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Synthesis, biological evaluation and molecular modelling studies of 1,3,7,8-tetrasubstituted xanthines as potent and selective A_{2A} AR ligands with *in vivo* efficacy against animal model of Parkinson's disease

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ABSTRACT

In the present study, an attempt has been made to develop a new series of 1,3,7,8-tetrasubstituted xanthine based potent and selective AR ligands for the treatment of Parkinson's disease. Antagonistic interactions between dopamine and A_{2A} adenosine receptors serve as the basis for the development of AR antagonists as potential drug candidates for PD. All the synthesized compounds have been evaluated for their affinity toward AR subtypes using *in vitro* radioligand binding assays. 1,3-Dipropylxanthine **7a** with a methyl substituent at N-7 position represents the most potent compound of the series and displayed highest affinity (A_{2A}, K_i = 0.108 μM), however incorporation of a propargyl group at 7-positon of the xanthine nucleus seems to be the most appropriate substitution to improve selectivity towards the A_{2A} subtype along with reasonable potency. Antiparkinsonian activity has been evaluated using perphenazine induced catatonia in rats. Most of the synthesized xanthines significantly lowered the catatonic score as compared to control and displayed antiparkinsonian effects comparable to standard drug. All the synthesized compounds were subjected to grid-based molecular docking studies to understand the key structural requirements for the development of new molecules well-endowed with intrinsic efficacy and selectivity as adenosine receptor ligands. *In silico* studies carried out on newly synthesized xanthines provided further support to the pharmacological results.

1. Introduction

Adenosine receptors represent a promising and unique target for the development of various drug candidates for the treatment of a wide range of biological disorders. Currently, four AR subtypes A_1, A_{2A}, A_{2B} and A_3 have been recognized and pharmacologically characterized [1,2]. The development of potent and selective ligands for adenosine receptors (ARs) has been a dynamic area of research for several decades. Among adenosine receptors, the A_{2A} receptor subtype participates as a major factor in the control of motor behaviour and management of dopamine-mediated responses [3,4]. Its distinctive restricted pattern of expression and antagonistic interactions with the dopamine receptor offer convincing reason for the development of effective and selective adenosine A_{2A} receptor antagonists as an innovative nondopaminergic approach for improved treatment of Parkinson's disease (PD). This tactic may prove advantageous in both the early and later stages of parkinsonism [5,6]. PD is characterized as a chronic, progressive neurological disorder which occurs due to the extensive loss of dopamine generating neurons in the striatum. It is a major neurochemical abnormality responsible for the onset of the cardinal motor symptoms of the PD such as tremor, muscular rigidity, akinesia/bradykinesia, postural instability, difficulty in movement and slow performance [7]. Current treatment strategies are principally based on dopaminergic substitution. Although levodopa is still considered the most effectual drug, serious side effects such as wearing-off, involuntary abnormal movements, dyskinesia and severe motor complications constitute some pitfalls. Also, these are all symptomatic approaches with no established disease modifying or neuroprotective effects [8]. For this reason, a great deal of current research has been focused on exploring potent and selective A_{2A} AR ligands as a novel protective, restorative and replacement non-dopaminergic therapy for PD.

Naturally occurring alkylxanthines are recognized as the most primitive class of adenosine receptor antagonists in the literature [9]. Caffeine is the most widely consumed xanthine for its mood-altering ability and high energy effects to enhance the capacity to work [10]. Interestingly, strong epidemiological evidence suggests an inverse

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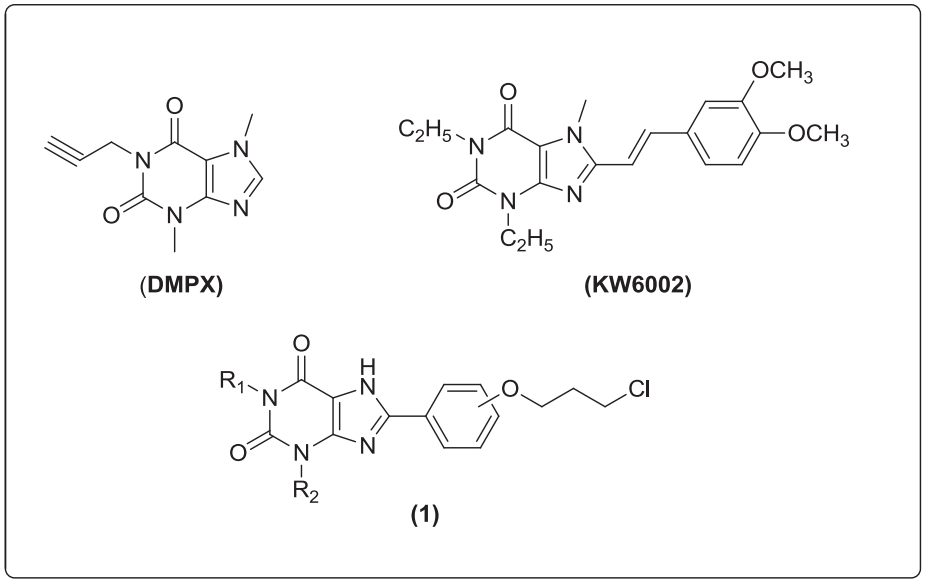


Fig. 1. Structures of some A_{2A} AR selective xanthine derivatives.

association between the consumption of coffee or other caffeinated beverages and a reduced risk of developing Parkinson's disease [11–13]. Although intensive efforts have been invested in the search of novel xanthine derivatives and a large number of structural modifications of this privileged nucleus have evolved into potent AR ligands so far, constant drawbacks such as poor selectivity and resultant high toxicity remains [14–16].

With the aim to develop novel, potent and selective A_{2A} adenosine receptor ligands, we synthesized a new series of 1,3-dialkylxanthines with synthetic modifications at 7- and 8-positions. Previous work conducted in our laboratory, well supported by preceding literature reports reveals that *meta/para*-chloropropoxy substituents on 8-phenylxanthines (**1**) result in potent and selective A_{2A} AR ligands [14,15]. Literature reports further indicate improved affinity for adenosine receptors by N^7-substitution in the 8-substituted xanthine derivatives (Fig. 1). The 1,3,7-trisubstituted xanthine derivative DMPX is the first A_{2A} AR antagonist reported in the literature having a hA_{2A} K_i = 4.1 μM [17]. Istradefylline (KW6002, K_i = 2.2 nM), a potent A_{2A} AR antagonist, has recently been approved for adjunctive treatment of PD in Japan [18].

A breakthrough in the AR field was the determination of a high-resolution 2.6 Å X-ray structure of the A_{2A}AR in complex with the AR antagonist 4-[2-[7-amino-2-(2-furyl)-1,2,4-triazolo-[1,5-*a*][1,3,5] triazin-5-ylamino]ethyl]phenol (ZM241385, PDB ID 3EML) which facilitated deeper understanding of binding mode and receptor-ligand interactions [19].

Considering the significance of appropriate substitutions at various positions of xanthine nucleus with respect to receptor binding, we decided to synthesize and evaluate some newer 1,3,7,8-tetrasubstituted xanthine derivatives as potent and selective AR ligands for the treatment of Parkinson's disease. To better understand the key structural requirements for effective receptor-ligand interactions in parallel with the biological evaluation, docking studies have also been performed.

2. Results and discussion

2.1. Chemistry

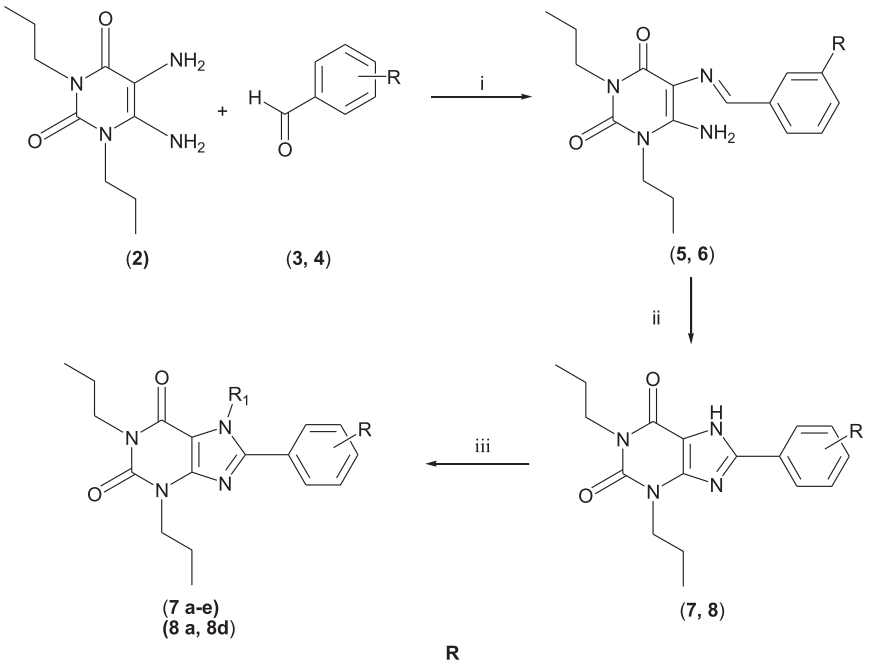
Synthesis of 1,3-dipropylxanthines was undertaken to observe the effect of elongation of the alkyl chain in naturally occurring xanthines at 1- and 3- positions. 5,6-Diamino-1,3-dipropyluracil (**2**), a key intermediate to the synthesis of all the desired 8-substituted xanthine derivatives was synthesized according to a general method with minor modifications in the synthetic procedure [20,21]. The crude intermediate 6-amino-1,3-dipropyl-5-nitrosouracil was dissolved in liquid ammonia at room temperature and the reaction was further carried out at room temperature. The requisite *m/p* substituted chloropropoxybenzaldehydes **3** and **4**, synthesized following a reported procedure were treated with diaminouracil **2** in a mixture of methanol and acetic acid (4:1) at room temperature to afford the quite unstable corresponding benzylidene derivatives **5** and **6** [22]. Subsequent cyclization of benzylidene derivatives **5** and **6** in thionyl chloride yielded the desired 8-(substituted phenyl)-1,3-dipropylxanthines **7** and **8**, respectively [23]. Highly downfield exchangeable N-*H* proton resonated as a broad singlet at ~δ 12.5 ppm for these compounds. 7-Substituted-1,3-dipropyl-8-phenylxanthines **7a-e**, **8a**, **8d** were synthesized by reacting requisite alkyl/aryl halide with respective xanthines **7** and **8** in dimethylformamide and anhydrous potassium carbonate as illustrated in Scheme 1 [24]. Based on initial *in vitro* adenosine receptor binding assay results, methyl and propargyl groups were selected for the N^7-substitution in case of *p*-chloropropoxy substituted xanthine **8**.

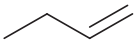
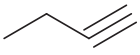
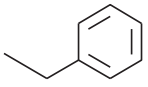
To further discover the significance of varying the alkyl groups on affinity and selectivity for adenosine receptor subtypes, another series of 1,3-unsymmetrically substituted dialkylxanthines was synthesized. Again, methyl and propargyl groups were selected for N^7-substitution. 5,6-Diamino-1-methyl-3-propyluracil (**9**) synthesized as reported [25,26] was used as the starting material for preparation of 8-(substituted phenyl)-1,3-unsymmetrically substituted dialkylxanthine **11** through an unstable benzylidene intermediate **10**. Further treatment of compound **11** with requisite alkyl halides gave the desired 7-substituted xanthines **11a** and **11b** as illustrated in Scheme 2.

2.2. Biological activity

2.2.1. Radioligand binding assays at adenosine receptors

Newly synthesized xanthines were evaluated using *in vitro* radioligand binding assays at cloned adenosine receptors. In this study all human AR subtypes were stably transfected into Chinese Hamster Ovary (CHO) cells to study their pharmacological profile in an identical cellular background utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}) [27,28]. Binding affinities and selectivity profile of various newly synthesized xanthine derivatives for adenosine A_1, A_{2A}, and A_3 receptors have been summarized in Table 1. No measurable interaction with A_{2B} AR was detected as no inhibition of



	a	b	c	d	e
R_1	$-CH_3$	$-C_3H_7$			

Scheme 1. Synthesis of 7-substituted-8-(substituted phenyl)xanthines (**7a-e, 8a, 8d**). Reagents and conditions: (i) MeOH/CH_3COOH, room temperature, 18 h; (ii) $SOCl_2$, reflux, 30–40 min; NH_4OH, (iii) RX, K_2CO_3, DMF.

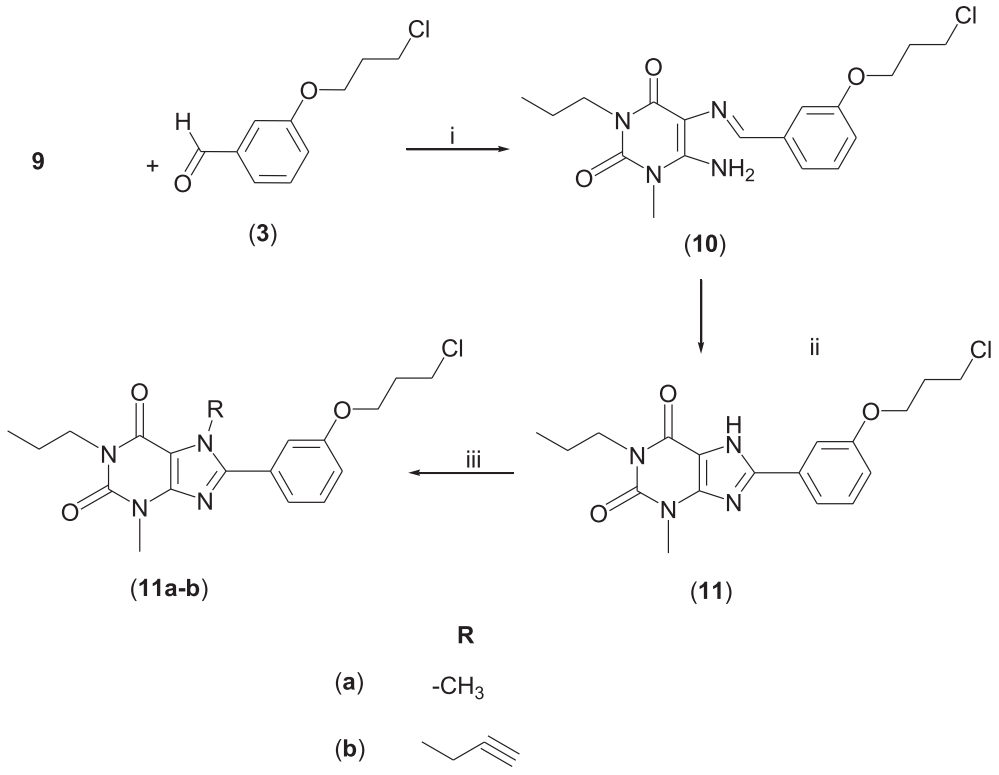
NECA-stimulated adenylyl cyclase activity was observed with concentrations up to 20 μM. It was observed that substituted xanthines displayed varying degrees of affinity and selectivity towards adenosine receptor subtypes in the radioligand binding studies.

In general, the effect of elongation of the alkyl chain from methyl (theophylline, K_i = 1710 nM) to propyl was evidently favorable for affinity toward all the AR subtypes. 1,3-Dipropyl substituted xanthines **7**, **7a-7e**, **8, 8a**, **8d** displayed good binding affinity for the three adenosine receptor subtypes A_1, A_{2A}, and A_3, however the effect was more pronounced at A_{2A} AR subtype. 8-[3-(3-Chloropropoxy)]-1,3-dipropylxanthine (**7**, K_i = 0.177 μM) exhibited improved affinity at A_{2A} receptor subtype compared to its previously reported 1,3-dimethyl analog [22] having A_{2A} K_i = 0.25 μM. Introduction of a methyl substituent at N^7-position resulted in further increase in binding affinity for A_{2A} receptors (K_i = 0.108 μM). Although a marginal decrease in A_{2A} affinity was observed by replacing methyl at N^7 position with other substituents as in case of compounds **7b-e** but overall better selectivity resulted for A_{2A} receptor subtype in comparison to other AR subtypes, especially in case of propargyl analogue **7d** (A_{2A} K_i = 0.278 μM), (A_1/A_{2A} = 17, A_3/A_{2A} = 12). Shifting the location of the *meta* substituent to *para* position of the 8-phenyl ring in xanthines **8**, **8a**, **8d** led to a slight decrease in affinity for A_{2A} AR with *para*-substituted 7-methylxanthine **8a** displaying a 20-fold decrease in comparison to its *meta*-substituted counterpart **7a**. However, a moderate increase in selectivity towards A_{2A} subtype was seen. No significant change in binding affinity but a drop in selectivity towards A_{2A} AR was noticed with 7-propargyl substitution in the *meta* (**7d**) and *para* (**8d**) substituted series. No noteworthy improvement in binding affinity and selectivity for AR subtypes was observed with 1,3-unsymmetrical substitution in 1-methyl-3-propylxanthines **11**, **11a** and **11b** in comparison to their symmetrically substituted dipropyl analogues **7**, **7a**, **7d**. Overall, substitution of propargyl group at N^7-position seems to be a good option for obtaining reasonably potent and selective A_{2A} ligands as is observed in the case of xanthines **7d**, **8d** and **11b** in the present study. Representative radioligand competition curves for the most potent compound **7a** and both 7-propargyl analogues **7d** and **8d** have been presented under Fig. 6A–C in the supporting information section.

2.2.2. Antiparkinsonian activity

All the newly synthesized xanthines were evaluated for antiparkinsonian activity using perphenazine (PPZ) induced catatonia in rats [29,30]. L-dopa was used as a standard 100 mg/kg in methylcellulose (0.5%) and dosed orally. Mean values of the treated groups were compared with that of the control group and analysed using analysis of variance followed by Dunnett's test of multiple comparisons (Fig. 2a–c).

Test compounds were injected at a fixed dose of 100 mg/kg thirty minutes prior to injecting perphenazine intraperitonially. Most of the synthesized xanthines significantly lowered the catatonic score as compared to control and displayed antiparkinsonian effects comparable to standard. Severity of tremors was negligible in animals pre-treated with test compounds. Reversal of drug induced catatonia was observed at various time intervals. The catatonic score of various animal groups



Scheme 2. Synthetic route to 8-(substituted phenyl)xanthines (**11a** and **11b**). Reagents and conditions: (i) MeOH/CH_3COOH, room temperature, 18 h; (ii) $SOCl_2$, reflux, 30–40 min; NH_4OH, (iii) RX, K_2CO_3, DMF.

have been summarized in Table 2. Out of 1,3-symmetrically substituted dialkylxanthines, compounds **7a** and **8a** produced significant anti-parkinsonian activity in comparison to that of control suggesting that substitution of hydrogen at 7-position of 1,3-dialkyl-8-phenylxanthines with methyl group increases anti-cataleptic activity. 7-Propargyl-substituted xanthines **7d, 8d, 11b** also noticeably reversed the signs of catatonia and showed very promising results.

2.2.3. Molecular docking analysis

In order to further validate the biological results, all the newly synthesized xanthines were docked to the putative binding site of the A_{2A} adenosine receptor using Maestro 10.5 module, Schrodinger. The antagonist-bound crystal structure of A_{2A}AR (PDB ID 3EML) was first analysed to identify receptor-ligand interactions responsible for receptor binding to validate the method used (Fig. 3a and b).

The co-crystallized A_{2A}AR antagonist ZM241385 is outlined by Met-177, Leu-85, Trp-246, Leu-249, Ile 80, Val-84, Phe-168, Tyr-271, Glu-169, Asn-253, His-264, Leu-167 residues which constitute the binding site. It consists of a core bicyclic triazolotriazine unit (located roughly in the middle of the binding cavity), a furan ring (located in the lower part of the binding cavity) and a 4-hydroxyphenylethyl side chain (located in the upper part of the binding cavity). The exocyclic nitrogen of the triazolotriazine unit displays hydrogen bonding interactions with the highly conserved Asn253 and Glu169 residues and hydrophobic aromatic π-π stacking interactions with the equally conserved Phe168 side chain. The 4-hydroxyphenylethyl side chain shares polar

Table 1
Binding affinity and selectivity profile of synthesized xanthine derivatives at adenosine receptor subtypes (A_1, A_{2A} and A_3).

COMP. NO.	K_i (μM)			A_{2A} Selectivity	
	$(A_1)^a$	$(A_{2A})^b$	$(A_3)^c$	A_1/A_{2A}	A_3/A_{2A}
7	0.201 (0.162–0.249)	0.177 (0.149–0.210)	0.208 (0.163–0.265)	1.1	1.2
7a	0.152 (0.109–0.212)	0.108 (0.081–0.143)	0.157 (0.149–0.166)	1.4	1.45
7b	3.49 (2.33–5.23)	0.857 (0.711–1.03)	2.57 (2.08–3.17)	4.1	3.0
7c	6.58 (5.89–7.36)	1.80 (1.65–1.97)	3.35 (2.63–4.26)	3.7	1.9
7d	4.75 (3.05–7.39)	0.278 (0.242–0.319)	3.35 (2.07–5.41)	17	12
7e	5.09 (4.04–6.42)	4.02 (3.23–5.00)	2.11 (1.22–3.65)	1.3	0.52
8	2.19 (1.81–2.64)	0.321 (0.184–0.559)	2.49 (1.41–4.38)	6.8	7.8
8a	11.7 (9.60–14.2)	1.94 (1.58–2.39)	2.54 (2.06–3.14)	6.0	1.3
8d	2.39 (2.15–2.65)	0.265 (0.238–0.295)	2.58 (2.05–3.25)	9.0	9.7
11	1.59 (9.48–2.65)	1.36 (1.01–1.81)	0.799 (0.639–1.00)	1.2	0.59
11a	21.0 (19.0–23.2)	5.44 (5.07 5.83)	12.4 (11.9–13.0)	3.9	2.3
11b	5.63 (3.62–8.77)	0.736 0.583–0.930	2.72 (1.62–4.57)	7.6	3.7

Shown values are geometric means from 3 to 5 experiments in μM with 95% confidence intervals in parentheses, where:
[a] Displacement of specific (^{3}H)CCPA binding in CHO cells, stably transfected with human recombinant A_1 adenosine receptor, expressed as K_i (μM).
[b] Displacement of specific (^{3}H)NECA binding in CHO cells, stably transfected with human recombinant A_{2A} adenosine receptor, expressed as K_i (μM).
[c] Displacement of specific (^{3}H)HEMADO binding in CHO cells, stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (μM).

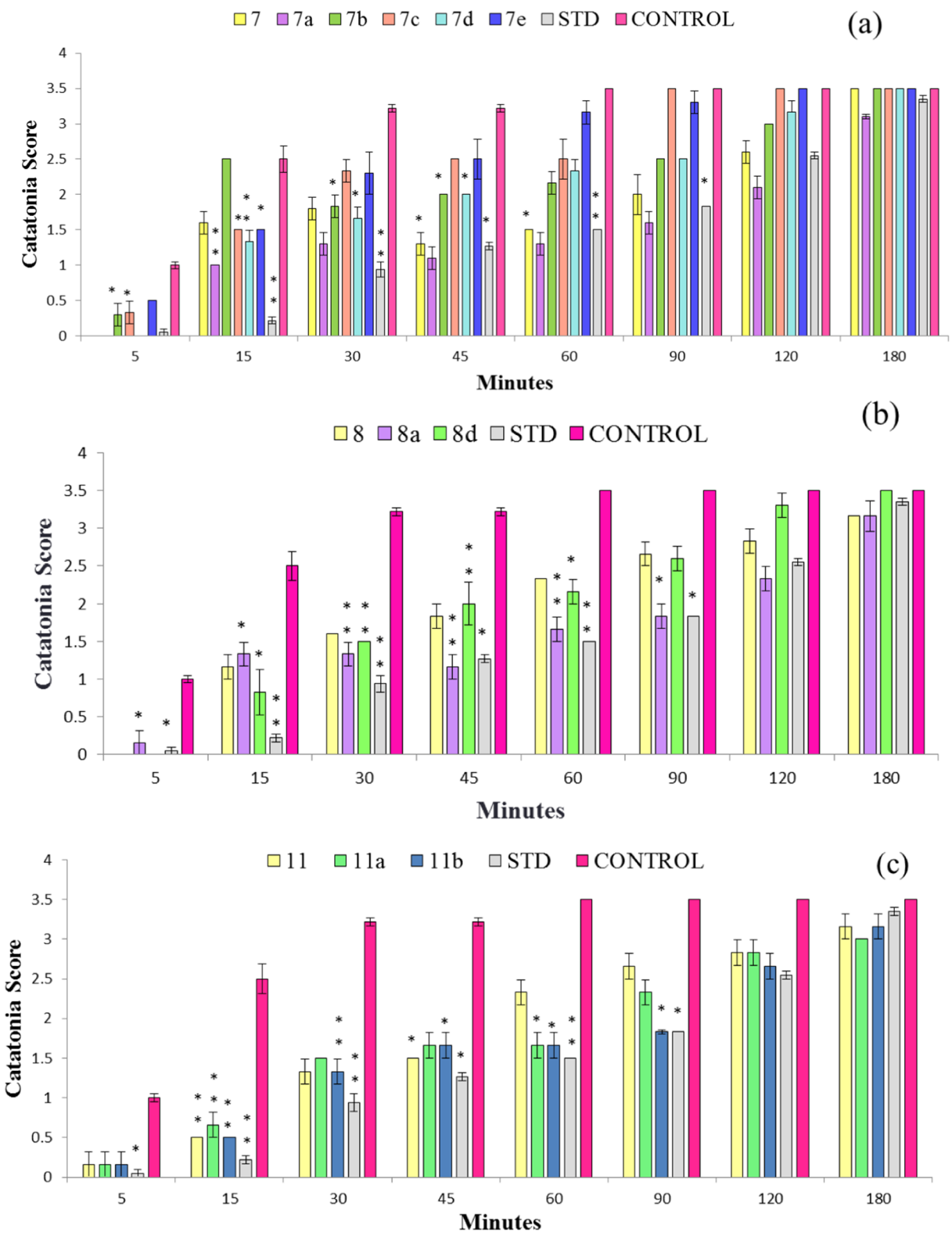


Fig. 2. (a)–(c). Effect of synthesized compounds (100 mg/kg, p.o.) on PPZ (5 mg/kg) induced catatonia in rats. The compounds were dosed 30 min before i.p. administration of PPZ. L-dopa was used as standard (100 mg/kg., p.o.).

interactions with Ile 80. Synthesized compounds were docked in order to find the type of interactions and major amino acid residues responsible for effective receptor-ligand interactions. Interestingly, substituted xanthines shared an identical binding motif and oriented themselves in the binding pocket to interact with the key recognition residues resulting in good binding affinity. Binding interactions of active compounds **7a**, **8d** are clearly illustrated in Figs. 4 and 5, respectively.

The xanthine nucleus interacts through hydrophobic aromatic π-π stacking interactions with the Phe168 side chain and hydrogen bonding with Asn253 residue. The exocyclic oxo group of xanthine ring displayed hydrogen bonding with Asn253 residue. The chlorine of the

Table 2
Effect of various compounds on severity of catatonia induced by PPZ in terms of catatonic scores.

COMP. NO.	Degree of catatonic response (score) (MEAN ± SEM) Min							
	5	15	30	45	60	90	120	180
7	0 ± 0.0	1.6 ± 0.16*	1.8 ± 0.16*	1.3 ± 0.16*	1.5 ± 0*	2.0 ± 0.28	2.6 ± 0.16	3.5 ± 0.0
7a	0 ± 0.0	1 ± 0.0**	1.3 ± 0.16**	1.1 ± 0.16**	1.3 ± 0.16*	1.6 ± 0.16*	2.1 ± 0.16*	3.1 ± 0.33*
7b	0.3 ± 0.16*	2.5 ± 0.0	1.83 ± 0.16*	2 ± 0.0*	2.16 ± 0.16	2.5 ± 0.0	3 ± 0.0	3.5 ± 0.0
7c	0.33 ± 0.16*	1.5 ± 0.0*	2.33 ± 0.16	2.5 ± 0.0	2.5 ± 0.28	3.5 ± 0.0	3.5 ± 0.0	3.5 ± 0.0
7d	0 ± 0.0	1.33 ± 0.16**	1.66 ± 0.16*	2 ± 0.0*	2.33 ± 0.16	2.5 ± 0.0	3.16 ± 0.16	3.5 ± 0.0
7e	0.5 ± 00	1.5 ± 00*	2.3 ± 0.33	2.5 ± 0.28	3.16 ± 0.16	3.3 ± 0.16	3.5 ± 00	3. 5 ± 00
8	0 ± 0.0	0.66 ± 0.16*	1.5 ± 0.0*	2.3 ± 0.16	2.5 ± 0.0	2.6 ± 0.16	3.1 ± 0.16	3.5 ± 0.0
8a	0.16 ± 0.16*	1.33 ± 0.16*	1.33 ± 0.16**	1.16 ± 0.16**	1.66 ± 0.16**	1.83 ± 0.16*	2.33 ± 0.16	3.16 ± 0.33
8d	0 ± 0.0	0.83 ± 0.33*	1.5 ± 0.0**	2.0 ± 0.28**	2.16 ± 0.16*	2.6 ± 0.16	3.3 ± 0.16	3.5 ± 0.0
11	0.16 ± 0.16	0.5 ± 0.0**	1.33 ± 0.16**	1.5 ± 0.0*	2.33 ± 0.16	2.66 ± 0.16	2.83 ± 0.16	3.16 ± 0.16
11a	0.16 ± 0.16	0.66 ± 0.16**	1.5 ± 0*	1.66 ± 0.16*	1.66 ± 0.16*	2.33 ± 0.16	2.83 ± 0.16	3 ± 0
11b	0.16 ± 0.16	0.5 ± 00**	1.33 ± 0.16**	1.66 ± 0.16*	1.66 ± 0.16*	1.83 ± 0.03*	2.66 ± 0.16	3.16 ± 0.16
Standard	0.05 ± 0.05*	0.22 ± 0.05**	0.94 ± 0.1**	1.27 ± 0.05*	1.5 ± 0**	1.83 ± 0*	2.55 ± 0.05	3.44 ± 0.05
Control	1 ± 0.05	2.5 ± 0.19	3.22 ± 0.05	3.22 ± 0.05	3.5 ± 0.0	3.5 ± 0.0	3.5 ± 0.0	3.5 ± 0.0

Significant differences compared with control treatment group (*$p < 0.05$, **$p < 0.001$) as compared to control. (ANOVA followed by Dunnett's test.

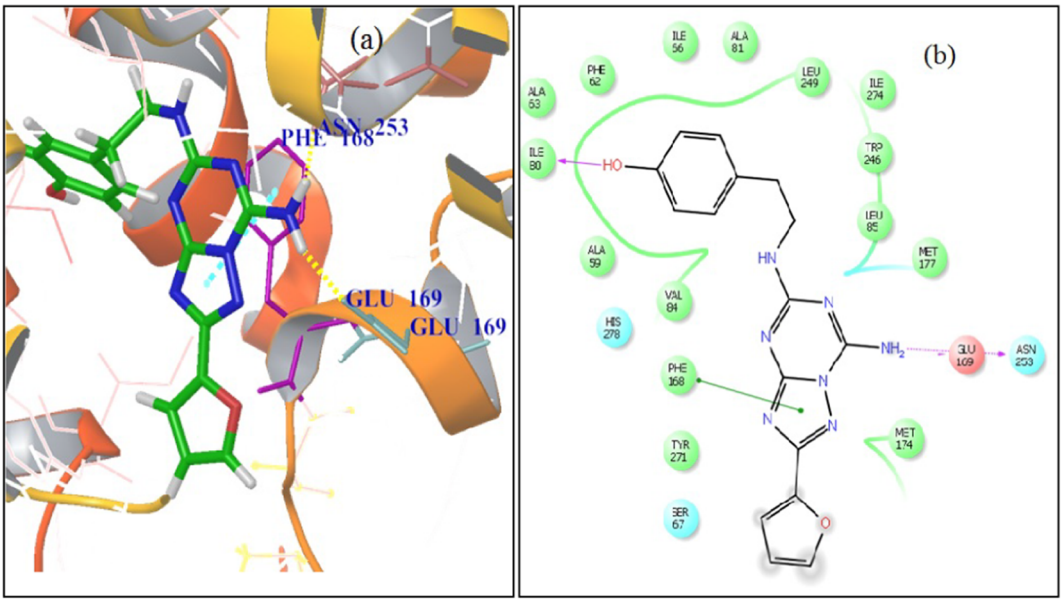


Fig 3. Binding orientation of redocked co-crystallized A_{2A} AR antagonist ZM241385 (docking score −10.11) in the binding site of crystal structure of A_{2A}AR (PDB ID 3EML) **(a):** 3D-interactions; hydrophobic aromatic π-π stacking interactions with Phe168 shown in blue, polar interactions with Asn 253 and Glu169 shown in yellow dotted lines (**b**): 2D-binding interactions diagram of ZM241385 in the binding pocket of A_{2A}AR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chloropropoxy side chain interacted with the highly conserved Glu169 residues which accounts for improved binding affinity. The docking score of the best poses docked into the receptor binding pocket for all the synthesized compounds is calculated to predict the strength of interaction. It was found that all the synthesized xanthines displayed appreciable binding interactions with the key amino acid residues in the binding pocket of A_{2A}AR. These observed favourable interactions between A_{2A}AR and the new xanthine derivatives might, at least in part, explain the observed *in vitro* and *in vivo* activity of these compounds.

3. Conclusions

A new series of 1,3,7,8-tetrasubstituted xanthine derivatives have been synthesized as potent and selective AR ligands. Synthesized xanthines displayed varying degrees of affinity towards adenosine receptor subtypes with selectivity for the A_{2A} subtype in *in vitro* radioligand binding studies. 1,3-Dipropylxanthine **7a** bearing a methyl substituent at N-7 position represents the most potent compound of the series and displayed highest A_{2A} affinity (K_i = 0.108 μM). The N^7-propargyl analogues **7d** and **8d** showed 9–17 fold selectivity towards the A_{2A} AR subtype *versus* A_1 and A_3 receptors along with reasonable affinity. All compounds are highly selective (more than 10–100 fold) towards A_{2A} when compared to A_{2B} AR subtype as no A_{2B} interaction was detectable in the tested concentrations. Most of the synthesized xanthines significantly lowered the catatonic score as compared to control and displayed antiparkinsonian effects comparable to the standard drug L-DOPA when assessed using perphenazine induced catatonia in rats. 8-[3-(3-Chloropropoxy)]-1,3-dipropyl-7-methylxanthine (**7a**, RB-531) produced maximum antiparkinsonian activity and produced a response comparable to that of standard treatment. The protective effect of **7a** in parkinsonism is conceived to be through the adenosine A_{2A} receptor antagonism. The findings were further validated by docking the newly synthesized xanthines to the putative binding site of the A_{2A} adenosine receptor using the antagonist-bound crystal structure of the A_{2A}AR (PDB ID 3EML). All the synthesized xanthines displayed favourable

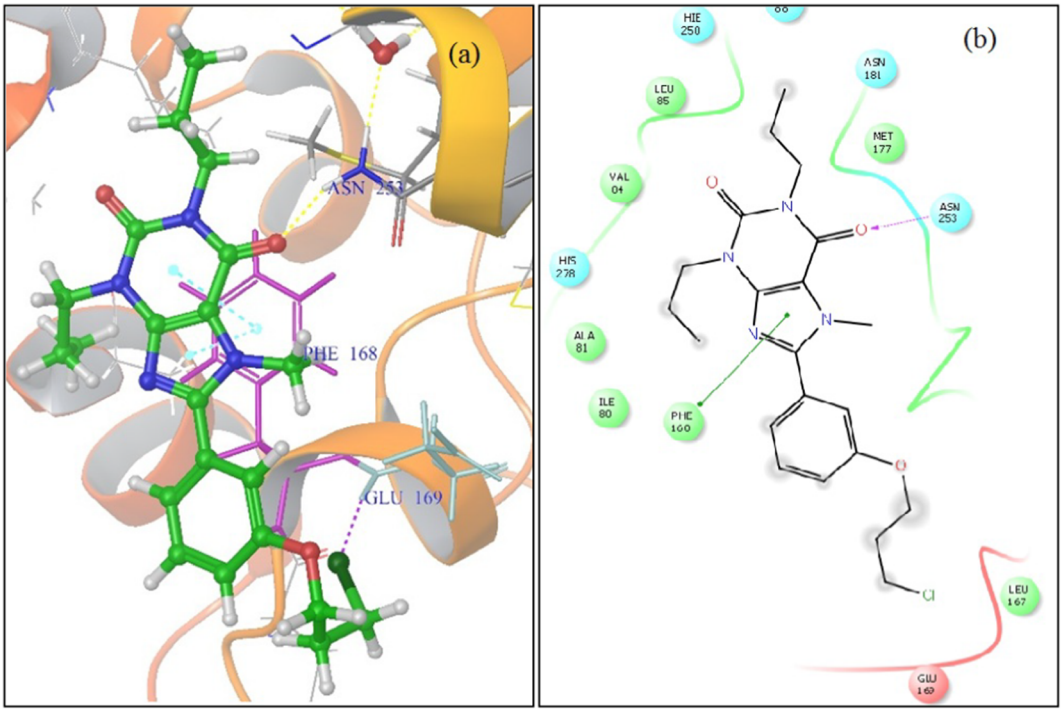


Fig. 4. Binding site interactions of compound **7a** in the binding site of crystal structure of $A_{2A}AR$ (PDB ID 3EML): (**a**) 3-D ligand binding interaction diagram of active compound **7a**; hydrophobic aromatic π-π stacking interactions with Phe168 shown in dotted blue lines, polar interactions with the amino acid residues Asn253 (yellow) and Glu169 are shown in purple dotted lines (**b**) 2-D ligand binding interaction diagram of compound **7a**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

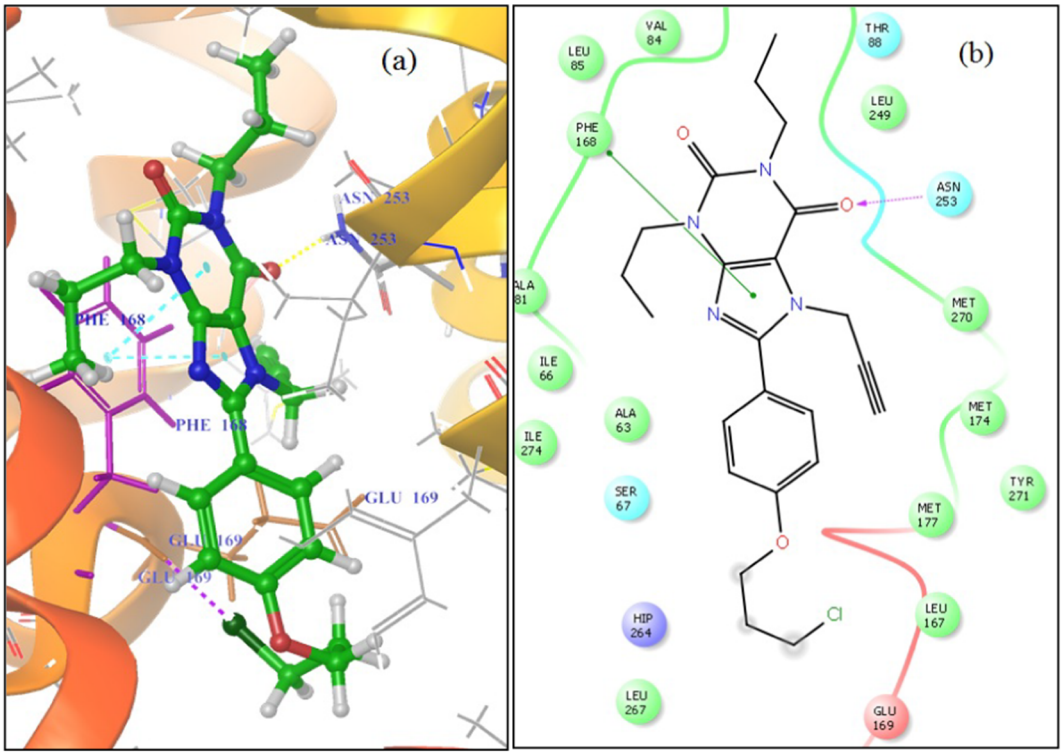


Fig. 5. Binding site interactions of compound **8d** in the binding site of crystal structure of $A_{2A}AR$ (PDB ID 3EML): (**a**) 3-D ligand binding interaction diagram of active compound **8d**; hydrophobic aromatic π-π stacking interactions with Phe168 shown in dotted blue lines, polar interactions with the amino acid residues Asn253 (yellow) and Glu169 are shown in purple dotted lines (**b**) 2-D ligand binding interaction diagram of compound **8d**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interactions with key amino acid residues which account for good receptor binding. High docking scores comparable to that of the co-crystallized ligand ZM241385 further supported the pharmacological data.

4. Experimental protocol

4.1. Chemistry

4.1.1. Material and method

All melting points were obtained using glass capillary tubes on Veego melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer RX1 Fourier Transform-Infrared (FT-IR) spectrophotometer model as potassium bromide pellets (υ_{max} in cm^{-1}). Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectroscopy was performed using a Brucker AV-400F spectrophotometer for solutions in deuteriochloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6) and are reported in parts per million (ppm) downfield from tetramethylsilane (Me_4Si) as internal reference. The spin multiplicities are indicated by the symbol, s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), sext (sextet), m (multiplet), br (broad) and coupling constants (*J*) are given in Hertz (Hz). Mass spectra were recorded on Waters Q-Tof Micro mass spectrometer. Elemental analyses were carried out on a Thermo Scientific (FLASH 2000) CHNS-O elemental analyser. Plates for thin layer chromatography (TLC) were prepared with silica gel G according to method of Stahl (E. Merck) using ethyl acetate as solvent and activated at 110 °C for 30 min. Chromatographic spots were visualized by exposure to iodine vapours in iodine chamber. Anhydrous sodium sulphate was utilized as drying agent. All solvents were freshly distilled and dried prior to use according to standard procedures.

4.1.2. General method for the synthesis of benzylidene derivatives **5** and **6**

To a stirred solution of 5,6-diamino-1,3-propyluracil, **2** (1.0 g, 4.41 mmol) in MeOH–AcOH (4:1, 40 ml) was slowly added the solution of oily residue of requisite *m/p* substituted aldehyde **3** and **4** in methanol (24 ml). The reaction mixture was further stirred overnight at room temperature and the completion of reaction was monitored by thin layer chromatography. The yellow colored precipitate obtained was filtered off, washed with methanol and dried to obtain corresponding quite unstable benzylidene derivatives **5** and **6** respectively which were used as such for further cyclization.

4.1.2.1. 6-Amino-5-[{3-(3-chloropropoxy)benzylidene}amino]-1,3-dipropyluracil (**5**).

(0.65 g, 36.31%), mp 119–121 °C. ^1H NMR ($CDCl_3$): δ 0.96 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.40 Hz), 1.03 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.40 Hz), 1.69 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.56 Hz), 1.79 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.68 Hz), 2.25 (p, 2H, $-OCH_2CH_2-$, J = 6.08 Hz), 3.76 (t, 2H, $-CH_2Cl$, J = 6.28 Hz), 3.90–3.96 (m, 4H, 2 X$-NCH_2-$), 4.16 (t, 2H, $-OCH_2-$, J = 5.84 Hz), 5.69 (br s, 2H, NH_2, exchangeable), 6.92 (d, 1H, 4-C*H*, aromatic, J_o = 7.6 Hz), 7.28–7.31 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic) and 9.78 ppm (s, 1H, N = C*H*).

4.1.2.2. 6-Amino-5-[{4-(3-chloropropoxy)benzylidene}amino]-1,3-dipropyluracil (**6**).

(0.65 g, 36.31%), ^1H NMR ($CDCl_3$): δ 0.94 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.4 Hz), 1.01 (t, 3H, $NCH_2CH_2CH_3$, J = 7.52 Hz), 1.69 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.6 Hz), 1.78 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.72 Hz), 2.17–2.31 (m, 4H, $-OCH_2CH_2-$ and CH_2Cl), 3.74–3.79 (m, 4H, 2 X$-NCH_2-$), 4.16 (t, 2H, $-OCH_2-$, J = 5.8 Hz), 5.70 (br s, 2H, NH_2, exchangeable), 6.94 (d, 2H, 3-C*H* and 5-C*H*, aromatic, J_o = 8.68 Hz), 7.83 (d, 2H, 2-C*H* and 4-C*H*, aromatic, J_o = 8.76 Hz) and 9.89 ppm (s, 1H, N = C*H*).

4.1.3. General method for synthesis of 8-substituted-1,3-dipropylxanthines **7** and **8**

Benzylidene derivatives **5** and **6** (1.0 g, 2.45 mmol) thus obtained were cyclized individually by refluxing in thionyl chloride (20 ml) for 30–40 min. The excess thionyl chloride was removed under reduced pressure to get a solid product. Ice cold water was added to it and resulting suspension was neutralized with ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried and recrystallized from ethanol to afford the desired product **7** and **8**, respectively.

4.1.3.1. 8-[3-(3-Chloropropoxy)phenyl]-1,3-dipropylxanthine (**7**).

(0.8 g, 80.80%), mp 198–200 °C. FTIRυ_{max} (KBr): 3180 (N−H), 2961 (C−H, aliphatic), 1700 (C═O), 1653 (C═C), 1514 (C═N), 1220 (*asym.* C−O−C), 1047 (*sym.* C−O−C) and 720 cm^{-1} (C−Cl). ^1H NMR ($CDCl_3$): δ 0.94 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.4 Hz), 1.01 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.5 Hz), 1.73 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.44 Hz), 1.85 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.36 Hz), 2.27 (p, 2H, $-OCH_2CH_2-$, J = 6.0 Hz), 3.79 (t, 2H, $-CH_2Cl$, J = 6.26 Hz), 4.11 (t, 2H, $-NCH_2-$, J = 7.48 Hz), 4.19 (t, 2H, $-NCH_2-$, J = 7.32 Hz), 4.23 (t, 2H, $-OCH_2-$, J = 5.8 Hz), 7.04 (dd, 1H, 4-C*H*, aromatic, J_o = 8.08 Hz, J_m = 2.2 Hz), 7.41 (t, 1H, 5-C*H*, aromatic, J_o = 7.98 Hz), 7.77 (d, 1H, 2-C*H*, aromatic, J_m = 2.24 Hz), 7.82 (d, 1H, 6-C*H*, aromatic, J_o = 7.74 Hz) and 12.76 ppm (br s, 1H, N−*H*, exchangeable). ^{13}C NMR ($CDCl_3$): δ 11.22 (1C, N−CH_2CH_2**C**H_3), 11.36 (1C, N−CH_2CH_2**C**H_3), 21.33 (1C, N−CH_2**C**H_2CH_3), 21.37 (1C, N−CH_2**C**H_2CH_3), 32.15 (1C, **C**H_2), 41.25 (1C, **C**H_2Cl), 43.37 (1C, N−CH_2), 45.43 (1C, N−CH_2), 64.55 (1C, O−**C**H_2), 108.06 (1C, Ar**C**), 113.84 (1C, Ar**C**H), 116.36 (1C, Ar**C**H), 119.36 (1C, Ar**C**H), 129.87 (1C, Ar**C**H), 130.11 (1C, Ar**C**), 149.49 (1C, Ar**C**), 150.98 (1C, Ar**C**), 151.43 (1C, Ar**C**), 155.73 (1C, **C**═O) and 159.15 ppm (1C, **C**═O). ESI-MS: 405 $[MH]^+$, 407 $[MH+2]^+$ Calcd. for $C_{20}H_{25}N_4O_3Cl$: C, 59.33; H, 6.22; N, 13.84%. Found C, 59.14; H, 6.13; N, 13.53%.

4.1.3.2. 8-[4-(3-Chloropropoxy)phenyl]-1,3-dipropylxanthine (**8**).

(0.78 g, 78.78%), mp 218–220 °C. FTIRυ_{max} (KBr): 3202 (N−H), 2961 (C−H, aliphatic), 1696 (C═O), 1654 (C═C), 1529 (C═N), 1248 (*asym.* C−O−C), 1032 (*sym.* C−O−C) and 729 cm^{-1} (C−Cl). ^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.48 Hz), 0.90 (t, 3H, $-NCH_2CH_2CH_3$, J = 7.36 Hz), 1.58 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.36 Hz), 1.74 (sext, 2H, $-NCH_2CH_2CH_3$, J = 7.16 Hz), 2.20 (p, 2H, $-OCH_2CH_2-$, J = 6.24 Hz), 3.81 (t, 2H, $-CH_2Cl$, J = 6.44 Hz), 3.87 (t, 2H, $-NCH_2-$, J = 7.24 Hz), 4.02 (t, 4H, $-NCH_2-$, J = 6.88 Hz), 4.17 (t, 4H, $-OCH_2-$, J = 6.03 Hz), 7.09 (d, 2H, 3-C*H* and 5-C*H* aromatic, J_o = 8.88 Hz), 8.07 ppm (d, 2H, 2-C*H* and 4-C*H* aromatic, J_o = 8.84 Hz) and 12.56 ppm (br s, 1H, N−*H*, exchangeable). ^{13}C NMR ($CDCl_3$): δ 11.23 (1C, N−CH_2CH_2**C**H_3), 11.50 (1C, N−CH_2CH_2**C**H_3), 21.39 (2C, 2 XN−CH_2**C**H_2CH_3), 32.14 (1C, **C**H_2), 41.34 (1C, **C**H_2Cl), 43.36 (1C, N−CH_2), 45.36 (1C, N−CH_2), 64.46 (1C, −O**C**H_2), 107.69 (1C, Ar**C**), 114.73 (2C, Ar**C**H), 121.84 (1C, Ar**C**), 128.76 (2C, Ar**C**H), 149.95 (1C, Ar**C**), 151.08 (1C, Ar**C**), 151.90 (1C, Ar**C**), 155.69 (1C, C═O) and 160.68 ppm (1C, C═O). ESI-MS: 405 $[MH]^+$, 407 $[MH+2]^+$ Calcd. for $C_{20}H_{25}N_4O_3Cl$: C, 59.33; H, 6.22; N, 13.84%. Found C, 59.51; H, 6.02; N, 13.75%.

4.1.4. General method for synthesis of various 7-substituted-1,3-dipropyl-8-phenylxanthines **7a-e** and **8a, 8d**

Requisite alkyl/aryl halide was added to the stirred slurry of **7** or **8** (0.8 g, 1.97 mmol) and anhydrous potassium carbonate (1.5 g, 10.85 mmol) in dimethylformamide (15 ml). The reaction mixture was further stirred and heated at 70–80 °C for 5 h. The completion of the reaction was monitored by TLC. The resultant suspension was filtered, cooled and water was added to it. The precipitate obtained was filtered, washed thoroughly with cold water, dried and recrystallized from methanol to afford **7a-e** and **8a**, **8d**, respectively.

*4.1.4.1. 8-[3-(3-Chloropropoxy)phenyl]-1,3-dipropyl-7-methylxanthine (**7a**).* (0.5 g, 60.97%), mp 98–100 °C. FTIRυ_{max} (KBr): 2959 (C—H, aliphatic), 1692 (C=O), 1656 (C=C), 1531 (C=N), 1249 (*asym.* C—O—C), 1034 (*sym.* C—O—C) and 754 cm^{-1} (C—Cl). ^{1}H NMR ($CDCl_3$): δ 0.96–1.00 (m, 6H, 2 X—$NCH_2CH_2CH_3$), 1.71 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.36 Hz), 1.84 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.44 Hz), 2.27 (p, 2H, —OCH_2CH_2—, J = 6.04 Hz), 3.77 (t, 2H, —CH_2Cl, J = 6.26 Hz), 4.00 (t, 2H, —NCH_2—, J = 7.58 Hz), 4.05 (s, 3H, N—CH_3), 4.11 (t, 2H, —NCH_2—, J = 7.48 Hz), 4.19 (t, 2H, —OCH_2—, J = 5.82 Hz), 7.04–7.06 (m, 1H, 4-C*H*, aromatic), 7.21–7.24 (m, 2H, 2-C*H* and 6-C*H*, aromatic) and 7.43 ppm (t, 1H, 5-C*H*, aromatic, J_o = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 11.18 (1C, N—CH_2CH_2**C**H_3), 11.38 (1C, N—CH_2CH_2**C**H_3), 21.37 (1C, N—CH_2**C**H_2CH_3), 21.42 (1C, N—CH_2**C**H_2CH_3), 32.12 (1C, N—CH_3), 33.92 (1C, **C**H_2), 41.38 (1C, **C**H_2Cl), 42.90 (1C, N—CH_2), 44.90 (1C, N—CH_2), 64.48 (1C, O—**C**H_2), 108.61 (1C, Ar**C**), 115.66 (1C, Ar**C**H), 116.52 (1C, Ar**C**H), 121.62 (1C, Ar**C**H), 129.44 (1C, Ar**C**H), 130.03 (1C, Ar**C**), 147.79 (1C, Ar**C**), 151.12 (1C, Ar**C**), 151.58 (1C, Ar**C**), 155.55 (1C, **C**=O) and 159.03 ppm (1C, **C**=O). ESI-MS: 419 $[MH]^+$, 421 $[MH+2]^+$ Calcd. for $C_{21}H_{27}N_4O_3Cl$: C, 60.21; H, 6.50; N, 13.37%. Found C, 59.99; H, 6.36; N, 13.27%.

*4.1.4.2. 8-[3-(3-Chloropropoxy)phenyl]-1,3,7-tripropylxanthine (**7b**).* (0.6 g, 68.18%), mp 70–71 °C. FTIRυ_{max} (KBr): 2959 (C—H, aliphatic), 1700 (C=O), 1658 (C=C), 1535 (C=N), 1263 (*asym.* C—O—C), 1031 (*sym.*C—O—C) and 800 cm^{-1} C—Cl). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, —$NCH_2CH_2CH_3$, J = 7.42 Hz), 0.96–0.98 (m, 6H, 2 X—$NCH_2CH_2CH_3$), 1.71 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.6 Hz), 1.84 (sext, 4H, 2 X—$NCH_2CH_2CH_3$, J = 7.48 Hz), 2.27 (p) and 2.36 (p) (3:1, 2H, —OCH_2CH_2—, J = 6.04 Hz), 3.6 (t) and 3.77 (t) (1:3, 2H, —CH_2Cl, J = 6.24 Hz), 4.00 (t, 2H, —NCH_2—, J = 7.64 Hz), 4.11 (t, 2H, —NCH_2—, J = 7.52 Hz), 4.18 (t, 2H, —OCH_2— J = 5.8 Hz), 4.29 (t, 2H, —NCH_2—, J = 7.68 Hz), 7.05 (dd, 1H, 4-C*H*, aromatic, J_o = 8.28 Hz, J_m = 2.52 Hz), 7.15 (d, 1H, 6-C*H*, J_o = 8.8 Hz), 7.17 (s, 1H, 2-C*H* aromatic) and 7.42 ppm (t, 1H, 5-C*H*, aromatic, J_o = 7.84 Hz). ^{13}C NMR ($CDCl_3$): δ 11.00 (1C, N—CH_2CH_2**C**H_3), 11.30 (1C, N—CH_2CH_2**C**H_3), 11.47 (1C, N—CH_2CH_2**C**H_3), 21.47 (1C, N—CH_2**C**H_2CH_3), 21.51(1C, N—CH_2**C**H_2CH_3), 24.79 (1C, N—CH_2**C**H_2CH_3), 29.94 (1C, **C**H_2), 41.46 (1C, **C**H_2Cl), 43.06 (1C, N—CH_2), 44.98 (1C, N—CH_2), 48.04 (1C, N—CH_2), 64.54 (1C, O—**C**H_2), 108.05 (1C, Ar**C**), 115.68 (1C, Ar**C**H), 116.47 (1C, Ar**C**H), 121.59 (1C, Ar**C**H), 130.15 (1C, Ar**C**H), 130.36 (1C, Ar**C**), 148.39 (1C, Ar**C**), 151.29 (1C, Ar**C**), 151.79 (1C, Ar**C**), 155.21 (1C, **C**=O) and 159.12 ppm (1C, **C**=O). ESI-MS: 447 $[MH]^+$, 449 $[MH+2]^+$ Calcd. for $C_{23}H_{31}N_4O_3Cl$: C, 61.80; H, 6.99; N, 12.53%. Found C, 61.56; H, 6.94; N, 12.38%.

*4.1.4.3. 7-Allyl-8-[3-(3-chloropropoxy)phenyl]-1,3-dipropylxanthine (**7c**).* (0.58 g, 65.98%), mp 78–80 °C. FTIRυ_{max} (KBr): 2962 (C—H, aliphatic), 1695 (C=O), 1659 (C=C), 1535 (C=N), 1253 (*asym.* C—O—C), 1045 (*sym.* C—O—C) and 801 cm^{-1} (C—Cl).^{1}H NMR ($CDCl_3$): δ 0.96 (m, 6H, 2 X—$NCH_2CH_2CH_3$), 1.70 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.56 Hz), 1.83 (sext, 2H-$NCH_2CH_2CH_3$, J = 7.44 Hz), 2.26 (p) and 2.34 (p) (9:1, 2H, —OCH_2CH_2—, J = 6.04 Hz), 3.76 (t, 2H, —CH_2Cl, J = 6.28 Hz), 3.99 (t, 2H, —NCH_2—, J = 7.6 Hz), 4.12, (t, 2H, —NCH_2—, J = 7.48 Hz), 4.16 (t, 2H, —OCH_2—, J = 5.84 Hz), 4.98 (d, 2H, —NCH_2—CH = CH_2, J = 4.84 Hz), 5.09 (d, 1H, —CH_2—CH = CH(*H*), J_{trans} = 17.08 Hz), 5.30 (dd, 1H, —CH_2—CH = C*H*(H), J_{cis} = 10.36 Hz, J_{gem} = 0.56 Hz), 6.11 (m, 1H, —CH_2—C*H* = CH_2, J = 4.88 Hz, J_{trans} = 17.08, J_{cis} = 10.44 Hz), 7.05 (dd, 1H, 4-C*H*, aromatic, J_o = 10.8 Hz, J_m = 2.52 Hz), 7.22–7.24 (m, 2H, 2-C*H* and 6-C*H*, aromatic) and 7.40 ppm (t, 1H, 5-C*H*, aromatic, J_o = 7.96 Hz). ^{13}C NMR ($CDCl_3$): δ 11.21 (1C, N—CH_2CH_2**C**H_3), 11.37 (1C, N—CH_2CH_2**C**H_3), 21.35 (1C, N—CH_2**C**H_2CH_3), 21.45 (1C, N—CH_2**C**H_2CH_3), 32.12 (1C, **C**H_2), 41.39 (1C, **C**H_2Cl), 42.96 (1C, N—CH_2), 44.97 (1C, N—CH_2), 48.43 (1C, N—CH_2), 64.45 (1C, O—**C**H_2), 107.94 (1C, Ar**C**), 115.30 (1C, Ar**C**H), 116.79 (1C, Ar**C**H), 117.45 (1C, —CH=**C**H_2), 121.53 (1C, Ar**C**H), 129.63 (1C, Ar**C**), 130.04 (1C, Ar**C**), 133.40 (1C, —**C**H=CH_2), 148.12 (1C, Ar**C**), 151.16 (1C, Ar**C**), 151.20 (1C, Ar**C**), 155.02 (1C, **C**=O) and 158.98 ppm (1C, **C**=O). ESI-MS: 381 $[M-CH_2CH_2Cl]^+$ Calcd. for $C_{23}H_{29}N_4O_3Cl$: C, 62.08; H, 6.57; N, 12.59%. Found C, 61.99; H, 6.49; N, 12.47%.

*4.1.4.4. 8-[3-(3-Chloropropoxy)phenyl]-1,3-dipropyl-7-(prop-2-ynyl) xanthine (**7d**).* (0.58 g, 66.66%), mp 110–112 °C. FTIRυ_{max} (KBr): 3236 (C—H, alkyne), 2933 (C—H, alkane), 2121 (—C≡C), 1691 (C=O), 1656 (C=C), 1541 (C=N), 1248 (*asym.* C—O—C), 1036 (*sym.* C—O—C) and 754 cm^{-1} (C—Cl). ^{1}H NMR (DMSO-d_6): δ 0.98 (m, 6H, 2 X—$NCH_2CH_2CH_3$), 1.72 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.6 Hz), 1.82 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.44 Hz), 2.28 (p, 1H, —OCH_2CH(*H*)—, J = 6.08 Hz), 2.34 (p, 1H, —OCH_2CH(H)—, J = 6.04 Hz), 2.53 (t, 1H, —C≡C*H*, J = 2.36 Hz), 3.63 (t, 1H, —CH(*H*)Cl, J = 6.36 Hz), 3.77 (t, 1H, —C*H*(H)Cl, J = 6.26 Hz), 4.01 (t, 2H, —NCH_2—, J = 7.6 Hz), 4.11 (t, 2H, —NCH_2—, J = 7.5 Hz), 4.19 (t, 2H, —OCH_2—, J = 5.8 Hz), 5.15 (d, 2H, —CH_2—C≡CH, J = 2.44 Hz), 7.09 (dd, 1H, 4-C*H*, aromatic, J_o = 7.72 Hz, J_m = 2.48 Hz) and 7.36–7.47 ppm (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic). ^{13}C NMR (DMSO-d_6): δ 11.21 (1C, N—CH_2CH_2**C**H_3), 11.38 (1C, N—CH_2CH_2**C**H_3), 21.36 (1C, N—CH_2**C**H_2CH_3), 21.45 (1C, N—CH_2**C**H_2CH_3), 29.83 (1C, **C**H_2), 36.30 (1C, N—CH_2), 41.39 (1C, **C**H_2Cl), 42.99 (1C, N—CH_2), 44.95 (1C, N—CH_2), 64.57 (1C, O—**C**H_2), 65.61 (1C, C≡**C**H), 100.00 (1C, **C**≡CH), 107.72 (1C, Ar**C**), 115.04 (1C, Ar**C**H), 117.29 (1C, Ar**C**H), 121.65 (1C, Ar**C**H), 129.28 (1C, Ar**C**H), 130.27 (1C, Ar**C**), 148.18 (1C, Ar**C**), 151.17 (1C, Ar**C**), 151.67 (1C, Ar**C**), 155.17 (1C, **C**=O) and 159.08 ppm (1C, **C**=O). ESI-MS: 443 $[MH]^+$, 445 $[MH+2]^+$ Calcd. for $C_{23}H_{27}N_4O_3Cl$: C, 62.37; H, 6.14; N, 12.65%. Found C, 61.93; H, 5.93; N, 12.39%.

*4.1.4.5. 7-Benzyl-8-[3-(3-chloropropoxy)phenyl]-1,3-dipropylxanthine (**7e**).* (0.63 g, 64.17%), mp 90–92 °C. FTIRυ_{max} (KBr): 2963 (C—H, aliphatic), 1696 (C=O), 1659 (C=C), 1536 (C=N), 1256 (*asym.* C—O—C), 1027 (*sym.* C—O—C) and 759 cm^{-1} (C—Cl). ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, —$NCH_2CH_2CH_3$, J = 7.44 Hz), 1.00 (t, 3H, —$NCH_2CH_2CH_3$, J = 7.44 Hz), 1.67 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.56 Hz), 1.84 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.48 Hz), 2.18 (p, 2H, —OCH_2CH_2—, J = 6.0 Hz), 3.71 (t, 2H, —CH_2Cl, J = 6.26 Hz), 3.95–4.00 (m, 4H, —NCH_2 and —OCH_2), 4.13 (t, 2H, —NCH_2—, J = 7.52 Hz), 5.64 (s, 2H, —NCH_2), 7.01–7.08 (m, 4H, aromatic), 7.16 (dd, 1H, 4-C*H*, aromatic, J_o = 7.64 Hz, J_m = 1.08 Hz) and 7.27–7.38 ppm (m, 4H, aromatic). ^{13}C NMR ($CDCl_3$): δ 11.24 (1C, N—CH_2CH_2**C**H_3), 11.34 (1C, N—CH_2CH_2**C**H_3), 21.34 (1C, N—CH_2**C**H_2CH_3), 21.47(1C, N—CH_2**C**H_2CH_3), 32.05 (1C, **C**H_2), 41.32 (1C, **C**H_2Cl), 42.92 (1C, N—CH_2), 44.96 (1C, N—CH_2), 49.35 (1C, N—CH_2), 64.28 (1C, O—**C**H_2), 108.09 (1C, Ar**C**), 114.99 (1C, Ar**C**H), 117.10 (1C, Ar**C**H), 121.71 (1C, Ar**C**H), 126.43 (2C, Ar**C**H), 127.85 (1C, Ar**C**H), 128.90 (2C, Ar**C**H), 129.83 (1C, Ar**C**H), 130.13 (1C, Ar**C**), 136.87 (1C, Ar**C**), 148.34 (1C, Ar**C**), 151.17 (1C, Ar**C**), 152.29 (1C, Ar**C**), 155.17 (1C, **C**=O) and 158.92 ppm (1C, **C**=O). ESI-MS: 495 $[MH]^+$, 497 $[MH+2]^+$ Calcd. for $C_{27}H_{31}N_4O_3Cl$: C, 65.51; H, 6.31; N, 11.32%. Found C, 65.50; H, 6.22; N, 11.27%.

*4.1.4.6. 8-[4-(3-Chloropropoxy)phenyl]-1,3-dipropyl-7-methylxanthine (**8a**).* (0.68 g, 82.92%), mp 72–74 °C. FTIRυ_{max} (KBr): 2960 (C—H, alkane), 1696 (C=O), 1656 (C=C), 1535 (C=N), 1255 (*asym.* C—O—C), 1054 (*sym.* C—O—C) and 754 cm^{-1} (C—Cl).^{1}H NMR ($CDCl_3$): δ 0.95–1.00 (m, 6H, 2 X—$NCH_2CH_2CH_3$), 1.69 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.48 Hz), 1.82 (sext, 2H, —$NCH_2CH_2CH_3$, J = 7.44 Hz), 2.29 (p, 2H, —OCH_2CH_2—, J = 6.08 Hz), 3.39 (t) and 3.77 (t) (3:2, 2H, —CH_2Cl, J = 6.66 Hz and J = 6.28 Hz), 3.99 (t, 2H, —NCH_2—, J = 7.6 Hz), 4.03 (s, 3H, N—CH_3), 4.09–4.13 (m, 2H, —NCH_2-), 4.12 (t, merged with —NCH_2—) and 4.19 (t) (1.3:0.7, 2H, —OCH_2—, J = 5.88 Hz), 7.02 (dd, 2H, 3-C*H* and 5-C*H* aromatic, J_o = 8.8 Hz,

J_m = 1.88 Hz) and 7.63 ppm (dd, 2H, 2-C*H* and 6-C*H* aromatic, J_o = 8.79 Hz, J_m = 1.92 Hz). ^{13}C NMR ($CDCl_3$): δ 9.02 (1C, N−CH_2CH_2**C**H_3), 9.21 (1C, N−CH_2CH_2**C**H_3), 19.21 (1C, N−CH_2**C**H_2CH_3), 19.24 (1C, N−CH_2**C**H_2CH_3), 29.90 (1C, N−CH_3), 30.52 (1C, **C**H_2), 39.14 (1C, **C**H_2Cl), 40.67 (1C, N−CH_2), 42.70 (1C, N−CH_2), 65.34 (1C, O−**C**H_2**)**, 106.25 (1C, Ar**C**), 112.72 (2C, Ar**C**H), 118.82 (1C, Ar**C**), 128.62 (2C, Ar**C**H), 145.89 (1C, Ar**C**), 149.02 (1C, Ar**C**), 149.79 (1C, Ar**C**), 153.37 (1C, **C**═O) and 158.15 ppm (1C, **C**═O). ESI-MS: 419 $[MH]^+$, 421 $[MH+2]^+$ Calcd. for $C_{21}H_{27}N_4O_3Cl$: C, 60.21; H, 6.50; N, 13.37%. Found C, 59.94; H, 6.39; N, 12.99%.

*4.1.4.7. 8-[4-(3-Chloropropoxy)phenyl]-1,3-dipropyl-7-(prop-2-ynyl) xanthine (**8d**).* (0.56 g, 64.36%) mp 176–178 °C. FTIRυ_{max} (KBr): 3225 (C−H, alkyne), 2951 (C−H, alkane), 2121 (C≡C) 1699 (C═O), 1658 (C═C), 1542 (C═N), 1223 (*asym.* C−O−C), 1044 (*sym.* C−O−C) and 755 cm^{-1} (C−Cl).^{1}H NMR ($CDCl_3$): δ 0.88–0.94 (m, 6H, 2 X−$NCH_2CH_2CH_3$), 1.63 (sext, 2H, −$NCH_2CH_2CH_3$, J = 7.56 Hz), 1.76 (sext, 2H, −$NCH_2CH_2CH_3$, J = 7.44 Hz), 2.21 (p, 1H, −OCH_2CH(*H*)−, J = 6 Hz), 2.29 (m, 1H, −OCH_2CH(H)− J = 6.04 Hz), 2.43 (t, 1H, −C≡C*H*, J = 2.39 Hz), 3.56 (t, 1H, −CH(*H*)Cl, J = 6.32 Hz), 3.70 (t, 1H, −C*H*(H)Cl, J = 6.24 Hz), 3.93 (t, 2H, −NCH_2−, J = 7.64 Hz), 4.03 (t, 2H, −NCH_2−, J = 7.2 Hz), 4.12 (q, 2H, −OCH_2−, J = 5.8 Hz), 5.06 (d, 2H, −CH_2−C≡CH, J = 2.35 Hz), 6.97 (dd, 2H, 3-C*H* and 5-C*H* aromatic, J_o = 8.76 Hz, J_m = 1.88 Hz) and 7.73 ppm (dd, 2H, 2-C*H* and 6-C*H* aromatic, J_o = 8.76 Hz, J_m = 1.92 Hz). ^{13}C NMR ($CDCl_3$): δ 11.21 (1C, N−CH_2CH_2**C**H_3), 11.38 (1C, N−CH_2CH_2**C**H_3), 21.36 (1C, N−CH_2**C**H_2CH_3), 21.44 (1C, N−CH_2**C**H_2CH_3), 29.74 (1C, **C**H_2), 36.21(1C, N−CH_2), 41.31 (1C, **C**H_2Cl), 42.94 (1C, N−CH_2), 44.90 (1C, N−CH_2), 64.51 (1C, O−**C**H_2), 65.54 (1C, C≡**C**H), 74.13 (1C, **C**≡CH), 107.47 (1C, Ar**C**), 115.04 (2C, Ar**C**H), 120.66 (1C, Ar**C**), 130.77 (2C, Ar**C**H), 148.25 (1C, Ar**C**), 151.18 (1C, Ar**C**), 151.89 (1C, Ar**C**), 155.11 (1C, **C**═O) and 160.59 ppm (1C, **C**═O). ESI-MS: 443 $[MH]^+$, 445 $[MH+2]^+$ Calcd. for $C_{23}H_{27}N_4O_3Cl$: C, 62.37; H, 6.14; N, 12.65%. Found C, 62.11; H, 5.83; N, 12.48%.

*4.1.5. Synthesis of 1,3-unsymmetrically substituted dialkylxanthine **11***

To a stirred solution of 5,6-diamino-1,3-unsymmetrically substituted dialkyluracil (**9**) in methanol-acetic acid (MeOH-AcOH) (4:1, 40 ml) was slowly added the solution of oily residue **3** in methanol (24 ml). The reaction mixture was further stirred overnight at room temperature and the completion of reaction was monitored by TLC. On completion, the reaction mixture was concentrated under vacuum and cold water was added to it. The precipitate obtained was filtered off, washed repeatedly with cold water and dried to obtain quite unstable intermediate benzylidene derivative **10**, which was sufficiently pure for further reaction.

*4.1.5.1. 6-Amino-5-[{3-(3-chloropropoxy)benzylidene}amino]-1-methyl-3-propyluracil (**10**).* (0.5 g, 26.17%), mp 195–197 °C, ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, −$NCH_2CH_2CH_3$, J = 7.46 Hz), 1.60 (sext, 2H, −$NCH_2CH_2CH_3$−, J = 7.52 Hz), 2.20 (p, 2H, −OCH_2CH_2−, J = 6.08 Hz), 3.45 (s, 3H, N−CH_3), 3.69 (t, 2H, −CH_2Cl, J = 6.28 Hz), 3.87 (t, 2H, −NCH_2−, J = 7.6 Hz), 4.11 (t, 2H, −OCH_2−, J = 5.84 Hz), 5.62 (s, 2H, NH_2, exchangeable), 6.85 (d, 1H, 4-C*H*, aromatic, J = 7.64 Hz), 7.21–7.28 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic) and 9.71 ppm (s, 1H, N═C*H*).

The Schiff base (**10**) (1.0 g, 2.63 mmol) was stirred in thionyl chloride (5 ml) for 5 min at room temperature to affect cyclization. The reaction mixture was poured on the crushed ice it and was neutralized with ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried and crystallized from methanol to obtain the desired dialkylxanthine **11**.

*4.1.5.2. 8-[3-(3-Chloropropoxy)phenyl]-3-methyl-1-propylxanthine (**11**).* (0.8 g, 80.80%), mp 238–240 °C. FTIRυ_{max} (KBr): 3166 (N−H), 2958 (C−H, aiphatic), 1697 (C═O), 1650 (C═C), 1520 (C═N), 1224 (*asym.* C−O−C), 1048 (*sym.* C−O−C) and 750 cm^{-1} (C−Cl). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, −$NCH_2CH_2CH_3$, J = 7.48 Hz), 1.71 (sext, 2H, −$NCH_2CH_2CH_3$, J = 7.4 Hz), 2.28 (p, 2H, −OCH_2CH_2−, J = 6.12 Hz), 3.70 (s, 3H, N−CH_3), 3.78 (t, 2H, −CH_2Cl, J = 6.28 Hz), 4.07 (t, 2H, −NCH_2−, J = 7.58 Hz), 4.23 (t, 2H, −OCH_2−, J = 5.88 Hz), 7.04 (dd, 1H, 4-C*H*, aromatic, J_o = 10.88, J_m = 2.55 Hz), 7.41 (t, 1H, 5-C*H*, aromatic, J_o = 8.00 Hz), 7.69–7.72 (m, 2H, 2-C*H* and 6-C*H*, aromatic) and 12.24 ppm (br s, 1H, N−*H*). ^{13}C NMR ($CDCl_3$): δ 11.08 (1C, N−CH_2CH_2**C**H_3), 20.81 (1C, N−CH_2**C**H_2CH_3), 29.50 (1C, **C**H_2), 31.68 (1C, N−CH_3), 41.42 (1C, **C**H_2Cl), 42.17 (1C, N−CH_2), 64.17 (1C, O−**C**H_2), 107.73 (1C, Ar**C**), 111.45 (1C, Ar**C**H), 116.69 (1C, Ar**C**H), 118.93 (1C, Ar**C**H), 129.66 (1C, Ar**C**H), 129.95 (1C, Ar**C**), 148.28 (1C, Ar**C**), 149.56 (1C, Ar**C**), 150.93 (1C, Ar**C**), 154.00 (1C, **C**═O) and 158.59 ppm (1C, **C**═O). ESI-MS: 375 $[M-H]^+$, 377 $[M+H]^+$ Calcd. for $C_{18}H_{21}N_4O_3Cl$: C, 57.37; H, 5.62; N, 14.87%. Found C, 57.12; H, 5.32; N, 14.63%.

*4.1.6. General method for the synthesis of 7-Substituted-1,3-unsymmetrically substituted dialkylxanthines **11a** and **11b***

Requisite alkyl halide was added to the stirred slurry of 1,3-unsymmetrically substituted dialkylxanthine **11** (1.0 g, 2.65 mmol) and anhydrous potassium carbonate (1.0 g, 7.23 mmol) in dimethylformamide (15 ml). The reaction mixture was further stirred and heated at 70–80 °C for 5 h. The completion of the reaction was monitored by TLC. The resultant suspension was filtered, cooled and water was added to it. The mixture was further cooled in ice for complete precipitation. The precipitate obtained was filtered, washed thoroughly with cold water, dried and recrystallized from hexane to afford 7-Substituted-1,3-unsymmetrically substituted dialkylxanthines **11a** and **11b.**

*4.1.6.1. 8-[3-(3-Chloropropoxy)phenyl]-3,7-dimethyl-1-propylxanthine (**11a**).* (0.79 g, 76.69%), mp 82–84 °C. FTIRυ_{max} (KBr): 2957 (C−H, aliphatic), 1696 (C═O), 1661 (C═C), 1539 (C═N), 1218 (*asym.* C−O−C), 1031 (*sym.* C−O−C) and 754 cm^{-1} (C−Cl). ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, −$NCH_2CH_2CH_3$, J = 7.46 Hz), 1.71 (sext, 2H, −$NCH_2CH_2CH_3$, J = 6.52 Hz), 2.30 (p, 2H, −OCH_2CH_2−, J = 6.64 Hz), 3.39 (t) and 3.77 (t) (2H, 2:1, −CH_2Cl, J = 6.64 Hz), 3.63 (s, 3H, N−CH_3), 4.00 (t, 2H, −NCH_2−, J = 7.58 Hz), 4.06 (s, 3H, N−CH_3), 4.11 (t) and 4.19 (t) (2H, 3:2, −OCH_2−, J = 5.8 Hz), 7.06 (dd, 1H, 4-C*H*, aromatic, J_o = 7.84 Hz, J_m = 2.19 Hz), 7.22–7.24 (m, 2H, 2-C*H* and 6-C*H*, aromatic) and 7.43 ppm (t, 1H, 5-C*H*, aromatic, J_o = 8.18 Hz). ^{13}C NMR ($CDCl_3$): δ 9.10 (1C, N−CH_2CH_2**C**H_3), 19.10 (1C, N−CH_2**C**H_2CH_3), 27.50 (1C, **C**H_2), 29.87 (1C, N−CH_3), 30.52 (1C, N−CH_3), 39.09 (1C, **C**H_2Cl), 40.69 (1C, N−CH_2), 65.25 (1C, O−**C**H_2), 106.39 (1C, Ar**C**), 113.20 (1C, Ar**C**H), 114.36 (1C, Ar**C**H), 119.26 (1C, Ar**C**H), 127.33 (1C, Ar**C**H), 127.76 (1C, Ar**C**), 145.91 (1C, Ar**C**), 149.22 (1C, Ar**C**), 149.47 (1C, Ar**C**), 153.24 (1C, **C**═O) and 156.79 (1C, **C**═O). ESI-MS: 391 $[MH]^+$, 393 $[MH+2]^+$ Calcd. for $C_{19}H_{23}N_4O_3Cl$: C, 58.38; H, 5.93; N, 14.33%. Found C, 57.96; H, 5.80; N, 14.19%.

*4.1.6.2. 8-[3-(3-Chloropropoxy)phenyl]-3-methyl-7-(prop-2-ynyl)-1-propylxanthine (**11b**).* (0.75 g, 68.18%), mp 135–137 °C. FTIRυ_{max} (KBr): 3231 (C−H, alkyne), 2955 (C−H, aliphatic), 2121 (C≡C) 1693 (C═O), 1654 (C═C), 1541 (C═N), 1227 (*asym.* C−O−C), 1034 (*sym.* C−O−C) and 757 cm^{-1} (C−Cl). ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, −$NCH_2CH_2CH_3$, J = 7.42 Hz), 1.71 (sext, 2H, −$NCH_2CH_2CH_3$, J = 5.76 Hz), 2.27 (p, 1H, −OCH_2CH(*H*)−, J = 6.04 Hz), 2.36 (p, 1H, −OCH_2CH(H)−, J = 6.08 Hz), 2.52 (td, 1H, −C≡C*H*, J = 2.32 Hz, J = 1.50 Hz), 3.63 (s, 3H, N−CH_3), 3.64 (t, (merged with N−CH_3), 1H, −C*H*(H)Cl, J = 6.34 Hz), 3.77 (t, 1H, −CH(*H*)Cl, J = 6.26 Hz), 4.01 (t, 2H, −NCH_2−, J = 7.6 Hz), 4.19 (qd, 2H, −OCH_2−, J = 4.36 Hz, J = 1.50 Hz), 5.15 (d, 2H, −CH_2−C≡CH, J = 2.44 Hz), 7.07 (dt, 1H, 4-C*H*, aromatic, J_o = 7.64 Hz, J_m = 2.36 Hz) and 7.38–7.47 ppm (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic).^{13}C NMR ($CDCl_3$): δ 11.37 (1C, N−CH_2CH_2**C**H_3), 21.34 (1C, N−CH_2**C**H_2CH_3), 29.82 (1C, N−CH_3), 32.14 (1C, **C**H_2), 36.34 (1C, **C**H_2Cl), 41.38 (1C, N−CH_2), 43.05 (1C, N−CH_2), 64.58 (1C, O−**C**H_2), 65.61 (1C, C≡**C**H), 74.31 (1C, **C**≡CH),

107.71 (1C, Ar**C**), 114.88 (1C, Ar**C**H), 117.45 (1C, Ar**C**H), 121.53 (1C, Ar**C**H), 129.16 (1C, Ar**C**H), 130.28 (1, C, Ar**C**), 148.30 (1C, Ar**C**), 151.46 (1C, Ar**C**), 151.68 (1C, Ar**C**), 155.06 (1C, **C**=O) and 159.11 ppm (1C, **C**=O). ESI-MS: 415 $[MH]^+$, 417 $[MH+2]^+$ Calcd. for $C_{21}H_{23}N_4O_3Cl$: C, 60.79; H, 5.59; N, 13.50%. Found C, 60.75; H, 5.35; N, 13.63%.

4.2. Biological activity

4.2.1. Radioligand binding assays at adenosine receptors

4.2.1.1. Binding studies at A_1, A_{2A} and A_3 receptor subtypes. Materials: [^{3}H]CCPA (2-chloro-*N*^{6}-cyclopentyladenosine), was purchased from GE Healthcare Europe, Freiburg, Germany, [^{3}H]NECA (5′-*N*-ethylcarboxamidoadenosine) and [α-^{32}P] ATP were obtained from Hartmann, Braunschweig, Germany. [^{3}H]HEMADO (2-hexyn-1-yl-*N*^{6}-methyladenosine) was from Tocris, Bristol, UK. The 96-well microplate filtration system (Multiscreen MAFC) was obtained from Millipore, Eschborn, Germany. Cell culture media and fetal calf serum were purchased from Pan Systems, Aidenbach, Germany. Penicillin (100 U/ml), streptomycin (100 mg/ml), L-glutamine and G418 were from Gibco-Life Technologies, Eggenstein, Germany.

Cell culture: The cells were grown adherently and maintained in Dulbecco's Modified Eagles Medium with nutrient mixture F12 (DMEM/F12) without nucleosides containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 mg/ml), L-glutamine (2 mM) and geneticin (G418, 0.2 mg/ml; A_{2B}, 0.5 mg/ml) at 37 °C in 5% CO_2/95% air. Cells were split 2 or 3 times weekly at a ratio between 1:5 and 1:20. For binding assays the culture medium was removed, cells were washed with PBS and frozen in the dishes until preparation of membranes. The cells utilized for cAMP determinations had a viability > 95%, as assessed by the exclusion of tryptan blue.

Membrane preparation: Crude membranes for radioligand binding experiments were prepared by thawing frozen cells followed by scraping them off the petridishes in ice-cold hypotonic buffer (5 mM Tris/HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized on ice (Ultra-Turrax, 2 × 15 s at full speed) and the homogenate was spun for 10 min (4 °C) at 1000 g. The supernatant was then centrifuged for 30 min at 100,000 g. The membrane pellet was resuspended in 50 mM Tris/HCl buffer pH 7.4 (for A_3 adenosine receptors: 50 mM Tris/HCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 8.25), frozen in liquid nitrogen at a protein concentration of 1–3 mg/ml and stored at −80 °C. For the measurement of adenylyl cyclase activity a slightly modified protocol with only one centrifugation step was used. Fresh cells were homogenized and the homogenate was sedimented for 30 min at 54,000 g. The resulting pellet was resuspended in 50 mM Tris/HCl pH 7.4 and used for the adenylyl cyclase assay immediately.

Radioligand binding: Dissociation constants of unlabeled compounds (K_i-values) were determined in radioligand competition experiments performed in a microplate format utilizing a 96-well microplate filtration system (Millipore Multiscreen MAFC). For binding experiments at A_1 adenosine receptors [^{3}H]CCPA (1 nM) was used as a radioligand, while [^{3}H]NECA (10 nM) and [^{3}H]HEMADO (1 nM) were used for A_{2A} and A_3 adenosine receptor binding, respectively. Membranes (10–50 μg of protein) from CHO cells stably transfected with the respective adenosine receptor subtype were incubated for 3 h at 25 °C, filtered through the built-in filter at the bottom of the wells and washed three times with 200 μl of ice-cold binding buffer. After addition of 20 μl of scintillator per well of the dried filter plates samples were counted in a Wallac Micro-Beta counter. Nonspecific binding was determined in the presence of 1 mM theophylline ([^{3}H]CCPA, A_1) or 100 mM R-PIA (R-*N*^{6}-phenylisopropyladenosine), ([^{3}H]NECA, A_{2A} and [^{3}H]HEMADO, A_3). All binding data were calculated by non-linear curve fitting with the program Prism (GraphPad Software, La Jolla, Ca, USA).

4.2.1.2. Adenylyl cyclase activity. Due to the lack of a suitable radioligand the affinity of ligands at A_{2B} adenosine receptors was determined in adenylyl cyclase experiments. Membranes were incubated with about 150,000 cpm of [α-^{32}P]ATP for 20 min in the incubation mixture as described previously [28]. No inhibition of NECA-stimulated adenylyl cyclase activity was observed with test compounds in concentrations up to 20 μM indicating that no interaction with the A_{2B} receptor occurred.

4.2.2. Antiparkinsonian activity (in vivo)

The antiparkinsonian activity of all the newly synthesized 1-,3-,7-,8-tetrasubstituted phenylxanthine derivatives was studied against perphenazine induced catatonia in rats using a reported procedure [29,30].

Animals: Male Wistar rats (fasted) weighing between 200 and 250 g, bred in Central Animal House (CAH) of Panjab University, Chandigarh were used. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 h. The experimental protocols have been approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh (PU/IAECS/14/119) and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals.

Drugs: Perphenazine (PPZ), (Sigma, MO, USA) for inducing catatonia and 3,4-dihydroxy-L-phenylalanine (L-dopa) (Sigma, MO, USA) as a standard were used. Perphenazine was dissolved in tartaric acid (0.3%) in saline (0.9%). The test compounds and L-dopa were suspended in 0.5% methylcellulose and dosed orally at 100 mg/kg of body weight 30 min before the animals were induced catatonia by injecting perphenazine. The drug doses were selected according to the reported literature.

Experimental protocol: The experimental protocol comprised of the following groups, each consisting minimum of 4 animals:

Group I: control group treated with vehicle (0.5% methylcellulose) followed by PPZ.
Group II: given protective dose of L-dopa (100 mg/kg, p.o.), followed by PPZ (standard group). Group III, (Test group), given test compounds to each group (100 mg/kg, p.o.), followed by PPZ.

Perphenazine (5 mg/kg, i.p.) was administered to induce catatonia. Severity of catatonic response was observed as follows:

Stage I: Rat moves normally when placed on the table, score = 0
Stage II: Rat moves only when touched or pushed, score = 0.5
Stage III: Rat placed on the table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 s, score = 0.5 for each paw with a total of 1 for this stage.
Stage IV: Rat fails to remove when the front paws were placed alternately on a 9 cm block, score = 1 for each paw with a total score of 2 for this stage.

Thus, for a single rat, the maximum possible score would be 3.5 reflecting total catatonia. Fewer score would mean an apparently lesser degree of catatonia. The severity of catatonia was observed at 5, 15, 30, 45, 60, 90, 120 and 180 min after PPZ administration. All the values were expressed as mean ± S.E.M. The data were analyzed by using analysis of variance followed by Dunnett's test. In all tests, the criterion for statistical significance was $p < 0.05$.

4.3. Molecular docking analysis

All the newly synthesized xanthines were studied in molecular docking analysis using Schrödinger glide module to predict the binding orientation and interactions with their protein target. Before starting docking, ligand and protein preparation was done as discussed below

Protein preparation: The protein data bank (PDB) a key resource in

areas of structural biology, is a key repository for 3D structure data of large molecules. For the present study, X-ray crystallographic structure of the A_{2A} adenosine receptor in complex with an antagonist having a resolution 2.6 Å (PDB ID: 3EML) was obtained from protein data bank (www.rscb.org), then refined and prepared using Schrodinger protein preparation wizard tool (Glide) which performs the following steps: assigning of bond orders, addition of hydrogens, optimization of hydrogen bonds, correction of charges and minimization of the protein complex. The chemistry of the protein was corrected for missing hydrogens and optimized the hydrogen bond network.. Following the above steps of preparation, the protein was subjected to energy minimization using the OPLS 2005 force field.

Ligand preparation: The structures of compounds under investigation were sketched in ChemDraw Ultra 8.0 software and saved as .sdf format. The ligand file was imported in the ligand preparation wizard. The LigPrep wizard is a utility of Schrodinger software suit that combines tools for generating 3D structures from 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. Energy was minimized using Molecular Mechanics Force Fields (OPLS_2005) with default settings.

Receptor Grid Generation: Prior to docking receptor grid was generated by displaying the prepared receptor in the workspace. The options in each tab of Maestro 10.5 module of Schrödinger molecular modeling interface allow defining the receptor structure by removing the co-crystallized ligand, determine the position and size of the active site as it will be represented by receptor grids, set up Glide constraints using constraint tab and set up flexible hydroxyl groups by running the receptor grid generation task [31,32].

Ligand Docking: The docking method used in this study is Glide XP docking program (Schrodinger Inc). Docking studies were carried out using the prepared protein of A_{2A} and ligands following the reported procedure with default settings without applying any constraints [31,32]. Using number of best 10 docking poses and per-residue interaction scores for each ligand have been evaluated in extra precision mode (Glide XP).

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Conflict of interest

Authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.032.

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