hand, the presence of GTP affects the competition curve of an agonist and shifts the curve to the right, as previously demonstrated by the A₁ AR agonist CPA [16, 18]. The sigmoidal-dose response curves were obtained via the Graphpad Software Inc. package and the K_i values determined as described above. The GTP shift was calculated by dividing the K_i value of a compound reported in the presence of GTP by the K_i value obtained in the absence of GTP [16, 18].

Results and Discussion

Chemistry

(► **Fig. 2**) The key starting material 4-hydroxy-2,3-dihydro-1H-inden-1-one (**2a**) was synthesised in a good yield by a rearrangement reaction. The crude product was used without further purification to synthesise compounds **2b–c** and **2f–q**. The starting material for compound **2d**, was synthesised via demethylation of 5-methoxy-2,3-dihydro-1H-inden-1-one using AlCl₃ to obtain 5-hydroxy-2,3-dihydro-1H-inden-1-one. Conversely, starting material for compound **2e**, namely 6-hydroxy-2,3-dihydro-1H-inden-1-one was commercially available and procured from Sigma–Aldrich. The target 2-benzylidene-1-indanones were synthesised through an acid catalysed aldol condensation reaction. The novel compounds **2b–q** were purified by recrystallization from a suitable solvent (yields of 10–85 %) and, in each instance, the structures and purity of these compounds were verified by ¹H-NMR, ¹³C-NMR, mass spectrometry and HPLC analysis.

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

Biology

The degree and type of binding affinity of the 2-benzylidene-1-indanone analogues (**2a–q**) at rat A₁ and A_{2A} AR's were determined by radioligand binding assays and GTP shift assays, respectively, and results are summarized in ► **Table 1**. Two reference compounds, namely CPA and DPCPX, were included in the study and the results are in accordance with literature values.

Previous studies identified the 5-hydroxy substituted 2-benzylidene-1-tetralone derivative, **1a**, as a lead compound to design novel and potent A₁ and A_{2A} AR antagonists [13]. The 4-hydroxy

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

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

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

Structural modifications to ring A

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

Structural modifications to ring B

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

In correlation to previous studies, A_1 and A_{2A} AR binding affinity favour OH-group substitution on meta (3') and para (4') positions

of ring B. For example, compound **2c** possessed a 1.7 fold increase in A_{2A} AR affinity compared to its unsubstituted counterpart **2b** ($A_1K_i > 100 \mu\text{M}$ and $A_{2A}K_i = 1.55 \mu\text{M}$).

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

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

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

Structural modifications to ring C

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

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

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

Conclusions

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

Acknowledgements

Financial support for this work was provided by the North-West University (NWU), the National Research Foundation (96135) and the Medical Research Council, South Africa. We are grateful to Dr. J. Jordaan of Chemical Research Beneficiation, NWU for NMR and MS analysis, as well as Prof. J. Du Preez of Pharmaceutics, School of Pharmacy, NWU for HPLC analysis.

Conflict of interest

The authors have no conflict of interest to declare.

References

- [1] Schwarzschild MA, Agnati L, Fuxe K et al. Targeting adenosine A_{2A} receptors in parkinson's disease. *Trends Neurosci* 2006; 29: 647–654
- [2] Kalia LV, Lang AE. Parkinson's disease. *Lancet* 2015; 386: 896–912
- [3] Ross GW, Abbott RD, Petrovitch H et al. Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA* 2000; 283: 2674–2679
- [4] Mihara T, Mihara K, Yarimizu J et al. Pharmacological characterization of a novel, potent adenosine A_1 and A_{2A} receptor dual antagonist, 5-[5-Amino-3-(4-fluorophenyl)pyrazin-2-yl]-1-isopropylpyridine-2(1H)-one (ASP5854), in models of Parkinson's disease and cognition. *J Pharm Exp Ther* 2007; 323: 708–719
- [5] Stehle JH, Rivkees SA, Lee JJ et al. Molecular cloning and expression of the cDNA for a novel A_2 -adenosine receptor subtype. *Mol. Endocrinol* 1992; 6: 384–393
- [6] Fredholm BB, Chen JF, Cunha RA et al. Adenosine and brain function. *Int Rev Neurobiol* 2005; 63: 191–270
- [7] Stockwell J, Jakova E, Cayabyab FS. Adenosine A_1 and A_{2A} receptors in the brain: current research and their role in neurodegeneration. *Molecules* 2017; 22: 676–694
- [8] El Yacoubi M, Ledent C, Parmentier M et al. Adenosine A_{2A} receptor antagonists are potential antidepressants: Evidence based on pharmacology and A_{2A} receptor knockout mice. *Br J Pharmacol* 2001; 134: 68–77
- [9] Yamada K, Kobayashi M, Mori A et al. Antidepressant-like activity of the adenosine A_{2A} receptor antagonist, istradefylline (KW-6002), in the forced swim test and the tail suspension test in rodents. *Pharmacol Biochem Behav* 2013; 114–115 23–30
- [10] Kanda T, Jackson MJ, Smith LA et al. Combined use of the adenosine A_{2A} antagonist KW-6002 with L-DOPA or with selective D_1 or D_2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. *Exp Neurol* 2000; 162: 321–327
- [11] Shook BC, Jackson PF. Adenosine A_{2A} receptor antagonists and Parkinson's disease. *ACS Chem Neurosci* 2011; 2: 555–567
- [12] Chen JF, Xu K, Petzer JP et al. Neuroprotection by caffeine and A_{2A} adenosine receptor inactivation in a model of Parkinson's disease. *J neurosci.* 2001; 21: RC143–RC143
- [13] Legoabe LJ, Van der Walt MM, Terre'Blanche G. Evaluation of 2-benzylidene-1-tetralone derivatives as antagonists of A_1 and A_{2A} adenosine receptors of hydroxy-substituted 2-benzylidene-1-tetralones as antagonist of A_1 and A_{2A} adenosine receptors. *Chem Biol Drug Des* 2018; 91: 234–244
- [14] Janse van Rensburg HD, Terre'Blanche G, Van der Walt MM et al. 5-Substituted 2-benzylidene-1-tetralone analogues as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions. *Bioorg Chem.* 2017; 74: 251–259
- [15] Jacobson KA, Moro S, Manthey JA et al. Interactions of flavones and other phytochemicals with adenosine receptors. *Adv Exp Med Biol* 2002; 505: 163–171
- [16] Van der Walt MM, Terre'Blanche G. 1,3,7-Triethyl-substituted xanthines – possess nanomolar affinity for the adenosine A_1 receptor. *Bioorg Med Chem* 2015; 23: 6641–6649
- [17] Van der Walt MM, Terre'Blanche G. Selected C8 two chain linkers enhance the adenosine A_1/A_{2A} receptor affinity and selectivity of caffeine. *Eur J Med Chem* 2016; 125: 652–656

- [18] Gütschow M, Schlenk M, Gäh J et al. Benzothiazinones: A novel class of adenosine receptor antagonists structurally unrelated to xanthine and adenine derivatives. *J Med Chem* 2012; 55: 3331–3341
- [19] Bruns RF, Fergus JH, Badger EW et al. Binding of the A₁-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Arch Pharmacol* 1987; 335: 59–63
- [20] Lohse MJ, Klotz KN, Lindenborn-Fotinos J et al. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) – a selective high affinity antagonist radioligand for A₁ adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1987; 336: 204–210