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The adenosine A_{2A} antagonistic properties of selected C8-substituted xanthines

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ABSTRACT

The adenosine A_{2A} receptor is considered to be an important target for the development of new therapies for Parkinson's disease. Several antagonists of the A_{2A} receptor have entered clinical trials for this purpose and many research groups have initiated programs to develop A_{2A} receptor antagonists. Most A_{2A} receptor antagonists belong to two different chemical classes, the xanthine derivatives and the amino-substituted heterocyclic compounds. In an attempt to discover high affinity A_{2A} receptor antagonists and to further explore the structure–activity relationships (SARs) of A_{2A} antagonism by the xanthine class of compounds, this study examines the A_{2A} antagonistic properties of series of (*E*)-8-styrylxanthines, 8-(phenoxymethyl)xanthines and 8-(3-phenylpropyl)xanthines. The results document that among these series, the (*E*)-8-styrylxanthines have the highest binding affinities with the most potent homologue, (*E*)-1,3-diethyl-7-methyl-8-[(3-trifluoromethyl)styryl]xanthine, exhibiting a K_i value of 11.9 nM. This compound was also effective in reversing haloperidol-induced catalepsy in rats, providing evidence that it is in fact an A_{2A} receptor antagonist. The importance of substitution at C8 with the styryl moiety was demonstrated by the finding that none of the 8-(phenoxymethyl)xanthines and 8-(3-phenylpropyl)xanthines exhibited high binding affinities for the A_{2A} receptor.

1. Introduction

The purine nucleoside, adenosine, is involved in numerous functions in the central nervous system (CNS) and mediates most of its effects through the activation of four guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) subtypes, A_1, A_{2A}, A_{2B} and A_3 [1]. Through its actions at these receptors, adenosine regulates a variety of physiological responses and adenosine receptors have become targets for the treatment of a variety of disease states [2]. Of particular interest is a potential role for the A_{2A} receptor subtype in the therapy of Parkinson's disease [3]. A_{2A} receptors are highly enriched in striatopallidal neurons in the striatum where they are colocalized with the dopamine D_2 receptors [4,5]. A_{2A} and D_2 receptors are thought to interact with each other, with A_{2A} receptor stimulation exerting a functional antagonistic effect on D_2 receptors [6,7]. This antagonistic relationship provides the basis for a role of A_{2A} receptors in the treatment of Parkinson's disease. Blockade of the A_{2A} receptor potentiates D_2 receptor-mediated neurotransmission and therefore reduces the effects of striatal dopamine depletion in Parkinson's disease [8,9]. These observations suggest that A_{2A} antagonism also may possibly potentiate the motor actions of levodopa and dopamine agonists [10–12]. An example of this behaviour is found with (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002, istradefylline, **1**), an A_{2A} antagonist which has entered clinical trials for the treatment of Parkinson's disease (Fig. 1) [11,12]. It was demonstrated that, in Parkinson's disease patients, KW-6002 potentiates the symptomatic benefits of a reduced dose of levodopa and produces motor enhancement that is comparable to that of an optimal levodopa dose [10]. In addition, KW-6002 prolongs the therapeutic action of a full dose of levodopa [13]. Pharmacological and epidemiological evidence suggest that A_{2A} antagonists may possess neuroprotective properties. For example, selective A_{2A} antagonists, and not A_1 antagonists, exert a protective effect against the neurotoxic actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [14,15] and 6-hydroxydopamine [15,16] in animals. Furthermore, the consumption of caffeine (**2**), a nonselective A_1/A_{2A} antagonist, has been correlated with a reduced risk of developing Parkinson's disease [17–20]. This protective effect is

Abbreviations: CNS, central nervous system; CPA, cyclopentyladenosine; CSC, (*E*)-8-(3-chlorostyryl)caffeine; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide; GPCR, guanine nucleotide-binding protein (G protein)-coupled receptor; MAO-B, monoamine oxidase B; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; [^{3}H]NECA, N-[^{3}H]ethyladenosin-5′-uronamide; [^{3}H]DPCPX, 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine; SAR, structure–activity relationship; SI, selectivity index.

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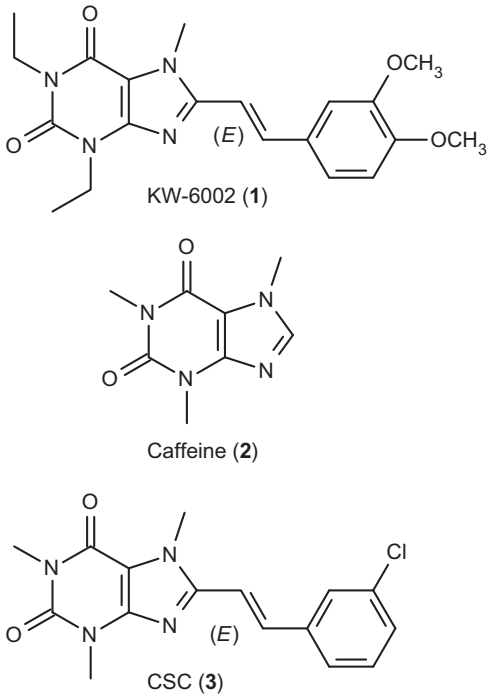


Fig. 1. The structures of KW-6002 (**1**), caffeine (**2**) and CSC (**3**).

thought to be related to blockade of A_{2A} receptors by caffeine [14]. The symptomatic and protective benefits of A_{2A} antagonists therefore make them attractive agents for the therapy of Parkinson's disease.

Based on these considerations, several research groups have initiated programs to develop A_{2A} receptor antagonists and at least five compounds have entered clinical trials for the treatment of Parkinson's disease [21]. Most A_{2A} receptor antagonists may be classified into two different chemical classes, the xanthine derivatives and the amino-substituted heterocyclic compounds [22]. KW-6002 and (*E*)-8-(3-chlorostyryl)caffeine (CSC, **3**; IC_{50} = 36–54 nM), a frequently used reference A_{2A} antagonist, are examples of xanthine derived A_{2A} antagonists [23,24]. It is noteworthy that certain xanthine derived A_{2A} antagonists also are highly potent monoamine oxidase B (MAO-B) inhibitors. For example, CSC is reported to inhibit MAO-B with a K_i value of 80.6 nM, a value that is comparable to that reported for its antagonism of A_{2A} receptors [25,26]. In Parkinson's disease, MAO-B inhibitors potentiate the motor restorative effects of levodopa by reducing the metabolic breakdown of dopamine in the central nervous system [27]. In addition, MAO-B inhibitors may also protect against the underlying neurodegenerative processes in Parkinson's disease. Since MAO-B inhibitors are considered useful in the therapy of Parkinson's disease, dual-target-directed compounds such as CSC may possess enhanced therapeutic value. The combination of A_{2A} antagonism and MAO-B inhibition in a single drug may be particularly advantageous as an adjunct to levodopa therapy [28].

An important structural feature of KW-6002 and CSC is the styryl moiety of the (*E*) configuration at C8 of the xanthine ring. While a wide variety of substituents on the styryl phenyl ring are tolerated, modification of the styryl double bond is usually associated with a loss of A_{2A} antagonistic activity. For example, (*E*)-8-styrylcaffeine displays a K_i value for the antagonism of A_{2A} receptors of 94 nM, while the corresponding phenyl substituted homologue is a weak A_{2A} receptor antagonist with a K_i value of 19 μM [24]. Structure–activity relationship (SAR) studies have further shown that a variety of substituents on the N1, N3 and N7 positions of the xanthine ring are appropriate for A_{2A} antagonism. These substituents include the methyl, ethyl, propyl and propargyl functional groups [23,24,29,30]. Based on these observations, the present study aims to discover high affinity xanthine derived A_{2A} antagonists and to further explore the SARs of A_{2A} antagonism by the xanthine class of compounds. For this purpose, the A_{2A} antagonistic properties of series of novel (*E*)-8-styrylxanthines (**4**–**7**) will be investigated and compared to those of 8-(phenoxymethyl) xanthines (**8**) and 8-(3-phenylpropyl)xanthines (**9**), chemical classes which have not previously been examined for potential A_{2A} antagonistic properties. The selection of compounds from series **8** and **9** is based on a recent report that these compounds also are highly potent MAO-B inhibitors [31]. Should they also possess A_{2A} antagonistic properties, these derivatives may be viewed as promising dual-target-directed antiparkinsonian agents. To explore chemical space, these series (**4**–**9**) will be comprised of homologues with different CH_3/C_2H_5 substitution patterns on N1, N3 and N7 of the xanthine ring. For the (*E*)-8-styrylxanthines (**4**–**7**), the effects on A_{2A} antagonism by different halogen (Cl, Br) and halogen containing (CF_3) substituents on the styryl phenyl ring will also be examined. This study also reports, for selected xanthines, the binding affinities to the adenosine A_1 receptor as well as the human MAO-B inhibitory potencies.

2. Results and discussion

2.1. Chemistry

The target xanthine derivatives, series **4**–**9**, were synthesized according to the literature procedure (Fig. 2) [32]. 1,3-Dimethyl- (**10a**) and 1,3-diethyl-5,6-diaminouracil (**10b**), synthesized according to literature, served as key starting materials [33]. The uracils were reacted with the appropriate carboxylic acids, the (*E*)-cinnamic acids (**11**), the phenoxyacetic acids (**12**) and the 4-phenylbutanoic acids (**13**), in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDAC) as dehydrating agent to yield the corresponding intermediary amides. Treatment of the amides with NaOH yielded the corresponding 1,3-dialkyl-7*H*-xanthine analogues (**14**). In order to obtain the desired 7-alkylated xanthine analogues (**4**–**9**), the xanthines **14** were reacted with an excess of iodomethane or iodoethane in the presence of K_2CO_3. The target compounds (yields of 7–93%) were purified by recrystallization from a suitable solvent, and in each instance, the structures and purities were verified by ^1H NMR, ^{13}C NMR, mass spectrometry and HPLC analysis as cited in the Supplementary material.

2.2. In vitro binding to A_{2A} and A_1 receptors

The affinities of compounds **4**–**9** for A_{2A} receptors were examined by the radiolig and binding protocol described in literature [34]. Binding of compounds from series **4**–**9** to the A_{2A} receptors were investigated by measuring the displacement of N-[^{3}H]ethyladenosin-5′-uronamide ([^{3}H]NECA) from rat striatal membranes. These studies were carried out in the presence of N^6-cyclopentyladenosine (CPA) to minimize the binding of [^{3}H]NECA to adenosine A_1 receptors. As positive controls the known A_{2A} antagonists, KW-6002 (**1**), CSC (**3**) and ZM 241385, were included in this study. As shown in Table 1, KW-6002, CSC and ZM 241385 displayed K_i values for the antagonism of A_{2A} receptors of 7.94 nM, 26.2 nM and 2.31 nM, respectively. These values correspond well with the literature values of 2.2 nM [29], 36–54 nM [23,24] and 2 nM [22] for these antagonists, respectively.

The affinities of the (*E*)-8-styrylxanthine (**4**–**7**), 8-(phenoxymethyl)xanthine (**8**) and 8-(3-phenylpropyl)xanthine (**9**) derivatives for A_{2A} receptors are given in Tables 2–4. From the results,

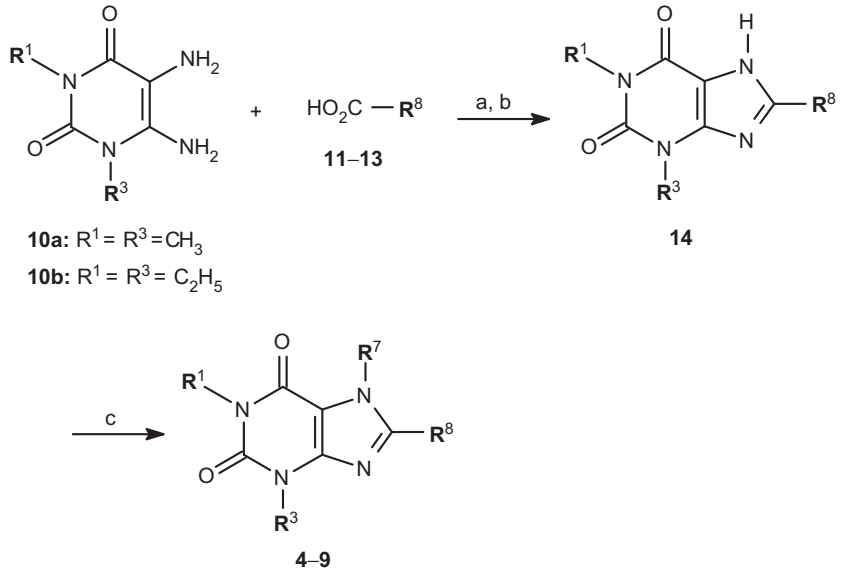


Fig. 2. Synthetic pathway to xanthine derivatives **4**–**9**. Reagents and conditions: (a) EDAC, dioxane:H_2O (1:1); (b) NaOH (aq), reflux; (c) CH_3I or C_2H_5I, K_2CO_3, DMF, ethanol or acetone.

Table 1
The K_i values for the competitive inhibition of [^{3}H]NECA binding to rat striatal adenosine A_{2A} receptors by KW-6002, CSC and ZM 241385.

	K_i values (nM)[a]
KW-6002	7.94 ± 2.05 (2.2)[b]
CSC	26.2 ± 3.49 (36)[c] (54)[d]
ZM 241385	2.31 ± 1.96 (2)[e]

[a] All K_i values were determined in duplicate and are expressed as mean ± SD.
[b] Value obtained from Ref. [29].
[c] Value obtained from Ref. [24].
[d] Value obtained from Ref. [23].
[e] Value obtained from Ref. [22].

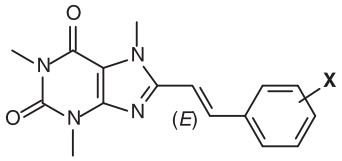
it is evident that the 8-(phenoxymethyl)xanthine (**8**) and (*E*)-8-(3-phenylpropyl)xanthine (**9**) derivatives do not possess affinities for A_{2A} receptors with only one homologue, compound **8a** (K_i = 137 nM), exhibiting moderate affinity. Since different CH_3/C_2H_5 substitution patterns on N1, N3 and N7 do not yield compounds with high binding affinities, it may be concluded that, in contrast to styryl substitution, phenoxymethyl and phenylpropyl substitution on C8 of the xanthine ring are in general not suitable for binding to A_{2A} receptors. In accordance with literature, the (*E*)-8-styrylxanthines were found to possess affinities for the A_{2A} receptor [23,24,32]. Compounds with good binding affinities for the A_{2A} receptor (K_i < 100 nM) were identified among all four series **4**–**7** of (*E*)-8-styrylxanthines examined. The compound with the highest binding affinity among the compounds examined was **6f**, the CF_3 substituted 1,3-diethyl-7-methylxanthine homologue. This compound exhibited a K_i value of 11.9 nM (Fig. 3), a value similar to that of KW-6002 (K_i = 7.94 nM). It is noteworthy that all of the 1,3-diethyl-7-methylxanthine homologues, **6a**–**f**, exhibited high affinities for A_{2A} receptors. This result suggests that the CH_3/C_2H_5 substitution pattern of **6a**–**f** is particularly suitable for binding to A_{2A} receptors by xanthine derived compounds. High affinity compounds were, however, also found among the 1,3,7-trimethylxanthines (**4a**–**e**) and the 1,3-dimethyl-7-ethylxanthines (**5a**–**f**) with K_i values as low as 28.4 nM (compound **4c**). The 1,3,7-triethylxanthines (**7a**–**f**) on the other hand displayed comparatively weaker affinities for A_{2A} receptors with three homologues (**7a**, **7b** and **7f**) exhibiting K_i values of >1 μM and only one homologue (**7e**) possessing a K_i value <100 nM. This analysis suggests that among the four series of (*E*)-8-styrylxanthines (**4**–**7**) examined, the 1,3-diethyl-7-methylxanthine substitution pattern, as found with compounds **6a**–**f**, is most appropriate for high affinity binding to A_{2A} receptors.

To examine the selectivities of the xanthines for A_{2A} receptors, the affinities of four homologues for adenosine A_1 receptors were examined. The xanthines selected for this purpose were compounds **4c**, **5c**, **6f** and **7e**. These compounds are representative of the four classes of xanthines (compounds **4**–**7**) which exhibited affinity for A_{2A} receptors and include the most potent compounds identified in this study. The binding of the test compounds to A_1 receptors were investigated by measuring the displacement of 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) from rat whole brain membranes [34]. As positive controls, the affinities of the antagonists, caffeine and ZM 241385, for A_1 receptors were examined. As shown in Table 5, caffeine and ZM 241385 displayed K_i values for the binding to A_1 receptors of 43,907 nM and 273 nM, respectively. These values correspond well with the literature values of 41,000 nM [24] and 225 nM [22] for these compounds, respectively. In addition, the recorded affinity of the agonist, CPA, for A_1 receptors corresponded to that reported in literature (Table 5). Compared to their affinities for binding to A_{2A} receptors, compounds **4c**, **5c** and **6f** displayed relatively weaker binding to A_1 receptors. Compound **4c** exhibited a K_i value >1 μM for binding to A_1 receptors while **5c** and **6f** were 16-and 17-fold, respectively, more selective for A_{2A} receptors. Interestingly, **7e** was found to bind to A_1 receptors (K_i = 56.9 nM) with higher affinity than to A_{2A} receptors (K_i = 83.0 nM). From these results it may be concluded that compound **4c** is a suitable lead were high potency and selective A_{2A} binding is desired, while **7e** is an example of a compound which exhibits high potency binding to both A_1 and A_{2A} receptors. Although not as selective as ZM 241385 (SI = 118), compounds **5c** and **6f** may still be considered to possess high potency and selectivity for A_{2A} receptors. These interesting SARs merit further investigation.

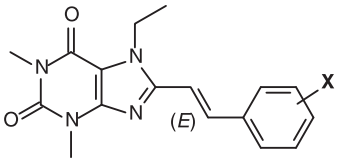
2.3. In vivo reversal of haloperidol-induced catalepsy

To obtain evidence of an antagonistic mechanism of action and of an in vivo efficacy, the abilities of two compounds with high A_{2A}

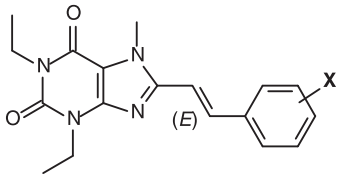
Table 2
The K_i values for the competitive inhibition of [^{3}H]NECA binding to rat striatal adenosine A_{2A} receptors by (*E*)-8-styrylxanthines **4**–**7**.



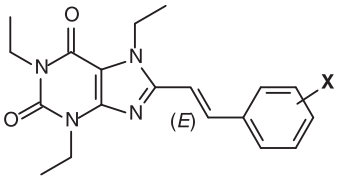
	X	K_i values (nM)[a]
4a	4-Cl	169 ± 61.4[b]
4b	3,4-Cl	44.8 ± 4.11
4c	3-Br	28.4 ± 2.40
4d	4-Br	127 ± 0.01
4e	3-CF_3	125 ± 24.2 (134)[c]



	X	K_i values (nM)[a]
5a	3-Cl	421 ± 105[b]
5b	4-Cl	95.3 ± 11.3
5c	3,4-Cl	32.9 ± 6.27
5d	3-Br	58.2 ± 2.91
5e	4-Br	546 ± 223[b]
5f	3-CF_3	93.8 ± 2.01



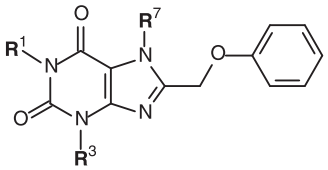
	X	K_i values (nM)[a]
6a	3-Cl	24.3 ± 1.57
6b	4-Cl	18.9 ± 1.88
6c	3,4-Cl	47.9 ± 8.55
6d	3-Br	17.2 ± 4.80
6e	4-Br	28.9 ± 4.93
6f	3-CF_3	11.9 ± 1.34



	X	K_i values (nM)[a]
7a	3-Cl	>1 μM
7b	4-Cl	>1 μM
7c	3,4-Cl	428 ± 491[b]
7d	3-Br	161 ± 223[b]
7e	4-Br	83.0 ± 50.9[b]
7f	3-CF_3	>1 μM

[a] All K_i values were determined in duplicate and are expressed as mean ± SD.
[b] Standard errors probably due to low aqueous solubility at concentrations approaching the K_i value.
[c] Value obtained from Ref. [23].

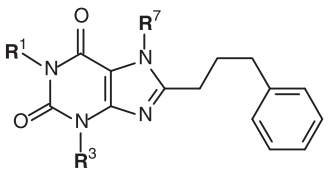
Table 3
The K_i values for the competitive inhibition of [^{3}H]NECA binding to rat striatal adenosine A_{2A} receptors by 8-(phenoxymethyl)xanthines **8**.



		K_i values (nM)[a]
8a	$R^1 = R^3 = R^7 = CH_3$	137 ± 50.8
8b	$R^1 = R^3 = CH_3$; $R^7 = C_2H_5$	>1 μM
8c	$R^1 = R^3 = C_2H_5$; $R^7 = CH_3$	>1 μM
8d	$R^1 = R^3 = R^7 = C_2H_5$	>1 μM

[a] All K_i values were determined in duplicate and are expressed as mean ± SD.

Table 4
The K_i values for the competitive inhibition of [^{3}H]NECA binding to rat striatal adenosine A_{2A} receptors by (*E*)-8-(3-phenylpropyl)xanthines **9**.



		K_i values (nM)[a]
9a	$R^1 = R^3 = R^7 = CH_3$	>1 μM
9b	$R^1 = R^3 = CH_3$; $R^7 = C_2H_5$	>1 μM
9c	$R^1 = R^3 = C_2H_5$; $R^7 = CH_3$	>1 μM
9d	$R^1 = R^3 = R^7 = C_2H_5$	>1 μM

[a] Determined in duplicate.

binding affinities, compounds **4c** and **6f**, to attenuate haloperidol-induced catalepsy in rats were examined. For this purpose rats (n = 6/group) were treated intraperitoneally (i.p.) with haloperidol (5 mg/kg) followed 30 min later with vehicle or test compound (**4c** or **6f**) at 0.1, 0.4, 1 and 2 mg/kg. At 90 min post haloperidol treatment, the degree of catalepsy of the rats were evaluated using the standard bar test according to the literature protocol [35,36]. Rats which were similarly treated with KW-6002 served as positive controls. The results presented in Fig. 4 show that both **4c** and **6f** reverse haloperidol-induced catalepsy in a concentration dependent manner. At doses of 0.1–2 mg/kg, **4c** significantly ($p < 0.05$) reduces mean catalepsy time by 20–42% compared to the vehicle. At doses of 0.4, 1 and 2 mg/kg, **6f** significantly ($p < 0.05$) reduces mean catalepsy time by 17–39% compared to the vehicle. For comparison, KW-6002 at doses of 0.1–2 mg/kg reduces mean catalepsy time by 7–44% compared to the vehicle. These data demonstrates that both **4c** and **6f** are in vivo active A_{2A} receptor antagonists. It should be noted that haloperidol-induced catalepsy may also be reversed by pharmacological agents acting at other biochemical targets such as dopamine and serotonin receptors. The current study cannot rule out that **6f** does not reverse haloperidol-induced catalepsy by acting at such alternative targets. It may, however, be expected that, if **6f** is an A_{2A} agonist (rather than an antagonist), its effect on the reversal of catalepsy would not be so pronounced since A_{2A} agonism would enhance haloperidol-induced catalepsy.

2.4. In vitro inhibition of MAO-B

The MAO-B inhibitory potencies of selected potent A_{2A} antagonists (compounds **4c**, **5c**, **6f**, **7e**) were examined using recombinant human MAO-B as enzyme source and kynuramine as enzyme substrate. For this purpose the amount of hydrogen peroxide that is produced in the MAO oxidation cycle was measured. In a horseradish peroxidase-coupled reaction, hydrogen peroxide reacts with

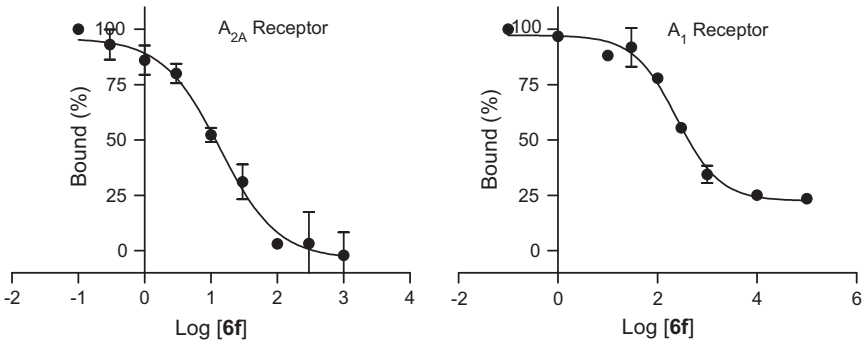


Fig. 3. The sigmoidal dose–response curve for the antagonism of [^{3}H]NECA binding to rat striatal A_{2A} receptors and [^{3}H]DPCPX binding to rat whole brain A_1 receptors by compound **6f** (expressed in nM). The bound cpm values were adjusted for nonspecific binding and are expressed as percentage of the cpm values recorded in the absence of **6f**.

Table 5
The K_i values for the competitive inhibition of [^{3}H]DPCPX binding to rat whole brain adenosine A_1 receptors by selected xanthines and reference compounds. The IC_{50} values for the inhibition of MAO-B are also listed.

	A_1 Antagonism		MAO-B inhibition
	K_i (nM)[a]	SI[b]	IC_{50} (μM)[c]
4c	>1 μM	–	0.112 ± 0.020
5c	562 ± 4.85	17	2.05 ± 1.03
6f	194 ± 17.3	16	No inhibition
7e	56.9 ± 2.45	0.7	No inhibition
Caffeine	43,907 ± 3151 (41,000)[d]	–	ND[g]
ZM 241385	273 ± 4.73 (225)[e]	118	ND[g]
CPA	10.4 ± 1.57 (7.9)[f]	–	ND[g]

[a] All K_i values were determined in duplicate and are expressed as mean ± SD.
[b] The selectivity index is the selectivity for the A_{2A} receptor isoform and is given as the ratio of K_i (A_1)/K_i (A_{2A}).
[c] All IC_{50} values were determined in triplicate and are expressed as mean ± SD.
[d] Value obtained from Ref. [24].
[e] Value obtained from Ref. [22].
[f] Value obtained from Ref. [41].
[g] Not determined.

Ampliflu Red to yield resorufin, a fluorescent compound, which is readily quantifiable via fluorescence spectrophotometry. The results, presented in Table 5, show that only **4c** and **5c** exhibit meaningful MAO-B inhibition with IC_{50} values of 0.112 μM and 2.05 μM, respectively. In fact, **4c** may be considered to be a highly potent MAO-B inhibitor since its IC_{50} value is in the nanomolar range. It is interesting to note that the active MAO-B inhibitors, compounds **4c** and **5c**, contain the di-CH_3 substitution pattern on N1 and N3 of the xanthine ring, while introduction of the C_2H_5 substituent on these positions (compounds **6f** and **7e**) eliminate MAO-B inhibition. In addition, a CH_3 on N7 is more suitable for MAO-B inhibition than the C_2H_5 substituent on this position since **4c** is approximately 18-fold more potent as a MAO-B inhibitor than **5c**. It should, however, be noted that different substitution on the phenyl rings of these compounds may also contribute to their different MAO-B inhibitory activities. These interesting relationships may be of value in future design studies and should be examined further.

Considering these results, compound **4c** is a particularly promising therapeutic compound for Parkinson's disease since it acts as a high affinity A_{2A} antagonist as well as a potent MAO-B inhibitor. In addition, **4c** displays a high degree of selectivity for A_{2A} receptors over A_1 receptors. A further point of interest is that, in addition to A_{2A} antagonism, MAO-B inhibition may also contribute to the in vivo anticataleptic activity of **4c**. Since compound **6f** is a selective A_{2A} antagonist with no affinity for MAO-B, its in vivo anticataleptic activity is, however, most likely not mediated by MAO-B inhibition.

3. Conclusion

In conclusion, this study shows that 8-(phenoxymethyl)xanthine and 8-(3-phenylpropyl)xanthine derivatives, in general, do not exhibit potent binding affinities to A_{2A} receptors. In accordance to literature, (*E*)-8-styrylxanthines, on the other hand, have high affinities for A_{2A} receptors, with particularly the 1,3-diethyl-7-methylxanthine substitution being most appropriate for high affinity binding. This behaviour is exemplified by compound **6f**, which binds to rat striatal A_{2A} receptors with a K_i value of 11.9 nM. An investigation of the affinities of selected compounds for A_1 receptors documents that **6f**, as well as **4c** and **5c**, display selective binding to A_{2A} receptors compared to A_1 receptors. Among these compounds **4c** is particularly selective for A_{2A} receptors. Interestingly **7e** was found to be bind relatively non-selectively to both A_1 and A_{2A} receptors while still displaying good affinities for both receptor subtypes. An examination of the MAO-B inhibitory potencies of selected potent A_{2A} antagonists reveals that **4c** also is a highly potent MAO-B inhibitor. This compound therefore represents a potential dual-acting antiparkinsonian agent with affinity for both A_{2A} receptors and MAO-B.

4. Experimental section

4.1. Chemicals and instrumentation

All reagents and solvents were obtained from Sigma–Aldrich and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer in $CDCl_3$. The chemical shifts are reported in parts per million (δ) relative to the signal of tetramethylsilane. Spin multiplicities are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), qn (quintet) or m (multiplet). High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI) mode or on a DFS high resolution magnetic sector mass spectrometer (Thermo Electron Corporation) in electrospray ionization (ESI) mode. Melting points (mp) were measured with a Buchi M-545 melting point apparatus and are uncorrected. Counting of radio activities were performed using a Packard Tri-Carb 2100 TR liquid scintillation counter. 1,3-Dimethyl-(**10a**) and 1,3-diethyl-5,6-diaminouracil (**10b**) were prepared according to a previously reported procedure [33]. [^{3}H]NECA (specific activity 25 Ci/mmol) and [^{3}H]DPCPX (specific activity 120 Ci/mmol) were obtained from Amersham Biosciences and PerkinElmer, respectively. Adenosine deaminase (type X from calf spleen; 250 units) and CPA were from Sigma–Aldrich. Whatman® GF/B 25 mm diameter filters and dimethyl sulfoxide (DMSO)

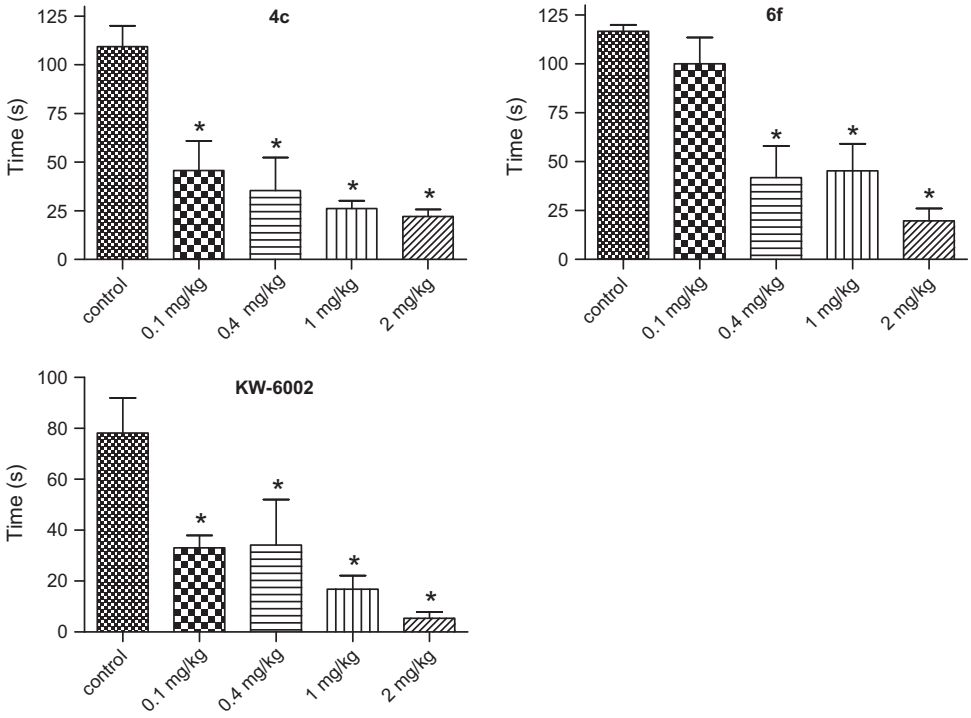


Fig. 4. Attenuation of haloperidol-induced catalepsy by **4c**, **6f** and KW-6002. Rats (n = 6/group) were treated with haloperidol (5 mg/kg) followed 30 min later with vehicle, compounds **4c**, **6f** or KW-6002 at 0.1, 0.4, 1 and 2 mg/kg. At 90 min post haloperidol treatment, catalepsy time were evaluated using the standard bar test. *One way ANOVA followed by Dunnet's multiple comparison test $p < 0.05$.

were obtained from Merck. Filter-count was purchased from Perkin Elmer. The reference compounds, KW-6002 (**1**) and CSC (**3**), were synthesized as previously reported [29,37], while ZM - 241385 was obtained from Ascent Scientific. The syntheses and characterizations of **4a** [38], **4b** [39] and **8a** [31] have been previously reported. For fluorescence spectrophotometry, a Varian Cary Eclipse fluorescence spectrophotometer was employed. The following chemical and biological agents were obtained from Sigma–Aldrich: microsomes from insect cells containing recombinant human MAO-B (5 mg/mL), kynuramine·2HBr, Ampliflu Red (10-acetyl-3,7-dihydroxyphenoxazine), horseradish peroxidase, (R)-deprenyl·HCl and H_2O_2 (3%).

4.2. General procedure for the synthesis of xanthine analogues (*4*–*9*)

*Synthesis of intermediary 1,3-dialkyl-7H-xanthine analogues (**14**):* 1,3-Dimethyl- (**10a**) or 1,3-diethyl-5,6-diaminouracil (**10b**) (10 mmol) was dissolved in a minimum amount of dioxane/H_2O (1:1). EDAC (13.4 mmol) and the appropriate carboxylic acid (10 mmol) was added and the pH was adjusted to 5 with 4 N HCl. The reaction was stirred for 2 h at room temperature. Except for the synthesis of compounds **8c**, **8d** and **9b**–**d**, the reaction was subsequently neutralized (pH 7) with the addition of 1 N NaOH. The precipitate was collected via filtration and suspended in 100 mL of dioxane/H_2O (1:1). The reaction was then heated at reflux for 2 h at room temperature. After cooling to 0 °C, the precipitate that formed was collected by filtration and washed with 50 mL H_2O. In the case of compounds **8c** and **8d**, H_2O (30 mL) was added to the reaction and the reaction was subsequently extracted to $CHCl_3$ (9 × 25 mL). The combined organic phases were washed with H_2O (4 × 80 mL) followed with brine (4 × 80 mL), dried over anhydrous $MgSO_4$ and finally concentrated under reduced pressure. The crude residue was dissolved in a mixture of 30 mL aqueous NaOH (1 M)/dioxane (1:1) and heated under reflux for 45 min. After cooling to 0 °C, the reaction was acidified with 4 N HCl and the resulting precipitate was collected by filtration and washed with 50 mL water. For the synthesis of **9b**–**d**, 1 g of sodium hydroxide was dissolved in H_2O (1 mL) and added to the reaction. The reaction was heated under reflux for 2 h, cooled to 0 °C and acidified with 4 N HCl. The resulting precipitate, the 7*H*-xanthine analogues (yields 29–75%), were collected via filtration and washed with 50 mL H_2O.

*Synthesis of **4–7**:* Without further purification, the crude 7*H*-xanthine analogue (**14**, 1.6 mmol) was dissolved in a minimum amount of N,N-dimethylformamide (DMF). K_2CO_3 (4 mmol) was added followed by iodomethane or iodoethane (3.2 mmol) and the reaction was stirred for 2 h at 90 °C. The progress of the reaction was monitored with neutral alumina thin layer chromatography (TLC). This was followed by adding 50 mL H_2O to the reaction, yielding a precipitate that was collected via filtration and dried overnight in a fume cupboard. Analytical pure samples were obtained after recrystallization from the appropriate solvent as cited below.

*Synthesis of **8b–d** and **9a–d**:* Without further purification, the crude 7*H*-xanthine analogue (**14**, 1.27 mmol) was dissolved in a minimum amount of either dry acetone (for the synthesis of **8b**–**d** and **9a**–**b**) or anhydrous ethanol (for the synthesis of **9c** and **9d**). K_2CO_3 (0.55 mmol) was added followed by iodomethane or iodoethane (1.5 mmol). The reaction was heated under reflux (90 °C) for 1–2 h. The progress of the reaction was monitored with neutral alumina TLC. The solvent was removed *in vacuo* and H_2O (30 mL) was added to the residue. The product was extracted to $CHCl_3$ (3 × 30 mL), the organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Analytical pure samples were obtained after recrystallization from the appropriate solvent as cited below.

4.2.1. (E)-8-(3-Bromostyryl)-1,3,7-trimethylxanthine (*4c*)

The title compound (yellow crystals) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3-bromocinnamic acid and iodo-

methane in a yield of 61%: mp 229.9 °C (lit. 229–231 °C) [39] (ethanol); ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.59 (s, 3H), 4.05 (s, 3H), 6.88 (d, 1H, J = 15.4 Hz), 7.25 (d, 1H, J = 6.4 Hz), 7.45 (dd, 2H, J = 1.5, 7.9 Hz), 7.70 (d, 1H, J = 15.4 Hz), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.9, 29.7, 31.6, 108.1, 112.5, 123.1, 126.2, 129.8, 130.4, 132.2, 136.4, 137.6, 148.5, 149.3, 151.6, 155.2; APCI-HRMS m/z: Calcd for $C_{16}H_{16}BrN_4O_2$ (MH^+), 375.0457. Found: 375.0440; Purity (HPLC): 100%.

*4.2.2. (E)-8-(4-Bromostyryl)-1,3,7-trimethylxanthine (**4d**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-4-bromocinnamic acid and iodomethane in a yield of 88%: mp 255.7 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 3H), 3.59 (s, 3H), 4.03 (s, 3H), 6.87 (d, 1H, J = 15.4 Hz), 7.41 (d, 2H, J = 8.7 Hz), 7.50 (d, 2H, J = 8.7 Hz), 7.70 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 27.9, 29.7, 31.5, 108.0, 111.7, 123.5, 128.7, 132.1, 134.3, 136.8, 148.5, 149.5, 151.6, 155.2; APCI-HRMS m/z: Calcd for $C_{16}H_{16}BrN_4O_2$ (MH^+), 375.0457. Found: 375.0442; Purity (HPLC): 100%.

*4.2.3. (E)-8-[3-(Trifluoromethyl)styryl]-1,3,7-trimethylxanthine (**4e**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3-(trifluoromethyl)cinnamic acid and iodomethane in a yield of 58%: mp 243.0 °C (lit. 243.7 °C) [38] (ethanol); ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 3H), 3.60 (s, 3H), 4.08 (s, 3H), 6.96 (d, 1H, J = 15.8 Hz), 7.51 (t, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 7.9 Hz), 7.72 (d, 1H, J = 7.9 Hz), 7.80 (s, 1H), 7.81 (d, 1H, J = 15.4 Hz); ^{13}C NMR ($CDCl_3$) δ 27.9, 29.7, 31.6, 108.2, 112.9, 123.0, 123.7, 123.7, 124.8, 125.8, 125.8, 129.5, 130.5 131.4, 131.6, 136.2, 136.4, 148.5, 149.2, 151.6, 155.3; APCI-HRMS m/z: Calcd for $C_{17}H_{16}F_3N_4O_2$ (MH^+), 365.1226. Found: 365.1218; Purity (HPLC): 100%.

*4.2.4. (E)-8-(3-Chlorostyryl)-1,3-dimethyl-7-ethylxanthine (**5a**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3-chlorocinnamic acid and iodoethane in a yield of 44%: mp 213.1 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, J = 7.2 Hz), 3.40 (s, 3H), 3.61 (s, 3H), 4.48 (q, 2H, J = 7.2 Hz), 6.88 (d, 1H, J = 15.8 Hz), 7.30–7.34 (m, 2H), 7.42 (d, 1H, J = 6.4 Hz), 7.55 (s, 1H), 7.74 (d, 1H, J = 15.4 Hz); ^{13}C NMR ($CDCl_3$) δ 16.7, 28.0, 29.8, 40.1, 107.3, 112.5, 125.8, 126.8, 129.3, 130.2, 134.9, 136.6, 137.3, 148.4, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{17}H_{18}ClN_4O_2$ (MH^+), 345.1119. Found: 345.1114; Purity (HPLC): 99%.

*4.2.5. (E)-8-(4-Chlorostyryl)-1,3-dimethyl-7-ethylxanthine (**5b**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-4-chlorocinnamic acid and iodoethane in a yield of 47%: mp 210.6 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, J = 7.2 Hz), 3.39 (s, 3H), 3.60 (s, 3H), 4.46 (q, 2H, J = 7.2 Hz), 6.84 (d, 1H, J = 15.8 Hz), 7.35 (d, 2H, J = 8.7 Hz), 7.48 (d, 2H, J = 8.3 Hz), 7.75 (d, 1H, J = 15.4 Hz); ^{13}C NMR ($CDCl_3$) δ 16.7, 27.9, 29.7, 40.0, 107.2, 111.6, 128.5, 129.2, 134.0, 135.2, 136.8, 148.6, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{17}H_{18}ClN_4O_2$ (MH^+), 345.1119. Found: 345.1105; Purity (HPLC): 100%.

*4.2.6. (E)-8-(3,4-Dichlorostyryl)-1,3-dimethyl-7-ethylxanthine (**5c**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3,4-dichlorocinnamic acid and iodoethane in a yield of 46%: mp 216.6 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, J = 7.2 Hz), 3.40 (s, 3H), 3.60 (s, 3H), 4.48 (q, 2H, J = 7.2 Hz), 6.86 (d, 1H, J = 15.8 Hz), 7.37 (dd, 1H, J = 1.9, 8.3 Hz), 7.45 (d, 1H, J = 8.3 Hz), 7.64 (d, 1H, J = 1.9 Hz), 7.70 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 16.8, 28.0, 29.8, 40.1, 107.4, 112.8, 126.5, 128.6, 130.9, 133.2, 133.2, 135.5, 135.6, 148.2, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{17}H_{17}Cl_2N_4O_2$ (MH^+), 379.0729. Found: 379.0715; Purity (HPLC): 98%.

*4.2.7. (E)-8-(3-Bromostyryl)-1,3-dimethyl-7-ethylxanthine (**5d**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3-bromocinnamic acid and iodoethane in a yield of 76%: mp 227.5 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, J = 7.2 Hz), 3.40 (s, 3H), 3.60 (s, 3H), 4.48 (q, 2H, J = 7.2 Hz), 6.87 (d, 1H, J = 15.8 Hz), 7.26 (d, 1H, J = 7.9 Hz), 7.45 (dd, 2H, J = 1.9, 7.9 Hz), 7.71 (s, 1H), 7.72 (d, 1H, J = 15.6 Hz); ^{13}C NMR ($CDCl_3$) δ 16.7, 28.0, 29.7, 40.1, 107.3, 112.5, 123.1, 126.2, 129.7, 130.4, 132.2, 136.5, 137.6, 148.4, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{17}H_{18}BrN_4O_2$ (MH^+), 389.0614. Found: 389.0599; Purity (HPLC): 99%.

*4.2.8. (E)-8-(4-Bromostyryl)-1,3-dimethyl-7-ethylxanthine (**5e**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-4-bromocinnamic acid and iodoethane in a yield of 63%: mp 233.7 °C (ethanol/toluene); ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 7.2 Hz), 3.38 (s, 3H), 3.59 (s, 3H), 4.46 (q, 2H, J = 7.2 Hz), 6.86 (d, 1H, J = 15.8 Hz), 7.41 (d, 2H, J = 8.3 Hz), 7.50 (d, 2H, J = 8.7 Hz), 7.73 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 16.7, 27.9, 29.7, 40.0, 107.2, 111.7, 123.5, 128.7, 132.1, 134.4, 136.8, 148.6, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{17}H_{18}BrN_4O_2$ (MH^+), 389.0614. Found: 389.0600; Purity (HPLC): 98%.

*4.2.9. (E)-8-[3-(Trifluoromethyl)styryl]-1,3-dimethyl-7-ethylxanthine (**5f**)*

The title compound (yellow needles) was prepared from 1,3-dimethyl-5,6-diaminouracil, (*E*)-3-(trifluoromethyl)cinnamic acid and iodoethane in a yield of 60%: mp 256.7 °C (ethanol/toluene); ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3H, J = 7.2 Hz), 3.40 (s, 3H), 3.61 (s, 3H), 4.50 (q, 2H, J = 7.2 Hz), 6.94 (d, 1H, J = 15.8 Hz), 7.52 (t, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 7.5 Hz), 7.72 (d, 1H, J = 7.5 Hz), 7.80 (s, 1H), 7.84 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 16.8, 28.0, 29.7, 40.1, 107.4, 112.9, 123.0, 123.7, 123.7, 124.8, 125.8, 129.5, 129.5, 130.5, 131.1, 131.3, 131.6, 136.3, 136.5, 148.3, 148.7, 151.7, 154.8; APCI-HRMS m/z: Calcd for $C_{18}H_{18}F_3N_4O_2$ (MH^+), 379.1383. Found: 379.1374; Purity (HPLC): 99%.

*4.2.10. (E)-8-(3-Chlorostyryl)-1,3-diethyl-7-methylxanthine (**6a**)*

The title compound (yellow powder) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3-chlorocinnamic acid and iodomethane in a yield of 55%: mp 178.6 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.2 Hz), 4.04 (s, 3H), 4.05 (q, 2H, J = 7.2 Hz), 4.18 (q, 2H, J = 7.2 Hz), 6.89 (d, 1H, J = 15.4 Hz), 7.28–7.32 (m, 2H), 7.41 (d, 1H, J = 6.8 Hz), 7.54 (s, 1H), 7.70 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.3, 38.4, 108.3, 112.6, 125.6, 126.9, 129.2, 130.1, 134.9, 136.4, 137.4, 148.0, 149.2, 150.7, 155.0; APCI-HRMS m/z: Calcd for $C_{18}H_{20}ClN_4O_2$ (MH^+), 359.1276. Found: 359.1262; Purity (HPLC): 100%.

*4.2.11. (E)-8-(4-Chlorostyryl)-1,3-diethyl-7-methylxanthine (**6b**)*

The title compound (yellow needles) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-4-chlorocinnamic acid and iodomethane in a yield of 48%: mp 214.6 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.2 Hz), 4.03 (s, 3H), 4.05 (q, 2H, J = 7.2 Hz), 4.18 (q, 2H, J = 7.2 Hz), 6.86 (d, 1H, J = 15.8 Hz), 7.35 (d, 2H, J = 8.3 Hz), 7.49 (d, 2H, J = 8.3 Hz), 7.71 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.3, 38.4, 108.2, 111.8, 128.5, 129.1, 134.0, 135.2, 136.6, 148.1, 149.5, 150.7, 155.0; APCI-HRMS m/z: Calcd for $C_{18}H_{20}ClN_4O_2$ (MH^+), 359.1276. Found: 359.1265; Purity (HPLC): 100%.

*4.2.12. (E)-8-(3,4-Dichlorostyryl)-1,3-diethyl-7-methylxanthine (**6c**)*

The title compound (yellow needles) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3,4-dichlorocinnamic acid and iodomethane in a yield of 65%: mp 215.5 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.2 Hz), 4.05 (s, 3H), 4.06 (q,

2H, J = 6.8 Hz), 4.17 (q, 2H, J = 7.2 Hz), 6.87 (d, 1H, J = 15.8 Hz), 7.37 (d, 1H, J = 6.8 Hz), 7.44 (d, 1H, J = 8.3 Hz), 7.64 (s, 1H), 7.66 (d, 1H, J = 15.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.4, 38.4, 108.4, 113.0, 126.4, 128.7, 130.9, 133.1, 133.2, 135.2, 135.6, 148.0, 149.0, 150.6, 155.0; APCI-HRMS m/z: Calcd for $C_{18}H_{19}Cl_2N_4O_2$ (MH^+), 393.0886. Found: 393.0873; Purity (HPLC): 100%.

*4.2.13. (E)-8-(3-Bromostyryl)-1,3-diethyl-7-methylxanthine (**6d**)*

The title compound (yellow powder) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3-bromocinnamic acid and iodomethane in a yield of 30%: mp 189.9 °C, (lit. mp 187.3–188.2 °C) [40] (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.2 Hz), 4.05 (s, 3H), 4.06 (q, 2H, J = 7.2 Hz), 4.18 (q, 2H, J = 7.2 Hz), 6.89 (d, 1H, J = 15.8 Hz), 7.26 (d, 1H, J = 7.9 Hz), 7.46 (t, 2H, J = 7.2 Hz), 7.69 (d, 1H, J = 15.8 Hz), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.4, 38.4, 108.3, 112.7, 123.1, 126.1, 129.8, 130.4, 132.1, 136.3, 137.7, 148.1, 149.2, 150.7, 155.1; APCI-HRMS m/z: Calcd for $C_{18}H_{20}BrN_4O_2$ (MH^+), 403.0770. Found: 403.0757; Purity (HPLC): 100%.

*4.2.14. (E)-8-(4-Bromostyryl)-1,3-diethyl-7-methylxanthine (**6e**)*

The title compound (yellow needles) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-4-bromocinnamic acid and iodomethane in a yield of 26%: mp 210.5 °C, (lit. mp 198.5–198.9 °C) [40] (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.2 Hz), 4.03 (s, 3H), 4.06 (q, 2H, J = 7.2 Hz), 4.18 (q, 2H, J = 7.2 Hz), 6.88 (d, 1H, J = 15.8 Hz), 7.42 (d, 2H, J = 8.3 Hz), 7.50 (d, 2H, J = 8.7 Hz), 7.70 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.4, 38.4, 108.3, 111.9, 123.4, 128.7, 132.1, 134.5, 136.7, 148.1, 149.5, 150.7, 155.1; APCI-HRMS m/z: Calcd for $C_{18}H_{20}BrN_4O_2$ (MH^+), 403.0770. Found: 403.0758; Purity (HPLC): 100%.

*4.2.15. (E)-8-[3-(Trifluoromethyl)styryl]-1,3-diethyl-7-methylxanthine (**6f**)*

The title compound (white powder) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3,4-(trifluoromethyl)cinnamic acid and iodomethane in a yield of 58%: mp 212.8 °C, (lit. mp 214.8–215.3 °C) [40] (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 4.06 (q, 2H, J = 7.2 Hz), 4.07 (s, 3H), 4.19 (q, 2H, J = 7.2 Hz), 6.96 (d, 1H, J = 15.8 Hz), 7.51 (t, 1H, J = 7.9 Hz), 7.58 (d, 1H, J = 7.9 Hz), 7.72 (d, 1H, J = 7.9 Hz), 7.80 (s, 1H), 7.80 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 31.5, 36.4, 38.4, 108.4, 113.1, 122.9, 123.7, 123.8, 124.8, 125.7, 125.7, 129.4, 130.4, 131.3, 131.5, 136.2, 136.3, 148.0, 149.1, 150.7, 155.1; APCI-HRMS m/z: Calcd for $C_{19}H_{20}F_3N_4O_2$ (MH^+), 393.1539. Found: 393.1525; Purity (HPLC): 100%.

*4.2.16. (E)-8-(3-Chlorostyryl)-1,3,7-triethylxanthine (**7a**)*

The title compound (light yellow crystals) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3-chlorocinnamic acid and iodoethane in a yield of 93%: mp 165.0 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz), 1.32 (t, 3H, J = 7.2 Hz), 1.41 (t, 3H, J = 7.2 Hz), 4.02 (q, 2H, J = 7.2 Hz), 4.14 (q, 2H, J = 7.2 Hz), 4.43 (q, 2H, J = 7.2 Hz), 6.83 (d, 1H, J = 15.8 Hz), 7.24–7.28 (m, 2H), 7.37 (d, 1H, J = 6.8 Hz), 7.50 (s, 1H), 7.68 (d, 1H, J = 15.4 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.7, 36.4, 38.4, 40.0, 107.6, 112.6, 125.7, 126.8, 129.2, 130.1, 134.9, 136.4, 137.4, 148.2, 148.3, 150.7, 154.6; APCI-HRMS m/z: Calcd for $C_{19}H_{22}ClN_4O_2$ (MH^+), 373.1432. Found: 373.1433; Purity (HPLC): 99%.

*4.2.17. (E)-8-(4-Chlorostyryl)-1,3,7-triethylxanthine (**7b**)*

The title compound (light yellow crystals) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-4-chlorocinnamic acid and iodoethane in a yield of 46%: mp 171.7 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 1.45 (t, 3H, J = 7.2 Hz), 4.07 (q, 2H, J = 7.2 Hz), 4.19 (q, 2H, J = 7.2 Hz), 4.47 (q, 2H, J = 7.2 Hz), 6.85 (d, 1H, J = 15.4 Hz), 7.36 (d, 2H, J = 8.3 Hz), 7.49 (d, 2H, J = 8.7 Hz), 7.75 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.4, 13.4, 16.7, 36.4, 38.4, 40.0, 107.5, 111.8, 128.5, 129.2, 134.1, 135.1, 136.6, 148.3, 148.6, 150.8, 154.6; APCI-HRMS m/z: Calcd for $C_{19}H_{22}ClN_4O_2$ (MH^+), 373.1432. Found: 373.1417; Purity (HPLC): 99%.

*4.2.18. (E)-8-(3,4-Dichlorostyryl)-1,3,7-triethylxanthine (**7c**)*

The title compound (light yellow powder) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3,4-dichlorocinnamic acid and iodoethane in a yield of 7%: mp 177.9 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz), 1.31 (t, 3H, J = 7.2 Hz), 1.41 (t, 3H, J = 7.2 Hz), 4.02 (q, 2H, J = 7.2 Hz), 4.13 (q, 2H, J = 7.2 Hz), 4.43 (q, 2H, J = 7.2 Hz), 6.81 (d, 1H, J = 15.4 Hz), 7.33 (dd, 1H, J = 1.8, 8.3 Hz), 7.40 (d, 1H, J = 8.3 Hz), 7.60 (d, 1H, J = 1.9 Hz), 7.65 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.8, 36.4, 38.4, 40.0, 107.6, 113.0, 126.5, 128.7, 130.8, 133.0, 133.2, 135.2, 135.7, 148.1, 148.2, 150.7, 154.6; APCI-HRMS m/z: Calcd for $C_{19}H_{21}Cl_2N_4O_2$ (MH^+), 407.1042. Found: 407.1026; Purity (HPLC): 98%.

*4.2.19. (E)-8-(3-Bromostyryl)-1,3,7-triethylxanthine (**7d**)*

The title compound (white needles) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3-bromocinnamic acid and iodoethane in a yield of 14%: mp 166.2 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 1.46 (t, 3H, J = 7.2 Hz), 4.07 (q, 2H, J = 7.2 Hz), 4.19 (q, 2H, J = 7.2 Hz), 4.48 (q, 2H, J = 7.2 Hz), 6.87 (d, 1H, J = 15.4 Hz), 7.26–7.27 (m, 1H), 7.46 (t, 2H, J = 7.2 Hz), 7.71–7.73 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 13.4, 13.4, 16.8, 36.4, 38.4, 40.0, 107.6, 112.6, 123.1, 126.2, 129.8, 130.4, 132.1, 136.3, 137.7, 148.3, 148.3, 150.7, 154.6; APCI-HRMS m/z: Calcd for $C_{19}H_{22}BrN_4O_2$ (MH^+), 417.0927. Found: 417.0914; Purity (HPLC): 99%.

*4.2.20. (E)-8-(4-Bromostyryl)-1,3,7-triethylxanthine (**7e**)*

The title compound (light yellow needles) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-4-bromocinnamic acid and iodoethane in a yield of 20%: mp 189.2 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 1.45 (t, 3H, J = 7.2 Hz), 4.07 (q, 2H, J = 7.2 Hz), 4.19 (q, 2H, J = 7.2 Hz), 4.46 (q, 2H, J = 7.2 Hz), 6.85 (d, 1H, J = 15.8 Hz), 7.42 (d, 2H, J = 8.3 Hz), 7.51 (d, 2H, J = 8.3 Hz), 7.73 (d, 1H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.7, 36.4, 38.4, 40.0, 107.5, 111.9, 123.4, 128.7, 132.1, 134.5, 136.7, 148.3, 148.6, 150.7, 154.6; APCI-HRMS m/z: Calcd for $C_{19}H_{22}BrN_4O_2$ (MH^+), 417.0927. Found: 417.0919; Purity (HPLC): 98%.

*4.2.21. (E)-8-[3-(Trifluoromethyl)styryl]-1,3,7-triethylxanthine (**7f**)*

The title compound (light yellow powder) was prepared from 1,3-diethyl-5,6-diaminouracil, (*E*)-3-(trifluoromethyl)cinnamic acid and iodoethane in a yield of 11%: mp 163.2 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz), 1.48 (t, 3H, J = 7.2 Hz), 4.07 (q, 2H, J = 7.2 Hz), 4.19 (q, 2H, J = 7.2 Hz), 4.50 (q, 2H, J = 7.2 Hz), 6.94 (d, 1H, J = 15.8 Hz), 7.52 (t, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 7.5 Hz), 7.73 (d, 1H, J = 7.5 Hz), 7.80 (s, 1H), 7.83 (d, 1H, J = 15.4 Hz); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.8, 36.4, 38.4, 40.0, 107.7, 113.0, 123.0, 123.8, 123.8, 124.8, 125.7, 125.7, 129.4, 130.4, 131.1, 131.3, 131.5, 136.3, 136.4, 148.2, 148.3, 150.8, 154.7; APCI-HRMS m/z: Calcd for $C_{20}H_{22}F_3N_4O_2$ (MH^+), 407.1695. Found: 407.1698; Purity (HPLC): 98%.

*4.2.22. 8-Phenoxymethyl-1,3-dimethyl-7-ethylxantine (**8b**)*

The title compound (white crystals) was prepared from 1,3-dimethyl-5,6-diaminouracil, phenoxyacetic acid and iodoethane in a yield of 93% mp 141.9 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, J = 7.2 Hz), 3.39 (s, 3H), 3.57 (s, 3H), 4.41 (q, 2H, J = 7.2 Hz), 5.16

(s, 2H), 6.98–7.00 (m, 3H), 7.28–7.31 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 16.5, 28.0, 29.7, 41.5, 61.9, 108.0, 114.6, 122.0, 129.7, 147.2, 147.7, 151.6, 154.9, 157.5; APCI-HRMS *m/z*: Calcd for $C_{16}H_{19}N_4O_3$ (MH^+), 315.1458. Found: 315.1461; Purity (HPLC): 93%.

*4.2.23. 8-Phenoxymethyl-1,3-diethyl-7-methylxanthine (**8c**)*

The title compound (white powder) was prepared from 1,3-diethyl-5,6-diaminouracil, phenoxyacetic acid and iodomethane in a yield of 91% mp 135.2 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, *J* = 7.2 Hz), 1.27 (t, 3H, *J* = 7.2 Hz), 3.98 (s, 3H), 3.99 (q, 2H, *J* = 7.2 Hz), 4.09 (q, 2H, *J* = 7.2 Hz), 5.12 (s, 2H), 6.92–6.94 (m, 3H), 7.19–7.24 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 12.3, 12.4, 31.4, 35.4, 37.5, 61.0, 107.9, 113.7, 121.0, 128.7, 145.9, 146.8, 149.6, 154.2, 156.5; APCI-HRMS *m/z*: Calcd for $C_{17}H_{21}N_4O_3$ (MH^+), 329.1614. Found: 329.1616; Purity (HPLC): 100%.

*4.2.24. 8-Phenoxymethyl-1,3,7-triethylxanthine (**8d**)*

The title compound (white crystals) was prepared from 1,3-diethyl-5,6-diaminouracil, phenoxyacetic acid and iodoethane in a yield of 58%: mp 95.0 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, *J* = 7.2 Hz), 1.33 (t, 3H, *J* = 7.2 Hz), 1.46 (t, 3H, *J* = 7.2 Hz), 4.05 (q, 2H, *J* = 7.2 Hz), 4.15 (q, 2H, *J* = 7.2 Hz), 4.41 (q, 2H, *J* = 7.2 Hz), 5.16 (s, 2H), 7.99–7.00 (m, 3H), 7.27–7.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.5, 36.5, 38.5, 41.4, 61.9, 108.2, 114.6, 121.9, 129.7, 147.1, 147.2, 150.7, 154.7, 157.6; APCI-HRMS *m/z*: Calcd for $C_{18}H_{23}N_4O_3$(MH^+), 343.1770. Found: 343.1722; Purity (HPLC): 89%.

*4.2.25. 8-(3-Phenylpropyl)-1,3,7-trimethylxanthine (**9a**)*

The title compound (white crystals) was prepared from 1,3-dimethyl-5,6-diaminouracil, 4-phenylbutanoic acid and iodomethane in a yield of 66%: mp 115–117 °C (methanol). ^{1}H NMR ($CDCl_3$) δ 2.09 (qn, 2H, *J* = 7.5 Hz), 2.71 (q, 4H, *J* = 7.5 Hz), 3.37 (s, 3H), 3.55 (s, 3H), 3.80 (s, 3H), 7.17 (m, 3H), 7.28 (t, 2H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 26.0, 27.8, 28.7, 29.7, 31.6, 35.1, 107.3, 126.2, 128.4, 128.5, 140.9, 148.0, 151.7, 153.9, 155.3; ESI-HRMS *m/z*: Calcd for $C_{17}H_{21}N_4O_2$ (M^+), 313.1665. Found: 313.1659; Purity (HPLC): 99%.

*4.2.26. 8-(3-Phenylpropyl)-1,3-dimethyl-7-ethylxanthine (**9b**)*

The title compound (white crystals) was prepared from 1,3-dimethyl-5,6-diaminouracil, 4-phenylbutanoic acid and iodoethane in a yield of 55%: mp 129.0 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, *J* = 7.2 Hz), 2.05 (qn, 2H, *J* = 7.5 Hz), 2.64–2.69 (m, 4H), 3.32 (s, 3H), 3.50 (s, 3H), 4.41 (q, 2H, *J* = 7.2 Hz), 7.11–7.14 (m, 3H), 7.20–7.24 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 16.3, 25.9, 27.8, 29.2, 29.7, 35.2, 40.2, 106.4, 126.1, 128.4, 128.5, 140.9, 148.2, 151.7, 153.1, 154.8; APCI-HRMS *m/z*: Calcd for $C_{18}H_{23}N_4O_2$ (MH^+), 327.1822. Found: 327.1823; Purity (HPLC): 94%.

*4.2.27. 8-(3-Phenylpropyl)-1,3-diethyl-7-methylxanthine (**9c**)*

The title compound (light yellow crystals) was prepared from 1,3-diethyl-5,6-diaminouracil, 4-phenylbutanoic acid and iodomethane in a yield of 80%: mp 61.7 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, *J* = 7.2 Hz), 1.27 (t, 3H, *J* = 7.2 Hz), 2.02 (qn, 2H, *J* = 7.5 Hz), 2.64–2.67 (m, 4H), 3.75 (s, 3H), 3.99 (q, 2H, *J* = 7.2 Hz), 4.08 (q, 2H, *J* = 7.2 Hz), 7.11–7.14 (m, 3H), 7.20–7.24 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 12.3, 12.5, 25.0, 27.9, 30.5, 34.1, 35.2, 37.3, 106.5, 125.1, 127.4, 127.5, 140.0, 146.5, 149.7, 152.8, 154.1; APCI-HRMS *m/z*: Calcd for $C_{19}H_{25}N_4O_2$ (MH^+), 341.1978. Found: 341.1980; Purity (HPLC): 100%.

*4.2.28. 8-(3-Phenylpropyl)-1,3,7-triethylxanthine (**9d**)*

The title compound (light red crystals) was prepared from 1,3-diethyl-5,6-diaminouracil, 4-phenylbutanoic acid and iodoethane in a yield of 46%: mp 73.6 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, *J* = 7.2 Hz), 1.31 (t, 3H, *J* = 7.2 Hz), 1.33 (t, 3H, *J* = 7.2 Hz), 2.08 (qn, 2H, *J* = 7.5 Hz), 2.69–2.73 (m, 4H), 4.04 (q, 2H, *J* = 7.2 Hz), 4.12 (q, 2H, *J* = 7.2 Hz), 4.19 (q, 2H, *J* = 7.2 Hz), 7.16–7.19 (m, 3H), 7.24–7.28 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.4, 16.3, 25.9, 29.4, 35.2, 36.2, 38.3, 40.1, 106.6, 126.1, 128.4, 128.4, 141.0, 147.7, 150.7, 153.0, 154.6; APCI-HRMS *m/z*: Calcd for $C_{20}H_{27}N_4O_2$ (MH^+), 355.2135. Found: 355.2138; Purity (HPLC): 87%.

4.3. Radioligand binding studies

The collection of tissue samples for the adenosine A_{2A} and A_1 receptor binding studies was approved by the Research Ethics Committee of the North-West University (application number NWU-0035-10-A5). The adenosine A_{2A} and A_1 receptor binding studies were carried out according to the procedure described in literature [34]. For studies with the A_{2A} receptor, the dissected striata of male Sprague–Dawley rats were used while for the studies with the A_1 receptor whole rat brain (excluding cerebellum and brain stem) were employed. After dissection, the tissues were snap frozen with liquid nitrogen and stored at −70 °C. The tissue were thawed on ice, weighed and disrupted for 30 s with the aid of a Polytron homogenizer (model: Polytron PT 10–35 GT) in 10 volumes of ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C). The resulting homogenate was centrifuged at 20,000*g* for 10 min at 4 °C and the pellet was resuspended in 10 volumes of ice-cold Tris buffer, again with the aid of a Polytron homogenizer as above. The resulting suspension was recentrifuged and the pellet obtained was suspended in Tris buffer (pH 7.7 at 25 °C) to a volume of 5 mL/g original striatal weight. The striatal and whole brain membranes were aliquoted into microcentrifuge tubes and stored at −70 °C until needed. The incubations were carried out in 4 mL polypropylene tubes that were precoated with Sigmacote® (Sigma–Aldrich). All incubations were prepared with Tris buffer (pH 7.7 at 25 °C) to a volume of 1 mL. For the A_{2A} receptor binding study, the incubations contained 10 mg of the original tissue weight of the striatal membranes, 4 nM [^{3}H]NECA, 50 nM CPA, 10 mM $MgCl_2$, 0.2 units/mL adenosine deaminase, the test compound and 1% DMSO. DMSO was used to prepare all stock solutions of the compounds to be tested. For the A_1 receptor binding study, the incubations contained 5 mg of the original tissue weight of the whole brain membranes, 0.1nM [^{3}H]DPCPX, 0.2 units/mL adenosine deaminase, the test compound and 1% DMSO. The incubations were vortexed and incubated for 60 min at 25 °C in a shaking water bath. Half an hour after incubation was started, the incubations were vortexed again. The incubations were terminated via filtration through a prewetted 2.5 cm Whatman glass microfiber filter (grade GF/B) under reduced pressure using a Hoffeler vacuum system. The tubes were washed twice with 4 mL ice-cold Tris buffer and the filters were washed once more with 4 mL ice-cold Tris buffer. The damp filters were placed in scintillation vials and 4 mL of scintillation fluid (Filter-Count) was added. The vials were shaken and incubated for 2 h before being counted (Packard Tri-CARB 2100 TR). An estimate of the nonspecific binding was obtained from binding studies in the presence of 100 μM CPA. The IC_{50} values were determined by plotting the count values (adjusted for nonspecific binding) vs. the logarithm of the inhibitor concentrations to obtain a sigmoidal dose–response curve. This kinetic data were fitted to the one site competition model incorporated into the Prism software package (GraphPad Software Inc.). The K_i values for the competitive inhibition of [^{3}H]NECA (K_d = 15.3 nM) [34] or [^{3}H]DPCPX (K_d = 0.36 nM) [41] binding by the test compounds were calculated from the IC_{50} values according to the Cheng and Prusoff equation [42]. All incubations were carried out in duplicate and the K_i values are expressed as mean ± standard deviation (SD).

4.4. Evaluation of the haloperidol-induced catalepsy

These studies were approved by the Research Ethics Committee of the North-West University (NWU-00035-10-S5). Male Sprague–Dawley rats (30 animals weighing 240–300 g) were housed 3 animals per cage with free access to food and water. To induce catalepsy, the animals received a single intraperitoneal (i.p.) injection of haloperidol (Serenace Injection; 5 mg/mL) at a dose of 5 mg/kg 90 min prior to catalepsy testing [36]. The test compounds (**4c** and **6f**) as well as the reference A_{2A} antagonist KW-6002 (**1**) were dissolved in a mixture of DMSO, Tween 80 and saline (1:1:4). Thirty min after treatment with haloperidol, the animals ($n = 6$/group) were treated i.p. with the test compounds or the reference A_{2A} antagonist at doses of 0.1, 0.4, 1 and 2 mg/kg. Animals ($n = 6$/group) treated with vehicle served as controls. The catalepsy was measured 60 min later. The animals were placed with their forepaws resting on the plastic bar and their hind quarters on the platform of a perspex chamber [35]. The time (up to a maximum of 120 s) required for each animal to touch the platform with one forepaw was recorded. One-way analyses of variances (ANOVA) followed by Dunnett's multiple comparison's test was used to determine if statistical differences exist between the mean catalepsy time of the control groups and those of the groups treated with the test compound and reference A_{2A} antagonist. A probability of $p < 0.05$ was used to declare statistically significant differences.

4.5. MAO-B inhibition studies

Microsomes expressing recombinant human MAO-B (5 mg/mL) were obtained from Sigma–Aldrich. The enzymatic reactions (500 μL final volume) were carried out in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) and contained kynuramine (30 μM) as substrate, various concentrations of the test inhibitor (0.003–100 μM), horseradish peroxidase (1 unit/mL) and Ampliflu Red (200 μM). Stock solutions of the test inhibitors were prepared in DMSO and added to the reactions to yield a final concentration of 4% (v/v) DMSO. MAO-B (0.0075 mg/mL) was added and the reactions were incubated at 37 °C for 20 min. The reactions were terminated with the addition of 10 μL (R)-deprenyl (5 mM) and distilled water (1400 μL) was added. The concentrations of resorufin in the reactions were subsequently determined by fluorescence spectrophotometry (λ_{ex} = 560 nm, λ_{em} = 590 nm) using a linear calibration curve, which was constructed from solutions of H_2O_2 (0.05–1.6 μM) [43,31]. The IC_{50} values were determined by fitting the sigmoidal curves to the one site competition model incorporated into the Prism 5 software package (GraphPad). All experiments were carried out in triplicate and the IC_{50} values are expressed as mean ± standard deviation (SD).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.06.006.

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