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Methoxy substituted 2-benzylidene-1-indanone derivatives as A_1 and/or A_{2A} AR antagonists for the potential treatment of neurological conditions†

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A prior study reported on hydroxy substituted 2-benzylidene-1-indanone derivatives as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions. A lead compound (**1a**) was identified with both A_1 and A_{2A} affinity in the micromolar range. The current study explored the structurally related methoxy substituted 2-benzylidene-1-indanone derivatives with various substitutions on ring A and B of the benzylidene indanone scaffold in order to enhance A_1 and A_{2A} affinity. This led to compounds with both A_1 and A_{2A} affinity in the nanomolar range, namely **2c** (A_1K_i (rat) = 41 nM; $A_{2A}K_i$ (rat) = 97 nM) with C4-OCH_3 substitution on ring A together with *meta* (3′) hydroxy substitution on ring B and **2e** (A_1K_i (rat) = 42 nM; $A_{2A}K_i$ (rat) = 78 nM) with C4-OCH_3 substitution on ring A together with *meta* (3′) and *para* (4′) dihydroxy substitution on ring B. Additionally, **2c** is an A_1 antagonist. Consequently, the methoxy substituted 2-benzylidene-1-indanone scaffold is highly promising for the design of novel A_1 and A_{2A} antagonists.

Introduction

The adenosine system – specifically adenosine A_1 and A_{2A} receptors – is a promising drug target for the non-dopaminergic treatment of the neurodegenerative disorder Parkinson's disease (PD).[1] These adenosine receptors (AR's), together with A_{2B} and A_3 AR's, are either inhibitory (A_1 and A_3) or stimulatory (A_{2A} and A_{2B}) G-protein coupled receptors.[2]

The spotlight first fell on the A_1 and A_{2A} AR's when an epidemiological study found that consumption of the non-selective AR antagonist caffeine – present in coffee and tea – is associated with a reduced risk of developing PD.[3] Since then, numerous preclinical studies in rodents and non-human primate models of PD have supported the potential of A_1 and A_{2A} AR antagonists for the treatment of PD.[4]

The structure of the xanthine derivative caffeine is similar to that of adenosine.[5] Caffeine and other A_1 and A_{2A} AR antagonists exert effects contrary to endogenous adenosine and, in so doing, affects various neurotransmitters, receptors and signalling pathways.[4,6]

There is growing evidence that selective A_{2A} AR antagonists, like KW-6002 (istradefylline), may be novel non-dopaminergic treatment for PD; KW-6002 is approved in Japan since 2013 for the treatment of wearing-off phenomenon associated with L-dopa treatment in PD.[7,8] Selective A_{2A} AR antagonists may possibly alleviate parkinsonian motor symptoms due to the close anatomical and functional relationship between A_{2A} AR's and dopamine D_2 receptors on the indirect striatopallidal GABAergic pathway.[7,8] In animal studies, agonists and antagonists of A_{2A} AR's produce behavioural effects similar to antagonists and agonists of dopamine D_2 receptors, respectively.[9] Thus, blockade of A_{2A} AR's on the indirect striatopallidal GABAergic pathway reduces postsynaptic effects of dopamine depletion (a pathological hallmark of PD) and, subsequently, reduces motor symptoms associated with PD.[10] Additionally, KW-6002 may address a non-motor symptom of PD, namely depression, evidenced by a decrease in immobility time during the forced swim test and tail suspension test in rodents (animal models of depression) when the said drug was administered.[11,12] Neurodegeneration may be stopped or, at least, slowed by A_{2A} AR antagonists as KW-6002 attenuated striatal dopamine depletion in the MPTP animal model of PD.[13]

Synaptic plasticity; the ability of synapses to strengthen or weaken in response to increases or decreases in their activity, is the basis for learning and memory.[14] Brain areas associated with cognition are the prefrontal cortex and hippocampus.[14] Endogenous adenosine *via* A_1 AR's—which are abundantly expressed in the prefrontal cortex and hippocampus—

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modulates synaptic plasticity phenomena long-term depression and long-term potentiation and, in so doing, inhibits learning and memory.[15] A_1 AR antagonists block A_1 AR mediated inhibitory modulation of synaptic plasticity in the prefrontal cortex and hippocampus: neurotransmitter release and synaptic transmission is increased, facilitating synaptic plasticity and, thus, learning and memory.[14] Pharmacological studies support the above, by means of the selective A_1 AR antagonists BIIP20 and FR194921 which are active in animal models of cognitive deficits.[16,17]

It proved to date challenging to translate findings on AR function into clinical studies and no other AR antagonists have been approved.[18]

The investigation of dual A_1/A_{2A} AR antagonists for the potential treatment of PD has been investigated in the past.[19–23] For example, 5-[5-amino-3-(4-fluorophenyl)pyrazin-2-yl]-1-isopropylpyridine-2(1*H*)-one (ASP5854) (see Fig. 1) showed promise in animal models of PD as well as cognition and has K_i values of 9.03 nM at the human A_1 AR and 1.76 at the human A_{2A} AR.[19] Additionally, 2-amino-8-[2-(4-morpholinyl)-ethoxy]-4-phenyl-5H-indeno-[1,2-d]pyrimidin-5-one (JNJ-40255293) (see Fig. 1) is also a dual A_1/A_{2A} AR antagonist with efficacy in animal models of PD (A_1K_i (human) = 48 nM; A_2AK_i (human) = 6.5 nM).[20,21] Of note, this compound is structurally related to the benzylidene indanones currently under investigation. 8-Substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinedione derivatives were evaluated as dual A_1/A_{2A} AR antagonist in a multitarget approach for the treatment of neurodegenerative disorders.[22] The 7-aminopyrazolo[4,3-*d*]pyrimidine derivatives were also explored as dual A_1/A_{2A} AR antagonists with several of these compounds possessing nanomolar affinity for the human A_{2A} AR and slightly lower human A_1 AR.[23]

Therefore, dual A_1 and A_{2A} AR antagonists may be non-dopaminergic drugs for the symptomatic treatment of both PD motor symptoms (for example bradykinesia, rigidity, resting tremor and postural instability) and PD non-motor symptoms (for example cognitive deficits such as cognitive dysfunction and depression) as well as exhibit neuroprotective properties.[4]

Benzopyrones are a class of compounds with significantly diverse biological activities[24] (including antiparkinsonian and neuroprotective properties)[25–30] and are, thus, considered a privileged scaffold in medicinal chemistry.[24] Benzopyrones constitute the basic framework of flavonoids,[31,32] and the structurally related isocoumarins[33] and coumarins.[24] It is reported that the flavonoid derivative 5,3′-dihydroxyflavone possess an A_1K_i (rat) value of 0.956 μM and an $A_{2A}K_i$ (rat) value of 1.44 μM (see Fig. 2).[34] In fact, the aurone derivatives (specifically hispidol (A_1K_i (rat) = 0.352 μM) and maritimetin (A_1K_i (rat) = 3.47 μM and A_{2A} K_i (rat) = 9.35 μM) (see Fig. 2)), which are members of the flavonoid family,[28] served as inspiration for previous work on the benzylidene tetralones[35,36] as well as benzylidene indanones[37] – leading to compounds with A_1 and A_{2A} AR affinity in the micromolar range. For example, the benzylidene tetralone derivative (*E*)-5-hydroxy-2-(3-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one has an A_1K_i (rat) value of 1.62 μM and an $A_{2A}K_i$ (rat) value of 5.46 μM (see Fig. 2).[36]

In recent times, hydroxy substituted 2-benzylidene-1-indanone analogues were explored as A_1 and/or A_{2A} AR antagonists for the potential treatment of neurological conditions.[37] Of note, is compound **1a** with K_i values for both the A_1 and A_{2A} AR below 1 μM (A_1K_i (rat) = 0.435 μM; $A_{2A}K_i$ (rat) = 0.903 μM). It was found that C4 hydroxy substitution on ring A of the benzylidene indanones in combination with *meta* (C3′) and *para* (C4′) dihydroxy substitution on ring B is preferable for both A_1 and A_{2A} AR binding.

These compounds comprise of a benzylidene indanone scaffold (*i.e.* fused 6- and 5-membered rings, namely ring A and ring C), where ring C bears a C2-phenyl substituted sidechain (namely ring B). Theoretically, these compounds may be either *E*- or *Z*-isomers.[38] The *E*-configuration is favourable for thermodynamic reasons because of steric interaction between the aryl and carbonyl groups in case of the *Z*-isomers.[38,39]

Based on the structure of **1a**, we investigated the structurally related methoxy substituted 2-benzylidene-1-indanone derivatives as potent A_1 and A_{2A} AR antagonists.

The 2-benzylidene-1-indanone scaffold was modified to include substituent changes to ring A and ring B (see Fig. 2). Firstly, C4-, C5- and/or C6-methoxy substitution on ring A was made, in order to determine whether hydroxy or methoxy substitution is preferable for A_1 and A_{2A} AR affinity and, secondly, the optimal position of the methoxy group for both A_1 and A_{2A} AR affinity was determined. Also, an unsubstituted ring A and methylenedioxy substitution on ring A was incorporated. Substitution at the *ortho* (C2′), *meta* (C3′), *para* (C4′) and/or C5 position(s) of phenyl ring B with hydroxy-, methoxy- or dimethylamino group(s) was investigated.

Accordingly, the 2-benzylidene-1-indanones was evaluated to ascertain which structure activity relationships govern A_1 and A_{2A} AR affinity.

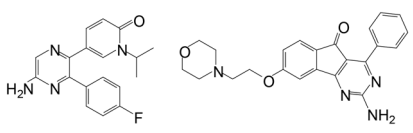


Fig. 1 The structures of ASP-5854 and JNJ-40255293.

Results and discussion

Chemistry

Reagents and test compounds were synthesised as depicted in Scheme 1. The key starting material for **2b–g**, namely **2a** was synthesised by methylation of 4-hydroxy-1-indanone, as described,[40] whereas, reagents for **2j**, **2l** and **2m** were commercially available. In turn, test compounds (**2b–g**, **2i**, **2k** & **2m**) were prepared *via* an acid catalysed aldol condensation

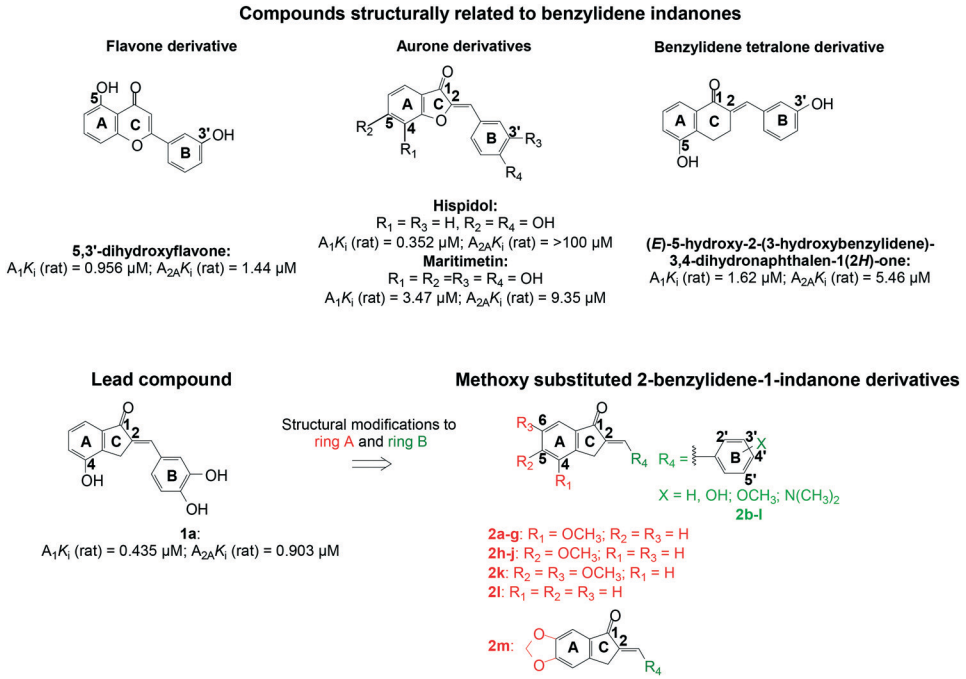


Fig. 2 The structures and K_i values of compounds structurally related to benzylidene indanones and the structural modifications to lead compound **1a** to determine features essential for dual A_1/A_{2A} AR affinity.

reaction.[35–37] Test compounds **2h**, **2j** and **2l** were available from a previous study.[41] The novel compounds **2b–g**, **2i**, **2k** and **2m** (fair yields) were purified by recrystallization from a suitable solvent (either MeOH or EtOH) and, in each instance, the structure, molecular mass and purity of these compounds were verified by ^{1}H NMR, ^{13}C NMR, MS and/or HPLC analysis.

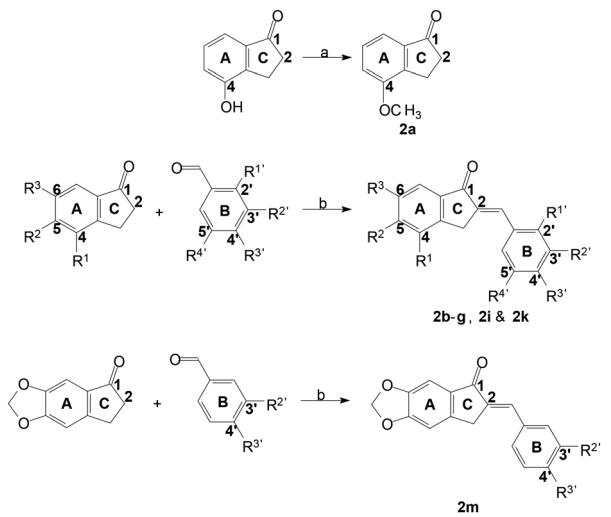
Biology

Radioligand binding assays. The affinities of the 2-benzylidene-1-indanone analogues (**2a–m**) at rat A_1 and A_{2A} AR subtypes were determined with radioligand competition experiments as described previously.[42,43] The A_1 and A_{2A} AR radioligand binding assay 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 (see Table 1).

A prior study identified the 4-hydroxy substituted 2-benzylidene-1-indanone analogue **1a** as a lead compound to design novel and potent A_1 and A_{2A} AR antagonists.[37] This study's parent scaffold is the 4-methoxy substituted compound **2a**, which is unsubstituted at position 2 and lacks both A_1 and A_{2A} AR affinity.

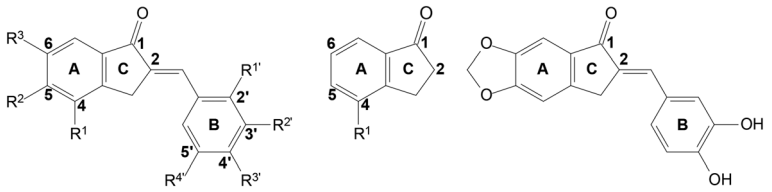
Structural modification to ring A. Firstly, in analogy to previous work on the 2-benzylidene-1-tetralones (which determined whether OH- or OCH_3-group substitution on ring A was preferable to attain both A_1 and A_{2A} AR affinity),[36] the current study investigated C4-OH substitution *versus* C4-OCH_3 substitution on ring A, as well as an unsubstituted ring A and 1,3-dioxolane substitution on ring A by comparing the K_i values of analogous compounds. Secondly, similar to a previous study of the 2-benzylidene-1-indanones – which determined optimal OH-substitution on ring A together with *meta* (3′) and *para* (4′) diOH-substitution on ring B[37] – the impact of OCH_3-substitution at either position C4, C5 and/or C6 of ring A, in combination with various ring B substitutions were evaluated by comparing the K_i values of analogous compounds.

C4-OH substitution vs. C4-OCH_3 substitution. Comparison of lead compound **1a** (A_1K_i (rat) = 0.435 μM; $A_{2A}K_i$ (rat) = 0.903 μM)[37] to its methoxy substituted counterpart **2e** (A_1K_i (rat) = 0.042 μM; $A_{2A}K_i$ (rat) = 0.078 μM) showed that in the



Scheme 1 Synthesis of **2a–g**, **2i**, **2k** & **2m**. Reagents and conditions: a) acetone, K_2CO_3, MeI, 50 °C (18 h); b) MeOH, HCl (32%), 120 °C (24 h).

Table 1 The dissociation constant (K_i) values for the binding of the 2-benyzlidene-1-indanone analogues at rat A_1 and A_{2A} AR's



#	Ring A			Ring B				K_i ± SEM (μM)[a] (% displacement)[b]		SI[d]
	R^1	R^2	R^3	$R^{1'}$	$R^{2'}$	$R^{3'}$	$R^{4'}$	A_1[c] *vs.* [^{3}H]DPCPX	A_{2A}[c] *vs.* [^{3}H]NECA	(A_1/A_{2A})
Lead compound										
1a	OH	H	H	H	OH	OH	H	0.435 ± 0.050[a,e]	0.903 ± 0.081[a,e]	0.5[d,e]
Methoxy substituted 2-benzylidene-1-indanones										
2a	OCH_3	H	H	—	—	—	—	>100 (78%)[b]	>100 (72%)[b]	—
2b	OCH_3	H	H	H	H	H	H	1.65 ± 0.15[a]	>100 (62%)[b]	—
2c	OCH_3	H	H	H	OH	H	H	0.041 ± 0.002[a]	0.097 ± 0.009[a]	0.4[d]
2d	OCH_3	H	H	H	H	OH	H	>100 (31%)[b]	>100 (65%)[b]	—
2e	OCH_3	H	H	H	OH	OH	H	0.042 ± 0.009[a]	0.078 ± 0.002[a]	0.5[d]
2f	OCH_3	H	H	OCH_3	H	OCH_3	OCH_3	>100 (46%)[b]	>100 (29%)[b]	—
2g	OCH_3	H	H	H	H	$N(CH_3)_2$	H	>100 (52%)[b]	>100 (23%)[b]	—
2h	H	OCH_3	H	H	H	H	H	>100 (42%)[b]	>100 (82%)[b]	—
2i	H	OCH_3	H	H	OH	OH	H	3.28 ± 0.31[a]	6.32 ± 0.32[a]	0.5[d]
2j	H	OCH_3	H	H	H	$N(CH_3)_2$	H	>100 (64%)[b]	>100 (47%)[b]	—
2k	H	OCH_3	OCH_3	H	OH	OH	H	4.29 ± 0.18[a]	18.02 ± 1.39[a]	0.2[d]
2l	H	H	H	H	H	$N(CH_3)_2$	H	>100 (51%)[b]	>100 (65%)[b]	—
2m	—	—	—	—	—	—	—	>100 (70%)[b]	1.07 ± 0.10[a]	—
Reference compounds										
CPA (A_1 agonist)								0.0068 ± 0.0001[a] (0.0079);[f] (0.015)[g]	0.163 ± 0.001[a] (0.331)[g]	24[d] (22)[g]
DPCPX (A_1 antagonist)								0.0004 ± 0.0002[a] (0.0005);[g] (0.0003)[h]	0.545 ± 0.204[a] (0.530);[g] (0.340)[h]	1363[d] (958)[g] (1130)[h]

[a] All K_i values determined in triplicate and expressed as mean ± SEM. [b] Percentage displacement of the radioligand at a maximum tested concentration (100 μM). [c] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes). [d] Selectivity index (SI) for the A_{2A} receptor isoform calculated as the ratio of $A_1K_i/A_{2A}K_i$. [e] Literature value obtained from reference.[37] [f] Literature value obtained from reference.[49] [g] Literature value obtained from reference.[42] [h] Literature value obtained from reference.[50]

2-benzylidene-1-indanone scaffold C4-OCH_3 substitution is favoured over C4-OH substitution on ring A; where a tenfold increase in A_1 AR affinity and a twelvefold increase in A_{2A} AR affinity were observed.

Interestingly, the structurally related 2-benzylidene-1-tetralone analogues generally prefer OH-substitution over OCH_3-substitution on ring A.[35,36]

An unsubstituted ring A in combination with a dimethylamino group in the *para* (4′) position (**2g**) diminished both A_1 and A_{2A} AR affinity as seen with comparison of **2g** to unsubstituted **2l**.

Methylenedioxy substitution on ring A (**2m**) reduced both A_1 AR affinity (A_1K_i (rat) = >100 μM) and A_{2A} AR affinity ($A_{2A}K_i$ (rat) = >1.07 μM) when compared to **2e**, however the said compound retained relatively good A_{2A} AR affinity.

Optimal position of OCH_3-group. Similar to the hydroxy substituted 2-benzylidene-1-indanone analogues, the position of the OCH_3-group on ring A modulates A_1 and A_{2A} AR binding affinity and C4-OCH_3 substitution on ring A is preferred over either C5-OCH_3 substitution or C5- and C6-diOCH_3 substitution, as evidenced when C4-OCH_3 substitution on ring A (**2e**; A_1K_i (rat) = 0.042 μM and $A_{2A}K_i$ (rat) = 0.078 μM) is compared to C5-OCH_3 substitution (**2i**; A_1K_i (rat) = 3.28 μM and $A_{2A}K_i$ (rat) = 6.32 μM) and C5 and C6-diOCH_3 substitution (**2k** A_1K_i (rat) = 4.29 μM and $A_{2A}K_i$ (rat) = 18.02 μM).

Comparison of 4-methoxy substituted **2b** (A_1K_i (rat) = 1.65 μM; $A_{2A}K_i$ (rat) = >100 μM) to 5-methoxy substituted **2h** (A_1K_i & $A_{2A}K_i$ (rat) = >100 μM), showed that C4-OCH_3 substitution on ring A, instead of C5-OCH_3 substitution, lead to increased A_1 AR affinity – yet, A_{2A} AR affinity was unaffected.

Additionally, both the A_1 and A_{2A} AR's favour C4-OCH_3 substitution on ring A to either C5-OCH_3 substitution or C5 and C6-diOCH_3 substitution when 4-methoxy substituted **2e** (A_1K_i (rat) = 0.042 μM; $A_{2A}K_i$ (rat) = 0.078 μM) was compared to 5-methoxy substituted **2i** (A_1K_i (rat) = 3.28 μM; $A_{2A}K_i$ (rat) = 6.32 μM) and 5,6-dimethoxy substituted **2k** (A_1K_i (rat) = 4.29 μM; $A_{2A}K_i$ (rat) = 18.02 μM); a dramatic increase in both A_1 and A_{2A} AR affinity was observed – seeing as **2e** possess A_1 and A_{2A} AR K_i values in the nanomolar range. In fact, the A_1 AR affinity of **2e** is 78 times better than that of **2i** and 102 times better than that of **2k**, whereas as the A_{2A} AR affinity of

2e is 81 times better than that of **2i** and 231 times better than that of **2k**. However, comparison of 4-methoxy substituted **2g**, 5-methoxy substituted **2j** and unsubstituted **2l** – all with K_i values >100 μM – such a trend could not be observed. All in all, it may be said that 4-methoxy substitution on ring A is preferred over either 5-methoxy substitution, 5,6-dimethoxy substitution or no substitution on ring A.

Additionally, when compound **2m** – containing a methylenedioxy on ring A – was compared to compounds **2e**, **2i** and **2k**, all with *meta* (3′) and *para* (4′) diOH-substitution on ring B, it was seen that A_1 AR affinity (>100 μM) was diminished, yet the compound retained relatively good A_{2A} AR affinity ($A_{2A}K_i$ (rat) = 1.07 μM).

An unsubstituted ring A in combination with *para* (4′) $N(CH_3)_2$-substitution on ring B (**2l**) was compared to C4-OCH_3 substitution on ring A (**2g**) and C5-OCH_3 substitution on ring A (**2j**) in combination with *para* (4′) $N(CH_3)_2$-substitution on ring B of the 2-benzylidene-1-indanones and, although, none of these compounds possess micromolar affinity for either the A_1 or A_{2A} AR, it seems that the A_1 AR prefers no substitution on ring A in combination with $N(CH_3)_2$-substitution (**2l**) on ring B, rather than OCH_3-substitution at position C4 or C5 of ring A. The A_{2A} AR again favours C4-OCH_3 substitution over C5-OCH_3 and then no substitution on ring A.

Structural modification to ring B. Comparison of compound **2a** (A_1K_i & $A_{2A}K_i$ (rat) = >100 μM) to compound **2b** (A_1K_i (rat) = 1.65 μM; $A_{2A}K_i$ (rat) = >100 μM) showed that the benzylidene linked at position C2 on ring C increased both A_1 and A_{2A} AR affinity – conveying the necessity of the benzylidene at position C2 on ring C – this trend was also observed with regards to 5-substituted 2-benzylidene-1-tetralones and hydroxy substituted 2-benzylidene-1-indanones.[36,37]

The A_1K_i (rat) value of compound **2b** (1.65 μM) when compared to the A_1K_i (rat) value of compound **2a** (>100 μM) suggests that phenyl ring B is valuable to A_1 AR affinity.

In correlation with previous studies[35–37] OH-group substitution on ring B favours A_1 and A_{2A} AR binding, especially *meta* (3′) hydroxy-group substitution (**2c**) and *meta* (3′) and *para* (4′) diOH-group substitution (**2e**).

It seems that both A_1 and A_{2A} AR's prefer either *meta* (3′) OH-group substitution (giving an A_1K_i (rat) value of 0.041 μM and an $A_{2A}K_i$ (rat) value of 0.097 μM) or *meta* (3′) and *para* (4′) diOH-group substitution (giving an A_1K_i (rat) value of 0.042 μM and an $A_{2A}K_i$ (rat) value of 0.078 μM); seeing as these K_i values are quite similar.

Interestingly, *para* (4′) hydroxy-substitution diminished both A_1 and A_{2A} AR affinity (**2d:** A_1 & A_{2A} (rat) K_i = >100 μM).

Other substitutions that diminish both A_1 and A_{2A} AR affinity include 2′,4′,5′-trimethoxy substitution on ring B (**2f**), as well as dimethylamino substitution (**2g** & **2j**) when compared to its unsubstituted counterpart **2b** (in the case of **2f** and **2g**) and **2h** (in the case of **2j**) (Fig. 3).

These compounds were found to be *E*-isomers. The *E*-configuration is favourable for thermodynamic reasons because of steric interaction between the aryl and carbonyl groups in case of the *Z*-isomers.[38,39]

The benzylidene indanone scaffold (**1a**, **2b**–**m**) contains an α,β-unsaturated ketone group perceived as a potential Michael acceptor.[44,45] Although, compounds that act as Michael acceptors are generally biologically active,[44,45] Michael acceptors are also notoriously reactive compound substructures.[46,47] These compound substructures, containing an electrophile, might show reactivity towards nucleophiles such as thiols.[47] Various biologically-relevant nucleophiles are thiols, for example glutathione, coenzyme A and protein cysteines.[47] Off-target effects are often due to such compound substructures reactivity.[47] Yet, a compound that acts as a Michael acceptor (and contains an electrophile) may still be useful after on-reaction with a suitable nucleophile to yield a lower energy compound.[48]

Compounds containing a catechol group, like **2e**, **2i**, **2k** & **2m**, are widespread in the literature as potential starting points to further explore structure activity relationships – yet, compounds that contain known reactive moieties, such as a catechol group, are also considered a liability;[46–48] as catechols potentially are chelators, redox-active and oxidizes to form protein-reactive quinones.[47,48] For example, the activity of **2e**, with relatively good A_1 and A_{2A} AR affinity, may be due to oxidation of the *ortho*-hydroquinone compound substructure (or catechol group) to *ortho*-quinone – a protein-reactive quinone.

Reactive compound substructures are a risk factor in early drug discovery and development; as the results of biological assays are subject to interference from reactive moieties,[47,48] like the α,β-unsaturated ketone group (present in all compounds in the current study, except **2a**) or the catechol group (present in **2e**, **2i**, **2k** & **2m**). Failure to identify these moieties may lead to wasted resources, as such, compounds have poor development potential.[47,48] Therefore a lack of reactivity interference, must be demonstrated,[47] before further development.

GTP shift assay

GTP shift experiments are performed to determine whether the test compounds that exhibit A_1 AR affinity function as agonists or antagonists. For this purpose, compound **2c** was selected as it exhibited the highest A_1 AR binding affinity among the investigated compounds. The affinities of the reference (CPA and DPCPX) and test compound **2c** were determined in the absence and presence of 100 μM GTP and are reported with the calculated GTP shifts in Table 2. The

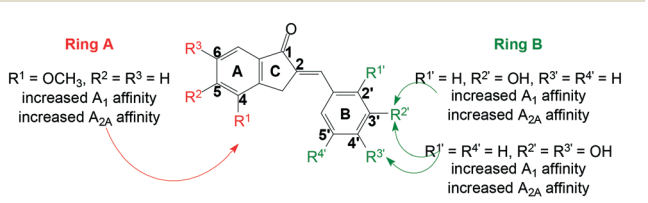


Fig. 3 Structural requirements of the 2-benzylidene-1-indanone scaffold for dual A_1/A_{2A} affinity.

Table 2 The A_1 AR affinities (in the absence and presence of GTP) and calculated GTP shifts of selected 2-benyzlidene-1-indanone analogues

#	K_i ± SEM (μM)[a]		GTP shift[d]
	A_1[b] *vs.* [^{3}H]DPCPX	A_1[b] + GTP[c] *vs.* [^{3}H]DPCPX	
Lead compound			
1a	0.435 ± 0.050[e]	0.339 ± 0.071[e]	0.90[e]
Methoxy substituted 2-benzylidene-1-indanones			
2c	0.041 ± 0.002[a,b]	0.060 ± 0.002[a,c]	1.46[d]
Reference compounds			
CPA (A_1 agonist)	0.0068 ± 0.0001[a] (0.0079);[f] (0.015)[g]	0.099 ± 0.015[a] (0.099)[g]	15 (14)[g]
DPCPX (A_1 antagonist)	0.0004 ± 0.0002[a] (0.0005);[g] (0.0003)[h]	0.0004 ± 0.0002[a] (0.0004)[g]	1.0 (1.0)[g]

[a] All K_i values determined in triplicate and expressed as mean ± SEM. [b] Rat receptors were used (A_1: rat whole brain membranes). [c] GTP shift assay, where the 100 μM GTP was added to the A_1 AR radioligand binding assay. [d] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP. [e] Literature value obtained from reference.[37] [f] Literature value obtained from reference.[49] [g] Literature value obtained from reference.[42] [h] Literature value obtained from reference.[50]

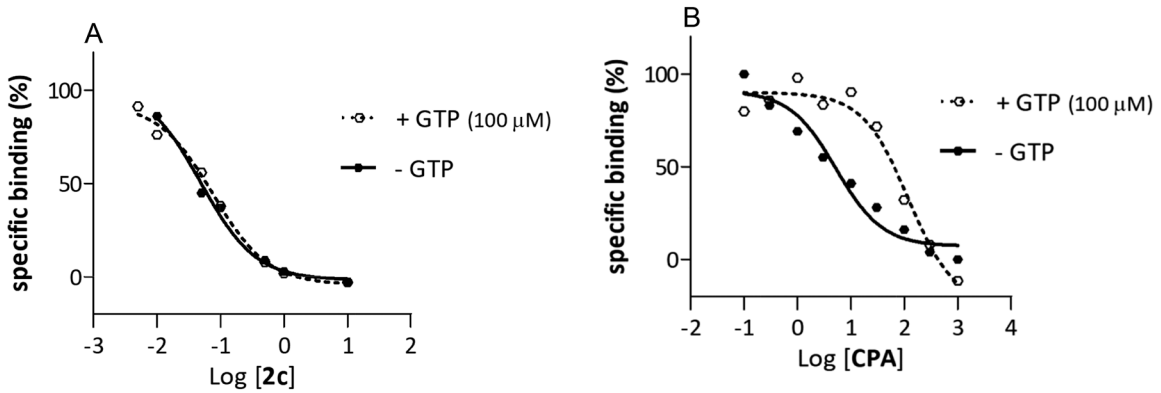


Fig. 4 The binding curves of compounds **2c** and **CPA** (reference compound) are examples of A_1 AR antagonistic action (A) and A_1 AR agonistic action (B), respectively, determined *via* a GTP shift assays (with and without 100 μM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. (A) GTP shift of 1.46 calculated for compound **2c**, (B) GTP shift of 15 calculated for compound **CPA**.

calculated GTP shift results for CPA and DPCPX (see Table 2) were found to correspond with literature values, where CPA acts as an agonist (see Fig. 4) and DPCPX as an antagonist. 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.[42,51] In the case of an A_1 AR antagonist no significant shift is anticipated in the presence of GTP.[42,51] The results suggest that compound **2c** act as A_1 AR antagonist – as no significant rightward shift of the binding curve was observed in the presence of GTP (see Fig. 4).

Conclusions

In summary, this study involved the synthesis, characterization and evaluation of methoxy substituted 2-benzylidene-1-indanone derivatives to understand the importance of structural modifications to ring A and B of the 2-benzylidene-1-indanone scaffold in gaining or even losing A_1 and/or A_{2A} AR affinity. Upon analysis, it was found that C4-OCH_3 substitution on ring A (**2e**) is preferred to C4-OH substitution (**1a**). Additionally, *meta* (3′) OH-group substitution (**2c**; A_1K_i (rat) = 41 nM; $A_{2A}K_i$ (rat) = 97 nM) and *meta* (3′) and *para* (4′) diOH-group substitution (**2e**; A_1K_i (rat) = 42 nM; $A_{2A}K_i$ (rat) = 78 nM) on ring B along with C4 OCH_3-substitution on ring A is complimentary to A_1 and A_{2A} AR affinity, affording these non-selective compounds K_i values in the nanomolar range for both the A_1 and A_{2A} AR. To reiterate the significance of the aforementioned, **2c** and **2e** showed an approximately ten-fold increase in A_1 and A_{2A} AR affinity when compared to lead compound **1a**. Other compounds showed A_1 affinity (**2b**) and A_{2A} affinity (**2m**) in the micromolar range, whereas compounds **2i** and **2k** showed dual A_1 and A_{2A} AR affinity. Yet, none of the other target compounds come close to **2c** and **2e** with regards to A_1 and A_{2A} AR affinity. Functional characterization of **2c** proved this compound to be an A_1 AR antagonist. In view of these findings, compounds **2c** and **2e** present the potential starting points to further explore the structure activity relationships for affinities of this class of compounds as ligands for A_1 and A_{2A} AR. However, since both **2c** and **2e**

contain potential reactive compound substructures, experiments that demonstrate the absence of reactivity interference using a variety of methods must be performed. If necessary, the undesirable reactive compound substructures may be removed or modified during medicinal chemistry optimization.

Experimental

Chemistry

General remarks. Unless otherwise noted, all starting materials and solvents were procured from Sigma-Aldrich and used without further purification. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively, with deuterated dimethylsulfoxide (DMSO-d_6) as solvent. Chemical shifts are reported in parts per million (δ) in relation to the signal of tetramethylsilane ($Si(CH_3)_4$). Spin multiplicities are indicated as: s (singlet), d (doublet), dd (doublet of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). 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) for **2a–g** were measured with a Buchi B545 melting point apparatus, whereas mp's for **2i**, **2k** & **2m** were measured by means of differential scanning calorimetry (DSC) with a Mettler DSC 3 Star System (Mettler Toledo, Greifensee, Switzerland) and are uncorrected and are uncorrected. Thin layer chromatography (TLC) was done using silica gel 60 (Merck) with UV254 fluorescent indicator.

Synthesis of 2a

*4-Methoxy-2,3-dihydro-1H-inden-1-one (**2a**).* To a solution of 4-hydroxy-1-indanone (2.00 g, 13.5 mmol) in acetone (70.0 mL), K_2CO_3 (7.46 g, 54.0 mmol) and then MeI (11.5 g, 81.0 mmol) were added and mechanically stirred at 50 °C under reflux for 18 h. Next, the reaction mixture was concentrated, extracted with EtOAc (3 × 100 mL), combined organic extracts dried ($MgSO_4$), filtered and concentrated to yield compound **2a** as brown crystals (2.00 g, 91%): R_f: 0.51 (PE/EtOAc 4 : 1); mp: 105.3–107.1 °C; ^{1}H NMR (600 MHz, DMSO) δ 2.61 (dd, J = 6.7, 4.6 Hz, 2H), 2.97–2.92 (m, 2H), 3.87 (s, 3H), 7.22 (dd, J = 18.8, 7.7 Hz, 2H), 7.40 (t, J = 7.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 22.31, 35.75, 55.52, 114.48, 115.48, 129.09, 138.20, 143.52, 156.89, 206.39. APCI-HRMS m/z calculated for $C_{10}H_{10}O_2$ (MH^+): 163.0753, found: 163.0754. Purity (HPLC): 98.6%.

Synthesis of 2b–g, 2i, 2k and 2m

*(E)-2-Benzylidene-4-methoxy-2,3-dihydro-1H-inden-1-one (**2b**).* Compound **2a** (0.200 g, 1.233 mmol) and benzaldehyde (0.131 g, 1.233 mmol) were suspended in MeOH (4 mL) and HCl (32%, 6 mL) and 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 (60 °C) and recrystallized from a suitable solvent (either MeOH or EtOH) to yield **2b** as light brown crystals (0.22 g, 71%): R_f: 0.68 (PE/EtOAc 3 : 1); mp: 385.3–385.5 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.96–3.90 (m, 5H), 7.29 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 7.5 Hz, 1H), 7.57–7.43 (m, 5H), 7.78 (d, J = 7.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 28.90, 55.56, 115.20, 116.00, 129.05, 129.44, 129.86, 130.79, 133.08, 134.71, 134.78, 138.05, 138.59, 156.50, 193.30. APCI-HRMS m/z calculated for $C_{17}H_{14}O_2$ (MH^+): 251.1067, found: 251.1088. Purity (HPLC): 98.1%.

*(E)-2-(3-Hydroxybenzylidene)-4-methoxy-2,3-dihydro-1H-inden-1-one (**2c**).* Prepared as for **2b** from compound **2a** (0.300 g, 1.850 mmol) and 3-hydroxybenzaldehyde (0.226 g, 1.850 mmol) to yield compound **2c** as light brown crystals (0.44 g, 90%): R_f: 0.29 (PE/EtOAc 3 : 1); mp: 104.3–104.4 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.90 (d, J = 14.8 Hz, 5H), 6.86 (dd, J = 8.0, 1.9 Hz, 1H), 7.20 (dd, J = 10.1, 4.7 Hz, 2H), 7.38–7.26 (m, 3H), 7.45 (dd, J = 15.6, 7.9 Hz, 2H), 9.73 (s, 1H); ^{13}C NMR (151 MHz, DMSO) δ 29.01, 55.56, 115.20, 115.98, 116.59, 117.24, 122.39, 129.45, 130.07, 133.30, 134.43, 135.95, 137.97, 138.65, 156.51, 157.73, 193.32. APCI-HRMS m/z calculated for $C_{17}H_{14}O_3$ (MH^+): 267.1016, found: 267.1017. Purity (HPLC): 100%.

*(E)-2-(4-Hydroxybenzylidene)-4-methoxy-2,3-dihydro-1H-inden-1-one (**2d**).* Prepared as for **2b** from compound **2a** (0.300 g, 1.850 mmol) and 4-hydroxybenzaldehyde (0.226 g, 1.850 mmol) to yield compound **2d** as mustard powder (0.45 g, 92%): R_f: 0.14 (PE : EtOAc 3 : 1); mp: 275.3–396.2 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.86 (d, J = 0.4 Hz, 2H), 3.91 (s, 3H), 6.91 (d, J = 8.6 Hz, 2H), 7.27 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 7.5 Hz, 1H), 7.44 (dd, J = 12.7, 4.8 Hz, 2H), 7.64 (d, J = 8.7 Hz, 2H), 10.15 (s, 1H); ^{13}C NMR (151 MHz, DMSO) δ 28.97, 55.53, 115.06, 115.63, 116.10, 125.89, 129.30, 131.13, 133.03, 133.61, 137.69, 139.03, 156.48, 159.50, 193.19. APCI-HRMS m/z calculated for $C_{17}H_{14}O_3$ (MH^+): 267.1016, found: 267.1020. Purity (HPLC): 100%.

*(E)-2-(3,4-Dihydroxybenzylidene)-4-methoxy-2,3-dihydro-1H-inden-1-one (**2e**).* Prepared as for **2b** from compound **2a** (0.300 g, 1.850 mmol) and 3,4-dihydroxybenzaldehyde (0.255 g, 1.850 mmol) to yield compound **2e** as light yellow crystals (0.47 g, 90%): R_f: 0.29 (PE/EtOAc 3 : 1); mp: 294.0–295.1 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.84 (s, 2H), 3.92 (s, 3H), 6.87 (d, J = 8.2 Hz, 1H), 7.10 (dd, J = 8.3, 1.9 Hz, 1H), 7.27 (t, J = 5.1 Hz, 2H), 7.33 (d, J = 7.5 Hz, 1H), 7.37 (s, 1H), 7.44 (t, J = 7.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 29.05, 55.54, 115.07, 115.63, 116.13, 117.08, 124.73, 126.32, 129.33, 130.89, 134.08, 137.62, 139.11, 145.69, 148.19, 156.49, 193.17. APCI-HRMS m/z calculated for $C_{17}H_{14}O_4$ (MH^+): 283.0965, found: 283.0959. Purity (HPLC): 100%.

*(E)-2-(2,4,5-Trimethoxybenzylidene)-4-methoxy-2,3-dihydro-1H-inden-1-one (**2f**).* Prepared as for **2b** from compound **2a** (0.300 g, 1.850 mmol) and 2,4,5-trimethoxybenzaldehyde (0.363 g, 1.850 mmol) to yield compound **2f** as green powder (0.34 g, 54%): R_f: 0.14 (PE/EtOAc 4 : 1); mp: 217.6–218.0 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.82 (s, 3H), 3.94–3.87 (m, 9H), 3.95 (d, J = 1.3 Hz, 2H), 6.79 (s, 1H), 7.37–7.27 (m, 3H), 7.45 (t, J = 7.8 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.89 (t, J = 1.9 Hz,

1H); ^{13}C NMR (151 MHz, DMSO) δ 28.52, 55.60, 55.86, 56.47, 97.64, 113.20, 114.49, 115.08, 115.66, 127.30, 129.29, 131.31, 137.70, 139.08, 142.73, 152.39, 154.91, 156.45, 167.00, 193.20. APCI-HRMS *m*/*z* calculated for $C_{20}H_{20}O_5$ (MH^+): 341.1384, found: 341.1393. Purity (HPLC): 95.1%.

(E)-2-(4-(Dimethylamino)benzylidene)-4-methoxy-2,3-dihydro-1H-inden-1-one (2g). Prepared as for **2b** from compound **2a** (0.300 g, 1.850 mmol) and 4-(dimethylamino)benzaldehyde (0.276 g, 1.850 mmol) to yield compound **2g** as brown crystals (0.33 g, 61%): R_f: 0.46 (PE/EtOAc 4 : 1); mp: 190.7–191.7 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.01 (s, 6H), 3.85 (s, 2H), 3.92 (s, 3H), 6.85–6.77 (m, 2H), 7.26 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 7.4 Hz, 1H), 7.44 (dd, J = 9.6, 5.0 Hz, 2H), 7.62 (d, J = 8.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 29.16, 55.53, 112.01, 114.93, 115.32, 122.13, 129.09, 129.20, 132.72, 134.31, 137.35, 139.45, 151.23, 156.43, 192.86. APCI-HRMS *m*/*z* calculated for $C_{19}H_{19}NO_2$ (MH^+): 294.1489, found: 294.1499 Purity (HPLC): 97.9%.

(E)-2-(3,4-Dihydroxybenzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (2i). Prepared as for **2b** from 5-methoxy-1-indanone (0.300 g, 1.850 mmol) and 3,4-dihydroxybenzaldehyde (0.255 g, 1.850 mmol) to yield compound **2i** as brown powder (0.33 g, 63%): R_f: 0.13 (DCM/EtOAc 10 : 1); mp: 281.26 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.88 (s, 3H), 3.96 (s, 2H), 6.85 (d, J = 8.2 Hz, 1H), 7.01 (dd, J = 8.5, 2.2 Hz, 1H), 7.08 (dd, J = 8.3, 2.0 Hz, 1H), 7.17 (dd, J = 12.5, 2.0 Hz, 2H), 7.29 (t, J = 1.7 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 9.24 (s, 1H), 9.64 (s, 1H); ^{13}C NMR (151 MHz, DMSO) δ 32.09, 55.76, 110.23, 115.15, 116.02, 117.44, 123.87, 125.20, 126.57, 130.91, 131.91, 132.43, 145.58, 147.75, 152.55, 164.61, 191.59. APCI-HRMS *m*/*z* calculated for $C_{17}H_{14}O_4$ (MH^+): 283.0965, found: 283.0972. Purity (HPLC): 95.4%.

(E)-2-(3,4-Dihydroxybenzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (2k). Prepared as for **2b** from 5,6-dimethoxy-1-indanone (0.300 g, 1.561 mmol) and 3,4-dihydroxybenzaldehyde (0.216 g, 1.561 mmol) to yield compound **2k** as dark green powder (0.41 g, 84%): R_f: 0.12 (DCM/EtOAc/PE 8 : 1 : 1); mp: 262.66 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.83 (s, 12H), 3.90 (s, 20H), 6.85 (d, J = 8.2 Hz, 4H), 7.07 (dd, J = 8.3, 2.0 Hz, 4H), 7.18 (dd, J = 12.3, 4.1 Hz, 12H), 7.27 (s, 4H), 9.43 (s, 7H); ^{13}C NMR (151 MHz, DMSO) δ 31.69, 55.65, 55.96, 104.50, 108.07, 116.01, 117.41, 123.75, 126.62, 130.32, 131.98, 132.19, 144.61, 145.58, 147.67, 149.25, 154.94, 191.88. APCI-HRMS *m*/*z* calculated for $C_{18}H_{16}O_5$ (MH^+): 313.1071, found: 313.0993. Purity (HPLC): 98.6%.

(E)-6-(3,4-Dihydroxybenzylidene)-6,7-dihydro-5H-indeno[5,6-d][1,3]dioxol-5-one (2m). Prepared as for **2b** from 5,6-methylenedioxy-1-indanone (0.300 g, 1.703 mmol) and 3,4-dihydroxybenzaldehyde (0.235 g, 1.703 mmol) to yield compound **2m** as green powder (0.45 g, 90%): R_f: 0.09 (DCM/EtOAc 10 : 1); mp: 317.96 °C; ^{1}H NMR (600 MHz, DMSO) δ 3.88 (s, 2H), 6.17 (s, 2H), 6.84 (d, J = 8.2 Hz, 1H), 7.06 (dd, J = 8.2, 1.7 Hz, 1H), 7.15 (dd, J = 9.0, 2.4 Hz, 3H), 7.25 (s, 1H), 9.25 (s, 1H), 9.63 (s, 1H); ^{13}C NMR (151 MHz, DMSO) δ 31.97, 101.97, 102.37, 105.92, 116.01, 117.33, 123.93, 126.49, 131.95, 132.18, 132.28, 145.59, 146.99, 147.77, 148.03, 153.48, 191.34. APCI-HRMS *m*/*z* calculated for $C_{17}H_{12}O_5$ (MH^+): 297.0758, found: 297.0755. Purity (HPLC): 87.2%.

Biology

General remarks. All commercially available reagents were obtained from various manufacturers: radioligands [^{3}H]NECA (specific activity 27.1 Ci mmol^{-1}) procured from PerkinElmer and [^{3}H]DPCPX (specific activity 120 Ci mmol^{-1}) from Amersham Biosciences, filter-count from PerkinElmer and Whatman GF/B 25 mm diameter filters from Merck. Radio activity was calculated by a Packard Tri-CARB 2810 TR liquid scintillation counter.

Radioligand binding assays. The collection of tissue samples for the A_1 and A_{2A} AR binding studies were approved by the Research Ethics Committee of the North-West University (application number NWU-0035-10-A5). The affinities of the 2-benzylidene-1-indanone analogues (**2a–m**) at rat A_1 and A_{2A} AR subtypes were determined with radioligand competition experiments as described previously.[22,23] The competition experiments were carried out in the presence of the radioligands [^{3}H]-8-cylcopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX; 0.1 nM; K_d = 0.36 nM) and 5′-N-[^{3}H]-ethylcarboxamideadenosine ([^{3}H]NECA; 4 nM; K_d = 15.3 nM) for the A_1 and A_{2A} AR radioligand binding assays, respectively.[22,24] In addition, the A_{2A} AR binding studies were determined in the presence of N^6-cyclopentyladenosine (CPA) to minimize the binding of [^{3}H]NECA to A_1 AR's. Non-specific binding was defined by the addition of a final concentration of 100 μM CPA. The sigmoidal-dose response curves, *via* Graphpad Software Inc. package, were obtained by plotting the specific binding *versus* 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 the 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 (see Table 1).

GTP shift assays. The GTP shift assay was performed as described previously with rat whole brain membranes and [^{3}H]DPCPX (0.1 nM; K_d = 0.36 nM) in the absence and presence of a final concentration of 100 μM GTP (see Table 2).[22,23] 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 A_1 AR agonist CPA.[22] 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.[22]

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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