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C2-substituted quinazolinone derivatives exhibit A_1 and/or A_{2A} adenosine receptor affinities in the low micromolar range

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Abstract

Antagonists of the adenosine receptors (A_1 and A_{2A} subtypes) are widely researched as potential drug candidates for their role in Parkinson's disease-related cognitive deficits (A_1 subtype), motor dysfunction (A_{2A} subtype) and to exhibit neuroprotective properties (A_{2A} subtype). Previously the benzo-α-pyrone based derivative, 3-phenyl-1H-2-benzopyran-1-one, was found to display both A_1 and A_{2A} adenosine receptor affinity in the low micromolar range. Prompted by this, the α-pyrone core was structurally modified to explore related benzoxazinone and quinazolinone homologues previously unknown as adenosine receptor antagonists. Overall, the C2-substituted quinazolinone analogues displayed superior A_1 and A_{2A} adenosine receptor affinity over their C2-substituted benzoxazinone homologues. The benzoxazinones were devoid of A_{2A} adenosine receptor binding, with only two compounds displaying A_1 adenosine receptor affinity. In turn, the quinazolinones displayed varying degrees of affinity (low micromolar range) towards the A_1 and A_{2A} adenosine receptor subtypes. The highest A_1 adenosine receptor affinity and selectivity were favoured by methyl *para*-substitution of phenyl ring B (A_1K_i = 2.50 μM). On the other hand, 3,4-dimethoxy substitution of phenyl ring B afforded the best A_{2A} adenosine receptor binding ($A_{2A}K_i$ = 2.81 μM) among the quinazolinones investigated. In conclusion, the quinazolinones are ideal lead compounds for further structural optimization to gain improved adenosine receptor affinity, which may find therapeutic relevance in Parkinson's disease-associated cognitive deficits and motor dysfunctions as well as exerting neuroprotective properties.

Keywords

Keywords: benzoxazinone; quinazolinone; benzo-α-pyrone; Adenosine A_{2A} receptor; Adenosine A_1 receptor; antagonist; GTP shift; radioligand binding assay; Parkinson's disease.

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Abbreviations: PD; Parkinson's Disease; AR, adenosine receptor; GTP, guanosine triphosphate; [^{3}H]DPCPX, [^{3}H]-dipropyl-8-cyclopentylxanthine; [^{3}H]NECA, N-[^{3}H]-ethyladenosin-5'-uronamide; CPA, N^6-cyclopentyladenosine.

Parkinson's disease (PD) is a complex neurodegenerative disorder and is characterized by the formation of Lewy bodies and the death of dopaminergic neurons along the nigrostriatal pathway [1, 2]. The loss of dopaminergic neurons results in a diminished amount of dopamine present in the corpus striatum [3]. In 2005 the prevalence of PD among individuals living in the United States, aged above 65 to 70 years, was documented as 9.5 per 1000 individuals [4] and it is estimated that between 2005 and 2030 the prevalence will have doubled [5]. Individuals suffering from PD have been found to present with a range of mainly motor features as first described by James Parkinson in 1817 [6, 7]. Four principal motor features were identified, namely tremors, rigidity, bradykinesia and postural instability [6]. Over time some non-motor symptoms may develop which are typically defined by cognitive impairment [8].

At present, the treatment of PD still follows a more traditional approach in the sense that current treatment is more symptomatic rather than curative [9]. Symptomatic treatment seeks to replenish dopamine stores or to disrupt the degradation of endogenous dopamine by inhibition of dopamine degrading enzymes [10]. The current treatment with regards to the motor symptoms of PD entails oral preparations of levodopa (L-3,4-dihydroxyphenylalanine) and dopamine receptor agonists as well as deep brain stimulation and apomorphine in severe cases [11]. Although levodopa remains the cornerstone treatment of PD-related motor symptoms [9], the development of dyskinesia with long-term use [12], along with the alarming prevalence and the need for neuroprotective and disease-modifying agents, demonstrate the necessity for novel drug development [1].

In the brain, adenosine functions as a neuromodulator and fulfils a physiological role opposite to that of dopamine [13, 14]. Four adenosine receptor (AR) subtypes (A_1, A_{2A}, A_{2B} and A_3) exist and of the four subtypes, A_1 and A_{2A} ARs have the highest density in the brain [15]. The A_1 ARs are found predominantly in the cortex, cerebellum and hippocampus of the brain [16, 17], and in turn, the A_{2A} ARs are generally more restricted to the dorsal striatum, nucleus accumbens, and olfactory tubercle [18]. Both the A_1 and A_{2A} ARs have been identified as possible targets for drug development in neurological disorders, such as PD [19], and are currently being examined for their potential benefit in PD [20]. The A_{2A} AR antagonists have been found to enhance motor activity with a reduced risk of developing dyskinesia and other associated side effects, making them viable candidates for the treatment of PD [21]. Preclinical data exists demonstrating that A_{2A} AR antagonists may possess neuroprotective potential [22], where neuroprotective qualities have been displayed by a selective A_{2A} AR antagonist, Istradefylline, in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced dopaminergic toxicity study [23]. More importantly, Istradefylline has recently been approved by the FDA as add-on therapy in PD under the trade name Nourianz [24].

A_1 ARs are important for cognitive function and antagonism of these receptors may improve cognition [25]. A selective A_1 AR antagonist, such as FR-194921, has been examined in animal models and appears to positively modulate memory processes while having a stimulating effect on the central nervous system [26]. Therefore, A_1 AR antagonists are being examined for use as cognitive enhancers in neurological disorders such as dementia, Alzheimer's disease and PD [27]. It was also observed that the blockade of both the A_1 and A_{2A} AR subtypes lead to a synergistic positive motor response, which could be ascribed to the release of dopamine triggered by the antagonism of the A_1 AR with the simultaneous enhancement of the postsynaptic response to dopamine potentiated by antagonism of the A_{2A} AR [28].

Thus, antagonists of the A_1 and/or A_{2A} AR are thought to exert neuroprotective properties and enhance motor function via A_{2A} AR antagonism, while A_1 AR antagonism improves PD-associated cognitive dysfunction. To date, an ideal drug candidate has not been identified for PD treatment. Therefore, considering the aforementioned potential of AR antagonists as prospective PD treatment and the lack of an ideal drug candidate, the need exists to discover high affinity A_1 and/or A_{2A} AR antagonists.

Recently, a series of benzopyrone derivatives were evaluated as AR antagonists [29]. The benzo-α-pyrone compound 3-phenyl-1H-2-benzopyran-1-one (**1**), possessed both A_1 and A_{2A} AR affinity (A_1K_i = 7.41 μM; $A_{2A}K_i$ = 3.35 μM) with a selectivity index (SI) of 2 towards the A_{2A} AR subtype (Figure 1; Table 1). Compound **1** consists of a basic benzo-α-pyrone skeleton (ring A and C is fused) with ring C bearing a C3-phenyl substituted side-chain (ring B) (Figure 1, A). The double bond between position C3 and C4 of ring C was found to be imperative for AR binding and the arrangement of the ketone and hetero oxygen in ring C was optimal for A_{2A} AR binding. In analogy to the structure of 3-phenyl-1H-2-benzopyran-1-one (**1**) the present study investigates the A_1 and A_{2A} AR binding properties of structurally related C2-substituted benzoxazinones (**2a**–**j**, **3**), 3-phenyl-isoquinolinone (**4**) and C2-substituted quinazolinones (**5a**–**j**) (Figure 1; Table 1).

The investigated C2-substituted benzoxazinones (**2a**–**j**) possess the basic scaffold of compound **1** with the addition of hetero nitrogen to ring C (Figure 1, B). This structural modification allows the structure-activity relationship (SAR) exploration of additional nitrogen to ring C, while retaining compound **1**'s aforementioned double bond (ring C) deemed essential for AR affinity. The benzoxazinones are further assessed by various substitutions on phenyl ring B (**2b**–**g**). The inclusion of compound **3** will provide insight into the necessity of the double bond in ring C of the benzoxazinone scaffold to govern AR binding (Figure 1, B). The comparison of the isoquinolinone derivative (**4**) to compound **1** will highlight the

importance of the hetero oxygen in ring C of the benzo-α-pyrone backbone to favour AR affinity (Figure 1, C). Furthermore, the C2-substituted quinazolinones (**5a**–**j**) is included to afford further SAR exploration of the AR affinity if ring C bears two hetero nitrogen atoms (Figure 1, D). The effect of other heterocyclic ring systems (e.g. furyl and thiophene) replacing phenyl ring B of the benzoxazinones (**2h** and **i**) and quinazolinones (**5h** and **i**), as well as the insertion of a styryl side-chain between ring C and B of the benzoxazinones (**2j**) and quinazolinones (**5j**) are additionally evaluated *in vitro*. Lastly, the rearrangement of the ketone and hetero oxygen of compound **1** is investigated by including compounds **6** and **7**, where this rearrangement was switched/flipped (Figure 2). Table 1 documents the A_1 and A_{2A} AR dissociation constant (K_i) values determined via radioligand binding assays.

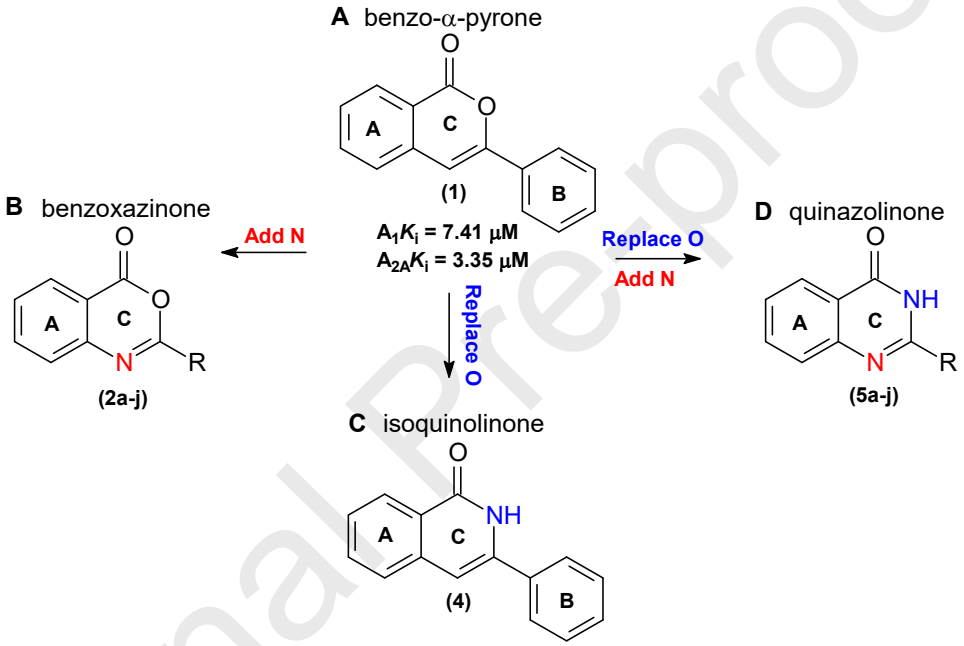


Figure 1: General structures of the various scaffolds explored in the current investigative study: (**A**) benzo-α-pyrone (**1**), (**B**) benzoxazinone (**2a-j**), (**C**) isoquinolinone (**4**) and (**D**) quinazolinones (**5a-j**).

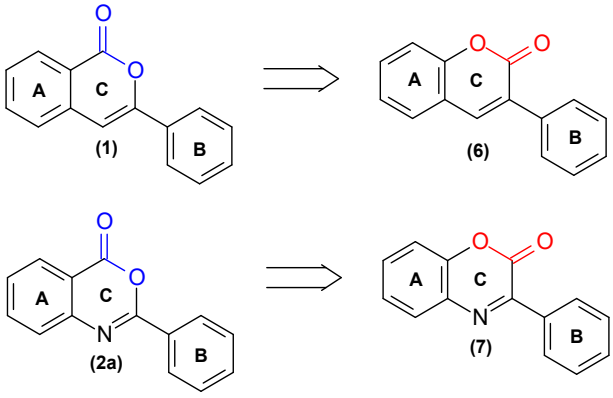
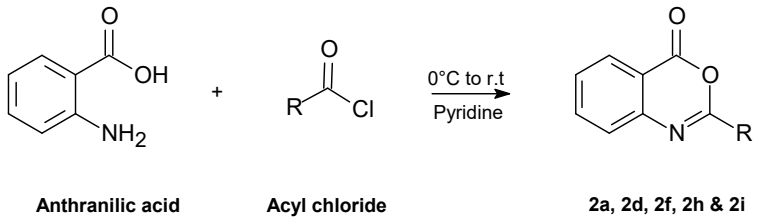


Figure 2: Depicting the rearrangement of the ketone and hetero oxygen of ring C by the benzo-α-pyrone (**1** vs **6**) and the benzoxazinones (**2a** vs **7**).

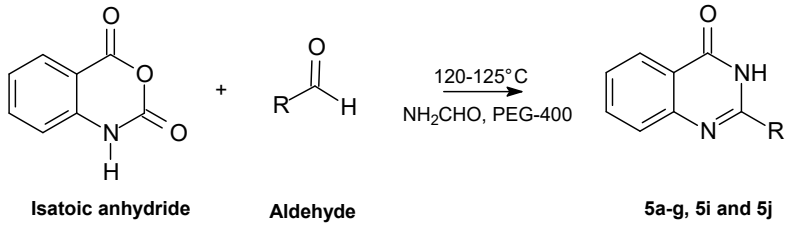
The test compounds were either obtained commercially (**1**, **2b**, **2c**, **2e**, **2g**, **2j**, **3**, **6** and **7**) or synthesized (**2a**, **2d**, **2f**, **2h**, **2i**, **4** and **5a**–**j**) according to literature procedures. The test compounds obtained from standard commercial sources (Sigma Aldrich) were used without further purification.

The benzoxazinone analogues (**2a**, **2d**, **2f**, **2h** and **2i**) that were not commercially obtained, were synthesized in an alkaline environment with commercially available acyl chlorides and anthranillic acid as key starting materials (Scheme 1) [30].

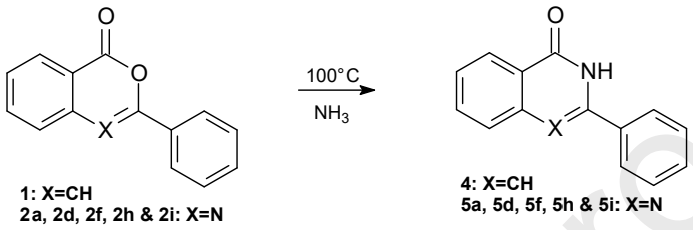
The synthesis of the proposed quinazolinone derivatives (**5a**–**j**) was performed by either method A and/or B. Method A involved a one-pot synthesis with the appropriate aldehyde, formamide and isatoic anhydride (Scheme 2) [31], while method B included treatment of the corresponding benzoxazinone with ammonia (Scheme 3) [32]. Isoquinolinone derivative **4** was synthesized by treating 3-phenyl-1H-2-benzopyran-1-one (**1**) with ammonia — a similar synthetic approach than outlined in method B (Scheme 3) [32]. In each instance, the structures of the proposed synthesized compounds (**2a**, **2d**, **2f**, **2h**, **2i**, **4** and **5a**–**j**) were verified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The reaction conditions and compound characterizations are detailed in the supplementary information.



Scheme 1: A synthetic pathway for C2-substituted benzoxazinone derivatives (**2a**, **2d**, **2f**, **2h** and **2i**).



Scheme 2: A synthetic pathway to the synthesized C2-substituted quinazolinone derivatives (**5a–g**, **5i** and **5j**), method A.



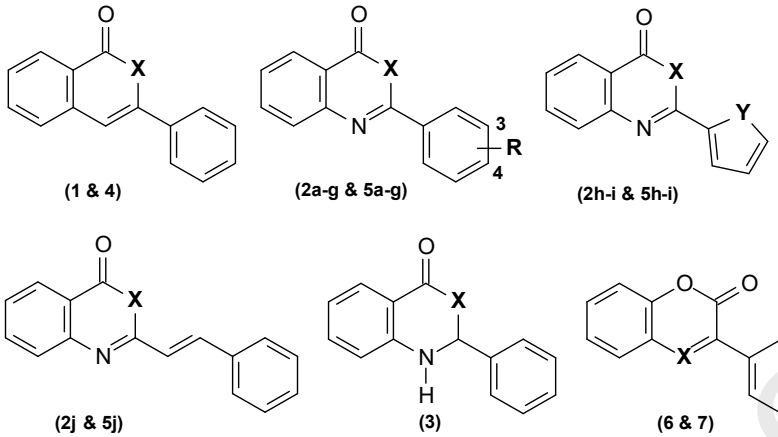
Scheme 3: A synthetic pathway for the isoquinolinone (**4**, X=CH) and C2-substituted quinazolinone derivatives (**5a**, **5d**, **5f**, **5h** and **5i**, X=N), method B.

Assays were completed using rat whole brain (A_1) and rat striatal membranes (A_{2A}). Noteworthy, a recently study investigated potential differences between human, rat and mouse ARs. Alnouri and co-workers calculated correlation coefficients and found that rat and humans share a 0.80 correlation of pK_i for the A_1 AR and 0.83 for the A_{2A} AR [33]. Furthermore, Maemoto and colleagues found that selected pyrazolopyridine derivatives exhibited a similar affinity in rat, human and guinea pig [34]. The A_1 and A_{2A} AR binding affinities of the test compounds (**1**, **2a**–**j**, **3**, **4**, **5a**–**j**, **6** and **7**) were evaluated *in vitro* with radioligand binding assays described previously [35, 36]. The Research Ethics Committee of North-West University approved the collection of tissue samples for the A_1 and A_{2A} AR radioligand binding assays (application number NWU-0035-10-A5). The AR binding affinities are expressed as the mean K_i values of triplicate determinations and are documented in Table 1. Additional information with regards to the radioligand binding assays are detailed in the supplementary information.

Introducing hetero nitrogen (ring C) to the α-pyrone core of compound **1** afforded the benzoxazinone derivative **2a** (Figure 1, B). The latter compound exhibited a similar A_1 AR affinity as documented for compound **1** (**1** A_1K_i = 7.41 µM vs **2a** A_1K_i = 6.38 µM), whereas the A_{2A} AR binding affinity was diminished (**1** $A_{2A}K_i$ = 3.35 µM vs **2a** $A_{2A}K_i$ = > 100 µM).

The SARs of the benzoxazinone scaffold was further explored at ring B by investigating several *para*-substituents on the phenyl ring (**2b**–**g**) and other heterocyclic ring systems which included a furyl (**2h**) and a thiophene (**2i**) ring. In addition, the insertion of a styryl side-chain

Table 1 Dissociation constant values (K_i values) for the binding of the test compounds to rat A_1 and A_{2A} adenosine receptors.



				K_i ± SEM (μM)[a] (% displacement)[b]				
Compd	**X**	**R**	**Y**	**A_1[c] vs [^{3}H]DPCPX**	**A_{2A}[c] vs [^{3}H]NECA**	**A_1[c] + GTP[d] vs [^{3}H]DPCPX**	**GTP shift[e]**	**SI[f] (A_{2A}/A)**
Benzo-α-pyrone scaffold								
1	O	-	-	7.41 ± 0.4[a,g]	3.35 ± 0.80[a,g]	6.49 ± 0.82[a,g]	0.9[g]	0.5[g]
Benzoxazinone scaffold								
2a	O	H	-	6.38 ± 0.40[a]	> 100 (49%) [b]	6.92 ± 1.16[a]	1.09	-
2b	O	4-F	-	> 100 (100%)[b]	> 100 (70%)[b]			-
2c	O	4-Cl	-	> 100 (86%)[b]	> 100 (81%)[b]			-
2d	O	4-Br	-	> 100 (95%)[b]	> 100 (92%)[b]			-
2e	O	4-CH_3	-	> 100 (84%)[b]	> 100 (77%)[b]			-
2f	O	4-OCH_3	-	> 100 (54%)[b]	> 100 (37%)[b]			-
2g	O	4-OCH_2CH_3	-	> 100 (99%)[b]	> 100 (77%)[b]			-
2h	O	-	O	> 100 (42%)[b]	> 100 (66%)[b]			-
2i	O	-	S	32.4 ± 0.58[a]	> 100 (59%)[b]			-
2j	O	-	-	> 100 (56%)[b]	> 100 (85%)[b]			-
3	O	-	-	5.06 ± 0.46[a]	> 100 (69%)[b]	5.72 ± 0.56[a]	1.12	-
Isoquinolinone scaffold								
4	NH	-	-	>100 (100%)[b]	> 100 (100%)[b]			-
Quinazolinone scaffold								
5a	NH	H	-	3.67 ± 0.02[a]	18.7 ± 1.99[a]	3.77 ± 0.28[a]	1.02	5.09
5b	NH	4-F	-	> 100 (50%)[b]	> 100 (62%)[b]			-
5c	NH	4-Cl	-	> 100 (54%)[b]	> 100 (82%)[b]			-
5d	NH	4-Br	-	> 100 (98%)[b]	> 100 (84%)[b]			-
5e	NH	4-CH_3	-	2.50 ± 0.47[a]	> 100 (55%)[b]	2.95 ± 0.18[a]	1.18	-
5f	NH	4-OCH_3	-	> 100 (44%)[b]	> 100 (79%)[b]			-
5g	NH	3,4-OCH_3		> 100 (67%)[b]	2.81 ± 0.40[a]			-
5h	NH	-	O	4.62 ± 0.63[a]	8.11 ± 0.03[a]	5.89 ± 0.42	1.23	1.76
5i	NH	-	S	> 100 (39%)[b]	> 100 (30%)[b]			-
5j	NH	-	-	> 100 (100%)[b]	> 100 (56%)[b]			-
Coumarin scaffold								
6	CH	-	-	> 100 (51%)[b,g]	> 100 (77%)[b,g]			-
Benzoxazinone scaffold								
7	N	-	-	17.3 ± 1.9[a]	> 100 (91%)[b]			-
Reference compounds								
CPA (A_1 agonist)				0.0051 ± 0.0002 (0.0079)[h]; (0.0059)[j]	0.557 ± 0.024 (0.460) [h]	29.5 ± 1.14 (35.2)[i]	5.8 (6)[i]	5784
ZM-241385 (A_{2A} antagonist)				0.420 ± 0.01 (0.225)[j]	0.0013 ± 0.00002 (0.002)[j]	0.510 ± 0.03	1.2	0.003

[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] GTP shift assay, where the 100 μM GTP was added to the A_1 AR radioligand binding assay; [e] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP; [f] Selectivity index (SI) for the A_1 AR isoform calculated as the ratio of K_i (A_{2A})/K_i (A_1); [g] Literature value obtained from reference [35]; [h] Literature value obtained from reference[37]; [i] Literature value obtained from reference[38]; [j] Literature value obtained from reference [39].

(**2j**) between ring C and phenyl ring B was also investigated. Compared to **2a**, p*ara*-substitution (**2b**–**g**) of phenyl ring B with F (**2b**), Cl (**2c**), Br (**2d**), CH_3 (**2e**), OCH_3 (**2f**) and OCH_2CH_3 (**2g**) diminished A_1 AR affinity, while A_{2A} AR activity remained elusive. The furyl compound (**2h**) and insertion of a styryl side-chain (**2j**) showed no A_1 or A_{2A} AR affinity. Thus, the C2-substituted benzoxazinones (**2a**–**j**) were generally found to be devoid of A_1 and A_{2A} AR affinity. However, the unsubstituted phenyl ring B (**2a**) and the thiophene ring B (**2i**) were the only benzoxazinones investigated to exhibited A_1 AR affinity with K_i values of 6.38 μM and 32.4 μM, respectively.

Upon further investigation, ring C of compound **2a** was saturated (absence of the double bond) to yield compound **3**. This structural modification led to a slight improvement of A_1 AR affinity compared to **2a** (**2a** A_1K_i = 6.38 μM vs **3** A_1K_i = 5.06 μM), but affinity at the A_{2A} AR subtype remained absent (Figure 1, B; Table 1).

Further structural modification to ring C included the replacement of the hetero oxygen of compound **1** with hetero nitrogen to yield compound **4** (Figure 1, C). Both the A_1 and A_{2A} AR affinity were diminished, emphasizing the preference of the benzo-α-pyrone scaffold (**1**) over the isoquinolinone moiety (**4**) for enhanced A_1 and A_{2A} AR activity.

Based on the aforementioned finding that the hetero oxygen (ring C) of the benzo-α-pyrone derivative **1** is essential for AR activity, the structurally related benzoxazinone (**2a**) and quinazolinone (**5a**) homologues were compared. It was found that replacing the hetero oxygen (ring C) of **2a** with hetero nitrogen resulted in **5a** (A_1K_i = 3.67 μM; $A_{2A}K_i$ = 18.7 μM) which exhibited superior A_1 and A_{2A} AR affinity over its benzoxazinone counterpart **2a** (A_1K_i = 6.38 μM; $A_{2A}K_i$ >100 μM). The latter observation (**2a** vs **5a**) is contrary to the above finding (**1** vs **4**). Thus, replacement of compound **1**'s hetero oxygen of ring C (benzo-α-pyrone) with hetero nitrogen (**5a**) in addition to a second hetero nitrogen led to improved A_1 AR affinity and reduced A_{2A} AR binding (Figure 1, D). The improved AR affinity of **5a** compared to **2a** may be ascribed, in part, to ring C containing two hetero nitrogens (**5a**) compared to one (**2a**). The latter observation is supported by the research of Gillespie and colleagues [40, 41]. In the study by Gillespie and co-workers, they compared the binding affinities of pyridine, pyrimidine and triazine to each other and found that two nitrogens in the heterocyclic ring are optimum to enhance both the A_1 and A_{2A} AR binding [40, 41]. The latter statement was based on their finding that the binding affinity of the pyridine scaffold was seven-fold less potent than the triazine and 45-fold less potent than the corresponding aminopyrimidine [40, 41].

Furthermore, unlike compounds **5a** and **1,** the benzoxazinone derivative **2a** only possessed affinity toward the A_1 AR. Noteworthy, an approximate two-fold A_1 AR affinity improvement

was documented for **5a** compared to compounds **1** and **2a**. On the other hand, compound **1** displayed a six-fold higher A_{2A} AR binding than **5a**.

Since compound **5a** favoured both A_1 and A_{2A} AR affinity (A_1K_i = 3.67 μM; $A_{2A}K_i$ = 18.7 μM), further SAR of the quinazolinones was explored (**5a**–**j**) at ring B. Several *para*-substituents on the phenyl ring (**5b**–**g**) and other heterocyclic ring systems which included a furyl (**5h**) and a thiophene (**5i**) ring were investigated. In addition, the insertion of a styryl side-chain (**5j**) between ring C and phenyl ring B was also explored. Halogen substitution (**5b**–**d**) exhibited no affinity towards either the A_1 or A_{2A} AR subtypes. In contrast, *para*-substitution with a CH_3 (**5e**) group exerted a favourable effect on A_1 AR affinity with a K_i value of 2.50 μM. This structural modification resulted in the highest A_1 AR activity documented for the current study. Interestingly, simultaneous *meta*- and *para*-substitution of ring B with a methoxy group led to compound **5g** with gained A_{2A} AR affinity ($A_{2A}K_i$ = 2.81 μM), whereas mono-substitution with an OCH_3 in the *para*-position (**5f**) led to diminished A_{2A} AR affinity. Noteworthy, compound **5g** displayed the best A_{2A} AR affinity amongst the test compounds. Surprisingly the C2-substituted furyl ring (**5h**) exhibited both A_1 and A_{2A} AR affinity in the low micromolar range (A_1K_i = 4.62 μM; $A_{2A}K_i$ = 8.11 μM).

In a previous study rearrangement of the ketone and hetero oxygen in ring C of compound **1** afforded compound **6** with diminished AR affinity of both AR subtypes [29]. Based on the latter finding, compound **2a** was directly compared to compound **7**. This structural modification resulted in a three-fold decrease in A_1 AR activity (**2a** A_1K_i = 6.38 μM vs **7** A_1K_i = 17.3 μM), while A_{2A} AR affinity remained elusive. Generally, the optimal arrangement of the ketone and hetero oxygen of ring C to favour A_1 AR affinity is exhibited by the benzo-α-pyrone (**1**) and benzoxazinone (**2a**) backbones.

Previously, the benzo-α-pyrone based derivative **1** was found to act as an A_1 AR antagonist [29]. In analogy to compound **1**, the agonistic or antagonistic activity at the A_1 AR subtype for compounds **2a**, **3**, **5a**, **5e** and **5h** were explored by performing guanosine triphosphate (GTP) shift experiments as described in the literature [42]. In the case of an A_1 AR agonist, 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. On the other hand, an A_1 AR antagonist is anticipated not to shift the binding curve significantly in the presence of GTP [35, 36, 43]. Generally, a GTP shift of approximately 6 is indicative of a full agonist and a GTP shift of 1, a full antagonist [38]. The GTP shift binding curve of N^6-cyclopentyladenosine (CPA) (Table 1, Figure 3) exhibited a GTP shift value of 5.8, thus, indicative of a full agonist. In contrast, in the presence of GTP, compounds **2a**, **3**, **5a, 5e** and **5h** was documented with no significant shift of the binding curve and they exhibited shifts of 1.09 (**2a**), 1.12 (**3**), 1.02 (**5a**), 1.18 (**5e**) and 1.23 (**5h**), respectively.

Thus, compounds **2a**, **3**, **5a**, **5e** and **5h** may be considered antagonists of the A_1 AR (Figure 3).

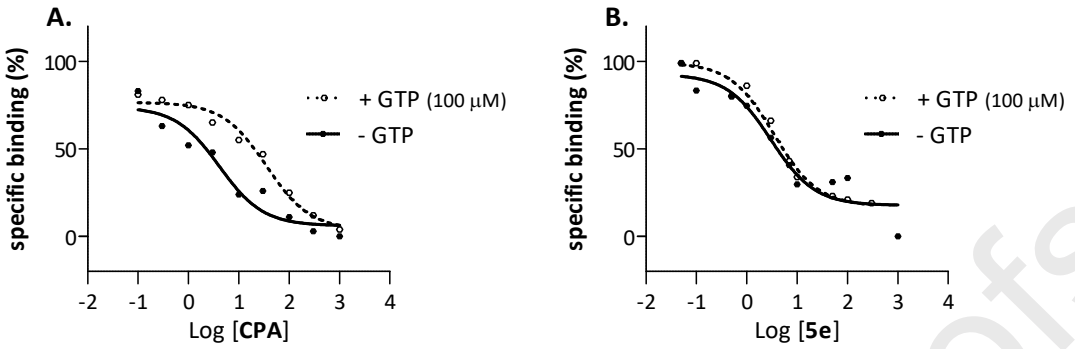


Figure 3: The binding curves of the reference compound CPA and **5e** are examples of A_1 AR agonistic and antagonistic action, respectively. The functionality was determined via GTP shift assays (with and without 100 µM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. (**A**) A GTP shift of 5.8 was calculated for CPA and (**B**) a GTP shift of 1.18 was calculated for compound **5e**.

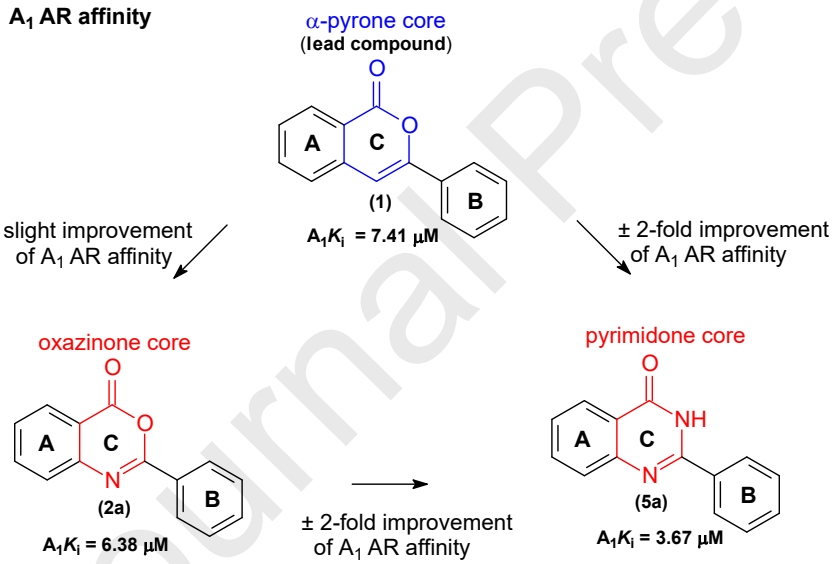


Figure 4: Structural changes to the α-pyrone core resulted in the pyrimidone core and oxazinone core. The pyrimidone core (**5a**) exhibited improved A_1 AR affinity in analogy to the oxazinone core (**2a**).

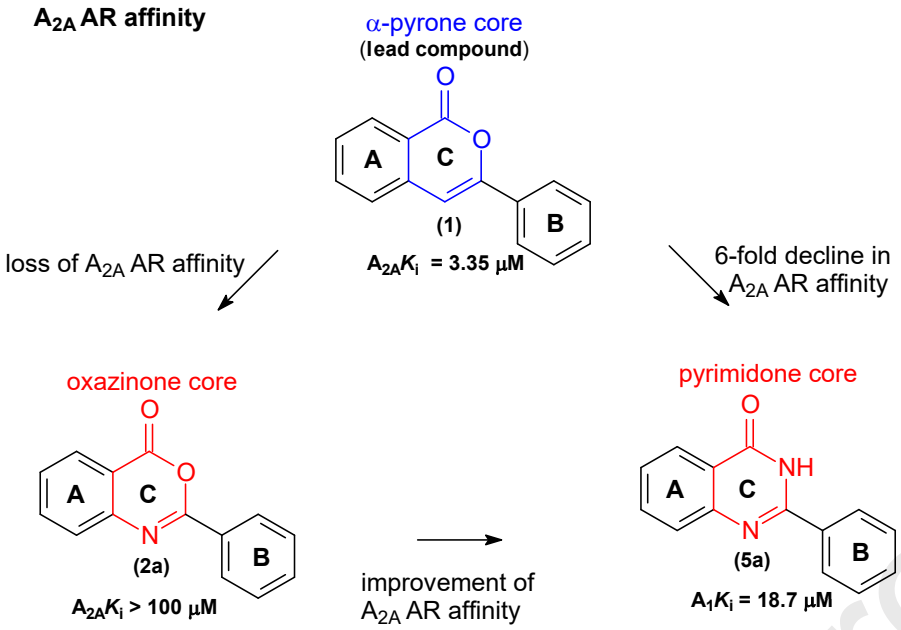


Figure 5: Structural changes to the α-pyrone core resulted in the pyrimidone core and oxazinone core. The pyrimidone core (**5a**) exhibited improved A_{2A} AR affinity in analogy to the oxazinone core (**2a**).

In conclusion, the newly proposed structural modifications of the benzo-α-pyrone based derivative 3-phenyl-1H-2-benzopyran-1-one (**1**) resulted in varying degrees of affinity and selectivity towards the A_1 and A_{2A} AR subtypes. The A_1 AR binding was improved two-fold after replacing the α-pyrone core (ring C) of compound **1** with a pyrimidone ring to afford the quinazolinone derivative **5a** (**1** A_1K_i = 7.41 μM vs **5a** A_1K_i = 3.67 μM) (Figure 4). However, replacement of the α-pyrone core (ring C) with an oxazinone afforded the benzoxazinone derivative **2a** which only showed a slight A_1 AR affinity improvement (**1** A_1K_i = 7.41 μM vs **2a** A_1K_i = 6.38 μM). Generally, the A_{2A} AR binding was best governed by ring C as the α-pyrone (**1** $A_{2A}K_i$ = 3.35 μM) compared to oxazinone (**2a** $A_{2A}K_i$ = > 100 μM) and pyrimidone (**5a** $A_{2A}K_i$ = 18.7 μM) (Figure 5).

Noteworthy, the benzoxazinone and quinazolinone based scaffolds were previously unknown to exhibit AR affinity and that analogues of these moieties may act as A_1 AR antagonists. Generally, the C2-substituted quinazolinone derivatives displayed superior A_1 and A_{2A} AR affinity over the C2-substituted benzoxazinone derivatives. Among the test compounds, the quinazolinone analogue **5a** exhibited the second-highest A_1 AR binding (A_1K_i = 3.67 μM). The introduction of a CH_3 group (*para*-position) to ring B of **5a** afforded compound **5e** (A_1K_i = 2.50 μM)) with the highest A_1 AR affinity among the compounds investigated. Furthermore, **5e** was found to be selective for the A_1 AR subtype. The 3,4-dimethoxy substituted quinazolinone **5g** exhibited the highest A_{2A} AR affinity and selectivity ($A_{2A}K_i$ = 2.81 μM). Thus, **5g** is an example

of a selective A_{2A} AR drug and may find therapeutic relevance to enhance PD-related motor dysfunction and exert neuroprotective properties, while **5e** may enhance cognitive dysfunction associated with PD. The identified drug candidates (**5a**, **5e** and **5g**) are ideal for future drug optimization and *in vivo* examinations as selective A_1 (**5a**, **5e**) and A_{2A} (**5g**) AR drugs in the treatment of neurological disorders.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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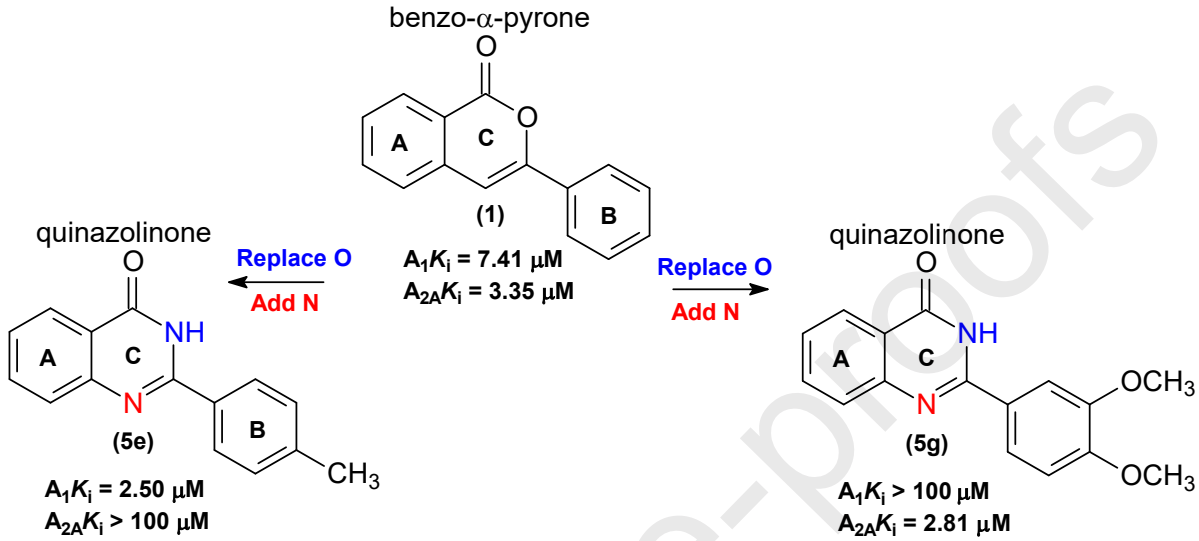

Highlights:

- The C2-substituted quinazolinones are ideal to identify novel AR antagonists.
- 3,4-OCH_3-substitution on ring B of quinazolinone modulate A_{2A} AR affinity.
- 4-CH_3-substitution on ring B of quinazolinone modulate A_1 AR affinity.
- Quinazolinones display superior AR affinity over benzoxazinones.
- A_{2A} AR binding is best governed by ring C as the α-pyrone.
- Further structural modifications are necessary.