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Discovery of 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine derivatives as novel adenosine A_1 and A_{2A} receptor antagonists.

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Abstract

Based on a previous report that a series of 8-(phenoxymethyl)-xanthines may be promising leads for the design of A_1 adenosine receptor antagonists, selected novel and known 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine and 1,3,7-trimethyl-8-(phenoxymethyl)-xanthine analogs were synthesized and evaluated for their A_1 and A_{2A} adenosine receptor affinity. Generally, the study compounds exhibited affinity for both the A_1 and A_{2A} adenosine receptors. Replacement of the 1,3-dimethyl-substition with a 1,3-diethyl-substition pattern increased A_1 and A_{2A} binding affinity. Overall it was found that *para*-substitution on the phenoxymethyl side-chain of the 1,3-diethyl-xanthines decreased A_1 affinity except for the 4-Br analog (**4f**) exhibiting the best A_1 affinity in the submicromolar range. On the other hand A_{2A} affinity for the 1,3-diethyl-xanthines were increased with *para*-substitution and the 4-OCH_3 (**4b**) analog showed the best A_{2A} affinity with a K_i value of 237 nM. The 1,3-diethyl-substituted analogs (**4a**, and **4f**) behaved as A_1 adenosine receptor antagonists in GTP shift assays performed with rat whole brain membranes expressing A_1 adenosine receptors. This study concludes that *para*-substituted 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine analogs represent novel A_1 and A_{2A} adenosine receptor antagonists that are appropriate for the design of therapies for neurodegenerative disorders such as Parkinson's and Alzheimer's disease.

Keywords: Caffeine, 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthines, 1,3,7-trimethyl-8-(phenoxymethyl)-xanthines, Adenosine A_1 and A_{2A} receptor antagonists

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The actions of adenosine may be mediated by four adenosine receptor (AR) subtypes [1], namely the A_1, A_{2A}, A_{2B} and A_3 ARs [2], all of which couple to G proteins [3]. The A_1 AR antagonists may potentially find therapeutic relevance as drug targets for numerous diseases including asthma [4], cardiovascular disorders [5], renal diseases [6] and cognitive deficits [7]. This wide range for therapeutic potential may be contributed to the wide distribution of the A_1 ARs throughout the human body, eg. lungs [4], heart [8], kidneys [6] and brain [9]. The ARs mediate bronchoconstriction, inflammation, increased endothelial cell permeability, and mucin production in the lung [4], while these receptors mediate negative chronotropic, dromotropic, and inotropic effects in the heart [8] and lead to vasoconstriction, reduction of glomerular filtration rate, inhibition of renin secretion, and inhibition of neurotransmitter release after activation in the kidneys [6]. Furthermore, the A_1 ARs are widely expressed throughout the human brain, including the hippocampus and prefrontal cortex that is important brain areas for cognitive function [10]. The A_1 AR antagonists depolarize postsynaptic neurons and presynaptically enhance the release of a number of neurotransmitters, e.g. acetylcholine, glutamate, serotonin and norepinephrine. Antagonists of the A_1 AR have been suggested as a potential treatment of cognitive deficits in animal models [11] and this release of neurotransmitters could find application in the treatment of cognitive deficits associated with Alzheimer's disease (AD) and Parkinson' disease (PD).

The expression of A_{2A} ARs are approximately 20 times greater in the basal ganglia, compared to the rest of the brain [12], with the highest abundance found in the striatum [13,14]. Furthermore, the A_{2A} ARs are localized exclusively in the dopamine enriched areas of the brain and mediate inhibition of locomotor activity [15]. PD is a neurodegenerative disorder and are pathologically defined by neuronal loss in the substantia nigra *pars compacta* (SNpc), followed by the loss of striatal dopamine content [16]. This dopamine deficiency within the basal ganglia, leads to the characteristic PD-associated impaired motor functions [17]. For this reason it is suggested that A_{2A} AR antagonists may show promise as a novel treatment of PD-related motor symptoms [18]. Further evidence proposes that A_{2A} AR antagonists exhibit a lower risk of dyskinesia when used in conjunction with L-DOPA [18]. In addition, these antagonists may also exhibit neuroprotective properties [19,20]. Therefore, selective A_{2A} AR antagonists present an attractive non-dopaminergic target to improve the PD-related motor symptoms [18].

Xanthine derivatives represent an important class of naturally occurring (e.g. caffeine, **1**) and/or synthetic (e.g. CSC, KW-6002) compounds that consist of a fused six- and five-membered nitrogen containing ring system. Some xanthine derivatives (e.g. caffeine, CSC and KW-6002) have previously been evaluated in the treatment of AD and PD by acting as AR antagonists (Figure 1) [21,22,23]. Caffeine (**1**) is a well-known 1,3,7-trimethyl-substituted xanthine that acts as a non-selective A_1 and A_{2A} AR antagonist (A_1K_i = 55 μM; $A_{2A}K_i$ = 50 μM) [24]. Substitution of caffeine at the C8 position has previously shown to result in gained A_1 and A_{2A} AR affinity. For example, 8-phenylcaffeine presented with an increased affinity for both the A_1 and A_{2A} ARs, with selectivity for the A_1 AR over the A_{2A} isoform (A_1K_i = 17 μM; $A_{2A}K_i$ = 27 μM) [24]. Furthermore, C8 substitution with a phenoxymethyl side-chain has resulted in 1,3,7-trimethyl-8-(phenoxymethyl)-xanthine (**2a**, A_1K_i = 3.09 μM; $A_{2A}K_i$ = 2.37 μM) [25] and displayed an approximate 17- and 21-fold enhancement towards the A_1 and A_{2A} AR affinity, respectively, when compared to caffeine (**1**). In addition, the 1,3-diethyl-substitution pattern, 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine (**4a**), lead to an approximate 63- and 26-fold increase towards A_1 and A_{2A} AR affinity (A_1K_i = 0.874 μM; $A_{2A}K_i$ = 1.91 μM) [25].

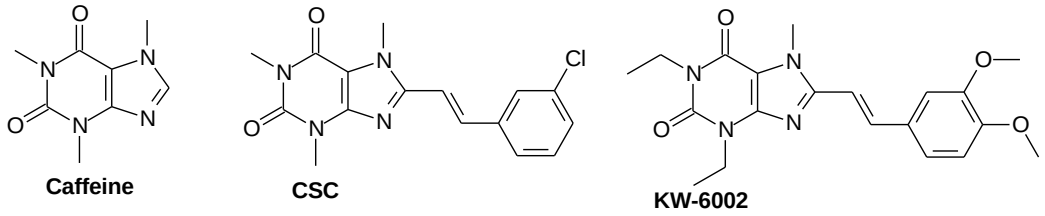
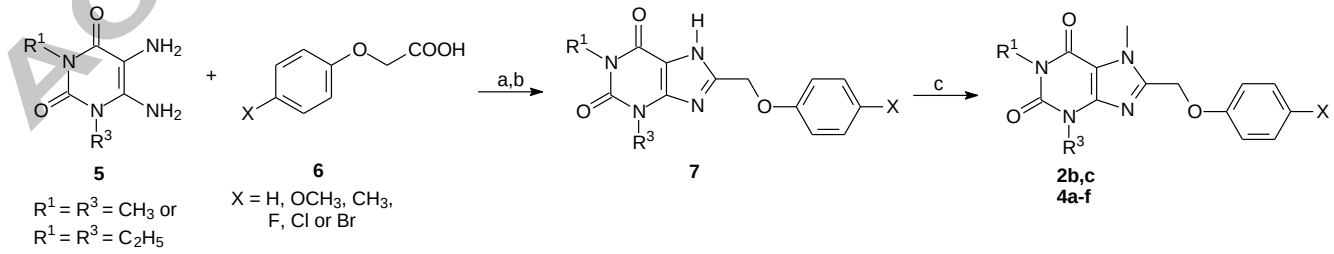


Figure 1. Xanthine structures of AR antagonists bearing 1,3-dimethyl-substitution (caffeine and CSC) and 1,3-diethyl-substitution (KW-6002).

To further explore the influence of 1,3-dimethyl-substitution and gain insight into the structure activity relationships, we compared 1,3-diethyl-7-methyl-xanthine (**3**) to its 1,3-dimethyl-substitution counterpart, namely caffeine (**1**), as the 1,3-diethyl-substitution pattern is expected to enhance AR affinity as shown by previously synthesized xanthine derivatives, CSC and KW-6002 (Figure 1) [26,27]. Based on recent research suggesting that 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthines represent an interesting class of xanthine derivatives that possess affinity for the A_1 and A_{2A} ARs [25] we synthesized and compared a series of *para*-substituted 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine derivatives (**4a–f**) to their 1,3,7-trimethyl-8-(phenoxymethyl)-xanthine counterparts (**2a–f**) as potential new A_1 and/or A_{2A} AR antagonists.

The starting materials, 1,3-dimethyl- and 1,3-diethyl-5,6-diaminouracil, was prepared as reported in the literature [25,28]. The phenoxyacetic acids that were not commercially available were synthesized as previously documented [29,30,31]. Overall, the desired target compounds were synthesized as described elsewhere [25,32]. In short, 1,3-dialkyl-5,6-diaminouracil (**5**) was reacted with an appropriately substituted phenoxyacetic acid (**6**) in the presence of the dehydrating reagent, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) (Scheme 1). The obtained intermediary amide was treated with aqueous NaOH (1 N) solution to yield the corresponding 1,3-dimethyl-8-phenoxymethyl-7*H*-xanthinyl or 1,3-diethyl-8-phenoxymethyl-7*H*-xanthinyl analogs [32]. These intermediary 1,3-dialkyl-8-phenoxymethyl-7*H*-xanthinyl analogs (**7**) were subsequently methylated in the presence of potassium carbonate and an excess iodomethane in order to obtain the desired 7-methylated novel test compounds (**2b**, **2c**, **4a–f**). The target compounds (yields of 79–90%) were purified by recrystallization from ethanol, and the structures and purities were verified by ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis (see *Supporting Information*). The known test compounds **2a** [25], **2d–2f** [33], **3** [32,34] and **4a** [25] were prepared as described in literature.

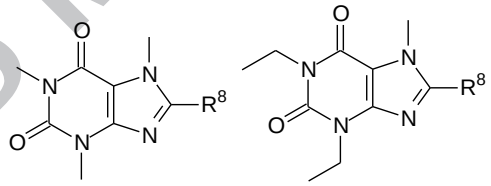


Scheme 1: Synthetic pathway to 8-(phenoxymethyl)-xanthine analogs (**2b**, **2c**, **4a-f**). Reagents and conditions: (a) EDAC, dioxane:H_2O (1:1), room temperature; (b) NaOH (aq), reflux; (c) CH_3I, K_2CO_3, DMF.

The A_1 and A_{2A} AR affinities of all the test compounds were determined with radioligand binding experiments (ethics number NWU-0035-10-A5) as described previously [25]. The A_1 AR radioligand binding assay was performed in the presence of the radioligand [^{3}H]-8-cylcopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) with rat whole brain membranes expressing the A_1 AR [25,35]. In turn the A_{2A} AR binding affinity was measured at rat striatal membranes with 5'-N-[^{3}H]-ethylcarboxamideadenosine ([^{3}H]NECA) as radioligand [25,36]. Selected compounds were further evaluated to assess if they act as agonists or antagonists of the A_1 AR in GTP shift assays with rat whole brain membranes in the absence and presence of 100 μM GTP [25]. The competition curves were obtained by plotting the percentage binding vs. the logarithm of the test compound's concentrations (ranging between 0 and 100 μM) by using the Prism software package (GraphPad Software Inc.). The radioligand binding assays were carried out in triplicate and the dissociation constant values (K_i) are expressed as mean ± standard error of mean (SEM). The K_i values were calculated by using the K_d values of 0.36 nM for [^{3}H]DPCPX at rat whole brain membranes [25,35], while 15.3 nM for [^{3}H]NECA at rat striata membrane [25,36] were used. Furthermore, GTP shifts are calculated by dividing the K_i value of a compound reported in the presence of GTP by the K_i value obtained in the absence of GTP [37]. A compound with a calculated GTP shift of approximately 1 is considered an antagonist, in turn the presence of GTP affects the competition curve of an agonist and shifts the curve to the right [25,38]. The A_1 and A_{2A} AR affinity and GTP shift results of the test compounds are summarized in Table 1.

Table 1

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



Compd	R^8	K_i ± SEM (μM)[a]				
		A_1	A_{2A}	A_1 + GTP[b]	GTP Shift[c]	SI[d] (A_{2A}/A_1)
1,3,7-trimethyl-xanthine (caffeine)						
1	-H	41[e]; 55[f]	43[e]; 50[f]	-	-	1.1
1,3,7-trimethyl-phenoxymethyl-xanthines						
2a	$-CH_2-O-C_6H_5$	3.09[g]	2.37[g]	-	-	0.8
2b	$-CH_2-O-[4-OCH_3-(C_6H_5)]$	4.67 ± 0.39	2.40 ± 0.66	-	-	0.5
2c	$-CH_2-O-[4-CH_3-(C_6H_5)]$	6.76 ± 1.11	3.64 ± 0.09	-	-	0.5
2d	$-CH_2-O-[4-F-(C_6H_5)]$	9.19 ± 0.90	2.57 ± 0.34	-	-	0.3
2e	$-CH_2-O-[4-Cl-(C_6H_5)]$	5.56 ± 0.69	0.94 ± 0.23	-	-	0.2
2f	$-CH_2-O-[4-Br-(C_6H_5)]$	3.69 ± 0.60	1.73 ± 0.92	-	-	0.5
1,3,7-triethyl-xanthine						
3	-H	5.61 ± 0.95	2.69 ± 0.29	-	-	0.5
1,3-diethyl-7-methyl-phenoxymethyl-xanthines						
4a	$-CH_2-O-C_6H_5$	0.874[g]	1.91[g]	0.892 ± 0.05	1	2.2
4b	$-CH_2-O-[4-OCH_3-(C_6H_5)]$	1.22 ± 0.20	0.237 ± 0.06	-	-	0.2
4c	$-CH_2-O-[4-CH_3-(C_6H_5)]$	1.42 ± 0.08	1.30 ± 0.42	-	-	0.9
4d	$-CH_2-O-[4-F-(C_6H_5)]$	1.23 ± 0.26	0.841 ± 0.24	-	-	0.7
4e	$-CH_2-O-[4-Cl-(C_6H_5)]$	0.974 ± 0.21	1.04 ± 0.19	-	-	1.1

4f	-CH_2-O-[4-Br-(C_6H_5)]	0.264 ± 0.08	1.36 ± 0.16	0.267 ± .030	1	5.2
CPA	(A_1 agonist)	0.015 [g]	-	0.99 [g]	6.48 [g]	-

[a] All K_i values determined in triplicate and expressed as mean ± SEM.
[b] GTP shift assay, where the 100 μM GTP was added to the A_1 AR radioligand binding assay.
[c] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP.
[d] Selectivity index (SI) for the A_1 receptor isoform calculated as the ratio of K_i (A_{2A})/K_i (A_1).
[e] Literature values obtained from reference [39].
[f] Literature values obtained from reference [24].
[g] Literature values obtained from reference [25].

Caffeine (**1**) is a non-selective A_1 and A_{2A} AR antagonist with beneficial effects towards both motor and non-motor symptoms associated with PD, but it is not considered as a treatment option due to its poor A_1 and A_{2A} AR binding affinities (A_1K_i = 41–55 μM; $A_{2A}K_i$ = 43–50 μM) [24,40]. Previous research [25] showed that substitution at position C8 of caffeine (**1**) with a phenoxymethyl side chain (**2a**) improved affinity for both A_1 and A_{2A} AR (A_1K_i = 3.09 μM; $A_{2A}K_i$ = 2.37 μM)(Table 1, Figure 2). Furthermore, the current study investigated the influence of *para*-substitution on compound **2a**. Overall *para*-substitution seemed to decrease affinity for A_1 AR as follow: **2f** (4-Br) > **2b** (4-OCH_3) > **2e** (4-Cl) > **2c** (4-CH_3) > **2d** (4-F). *Para*-substitution only slightly decreased A_{2A} AR affinity (**2b**, **2c**, **2d**) with the exception of compound **2e** (4-Cl) and **2f** (4-Br) where a slight increase was observed: **2e** (4-Cl) > **2f** (4-Br) > **2b** (4-OCH_3) > **2d** (4F) >**2c** (4-CH_3) (Table 1).

The influence of the 1,3-diethyl-substitution pattern was investigated by comparing caffeine (1,3-dimethyl-substitution, **1**) to compound **3** (1,3-diethyl-substitution). 1,3-Diethyl-substitution improved affinity for rat A_1 and A_{2A} ARs, with K_i values in the low micromolar range (A_1K_i = 5.61 μM; $A_{2A}K_i$ = 2.69 μM) and a slight selectivity towards the A_{2A} AR isoform was observed. Thus, AR affinity seems to be governed by replacement of the 1,3-dimethyl-substitution with a 1,3-diethyl-substitution pattern, which support previous findings that 1,3-diethyl-substitution compared to 1,3-dimethyl-substitution enhances A_1/A_{2A} AR affinity [25,40].

Furthermore, C8 substitution of compound **3** (1,3-diethyl-7-methyl-xanthine) with a phenoxymethyl side-chain, yielded 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine (**4a**), which showed the same trend as its counterpart caffeine (**1**). Compared to compound **3**, the 8-(phenoxymethyl)-xanthine analog **4a** showed a 6-fold enhancement in A_1 AR affinity (A_1K_i = 0.892 μM) and a slight increase (1.4 fold) in A_{2A} AR affinity ($A_{2A}K_i$ = 1.91 μM) [25]. In order to further investigate the A_1/A_{2A} AR structure-activity relationships (SARs) of the 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine scaffold, a number *para*-substituted 1,3-diethyl-7-methyl-phenoxymethyl-xanthine analogs were synthesized (**4b**, **4c**, **4d**, **4e**, **4f**) to compare with the 1,3,7-trimethyl-xanthine analogs (**2b**, **2c**, **2d**, **2e**, **2f**) (Table 1).

Generally *para*-substitution on the 1,3-diethyl-7-methyl-xanthine analogs (**4a–f**) showed the same pattern as the 1,3,7-trimethyl-xanthine analogs on A_1 AR affinity. *Para*-substitution decreased A_1 AR affinity with the exception of compound **4f** (4-Br) which showed an increase in A_1 AR affinity with a submicromolar K_i value of 264 nM. Affinity for the A_1 AR was in the following order: **4f** (4-Br) > **4e** (4-Cl) > **4b** (4-OCH_3) > **4d** (4-F) > **4c** (4-CH_3). In contrast to the 1,3,7-trimethyl analogs, *para*-substitution to the 1,3-diethyl analogs increased A_{2A} AR affinity overall with compound **4b** showing an 8-fold improved A_{2A} AR affinity (**4a** vs. **4b**) with a submicromolar K_i value of 237 nM. The order

of A_{2A} binding affinity for the 1,3-diethyl-analogs (**4a–4f**) may be summarized as follow: **4b** (4-OCH_3) > **4d** (4-F) > **4e** (4-Cl) > **4c** (CH_3) > **4f** (4-Br) > **4a** (4-H).

Comparing the 1,3,7-trimethyl-phenoxymethyl-xanthines to their counterparts, 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthines (**1** vs **3**; **2a** vs **4a**; **2b** vs **4b**; **2c** vs **4c**; **2d** vs **4d**; **2e** vs **4e**; **2f** vs **4f**), we found an overall increase in both the A_1 and A_{2A} affinity (Table 1), thus concluding that AR affinity may be governed by 1,3-diethyl-substitution at the xanthine core (Figure 2).

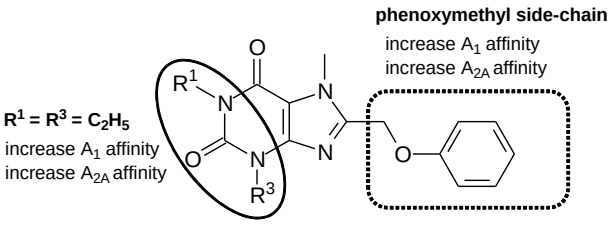


Figure 2: A general depiction showing the influence of 1,3-diethyl-substitution and C8-phenoxymethyl-substitution at the xanthine core on the A_1 and A_{2A} AR affinity.

In order to demonstrate if the two compounds possessing the highest A_1 AR binding affinity (**4a** and **4f**) acted as antagonists or agonists, GTP shift experiments were performed. Compounds **4a** and **4f** showed no significant shifts of the binding curves in the presence of GTP and these compounds may be considered as antagonists of the A_1 AR (Table 1, Figure 3).

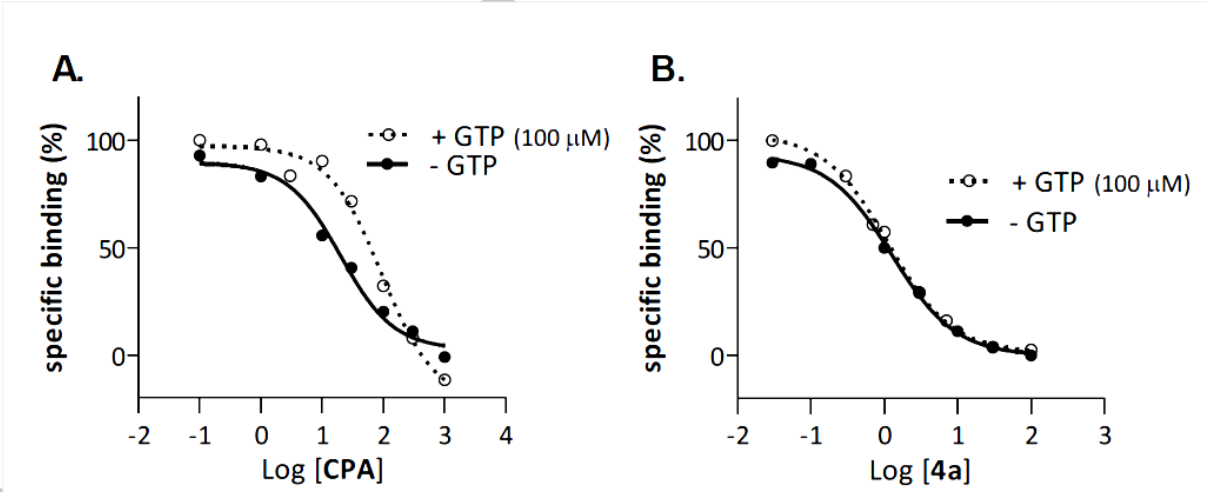


Figure 3. The binding curves of compounds CPA (reference agonist) and **4a** (test compound), indicating their A_1 AR agonist/antagonistic action as determined via GTP shift assays (with and without 100 µM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. (A) GTP shift of 6.48 calculated for the A_1 AR agonist CPA [25]. (B) GTP shift of 1.2 calculated for the A_1 AR antagonist **4a**.

The current study shows that the novel 8-(phenoxymethyl)-xanthines (**2b**, **2c**, **4b–f**) as well as the previously synthesized 8-(phenoxymethyl)-xanthine derivatives (**2d–f**) exhibited potential as potent AR antagonists with K_i values in the low micromolar to nanomolar range (A_1K_i = 6.76 – 0.264 µM; $A_{2A}K_i$ = 2.40 – 0.237 µM). Replacement of the 1,3-dimethyl-substition with a 1,3-diethyl-pattern is important for increased A_1 and A_{2A} binding affinity. Overall it was found that *para*-substitution on the phenoxymethyl side-chain decreased A_1 affinity except for the 4-Br (**4f**) analog which exhibited the

best and enhanced A_1 affinity. On the other hand A_{2A} affinity was increased with *para*-substitution and compound **4b** showed the best A_{2A} affinity. Compounds **4a** and **4f** was characterized as antagonists of the A_1 AR and due to the structural similarity of the test compounds it is reasonable to assume that the 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine (**4b–f**) and 1,3,7-trimethyl-8-(phenoxymethyl)-xanthine (**2b–e**) derivatives may act as A_1 AR antagonists. In conclusion, compounds **4b** and **4f** was found to possess the highest A_{2A} and A_1 AR binding affinities respectively, among the investigated compounds and therefore these 8-(phenoxymethyl)-xanthine analogs are ideal drug candidates for future *in vivo* investigation as adenosine receptor antagonists in neurodegenerative disorders, such as AD and PD.

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Discovery of 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine derivatives as novel adenosine A_1 and A_{2A} receptor antagonists

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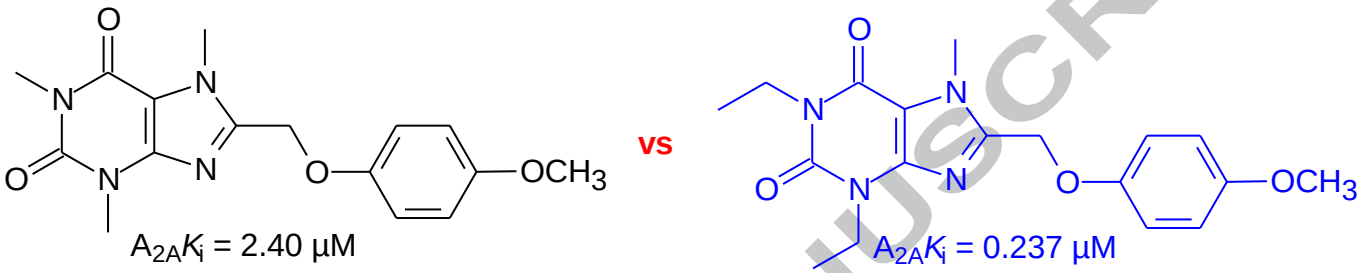
Graphical abstract



Discovery of 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthine derivatives as novel adenosine A1 and A2A receptor antagonists.

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Highlights:

- A_1 receptors are considered drug targets for Alzheimer's and Parkinson's disease.
- A_{2A} receptors are considered drug targets for Alzheimer's and Parkinson's disease.
- Novel 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthines were synthesized.
- 1,3-diethyl-7-methyl-8-(phenoxymethyl)-xanthines possess A_1 and A_{2A} affinity.