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BRONCHOSPASMOLYTIC ACTIVITY AND ADENOSINE RECEPTOR BINDING OF SOME NEWER 1,3-DIPROPYL-8-PHENYL SUBSTITUTED XANTHINE DERIVATIVES

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ABSTRACT

The aldehyde derivatives of 1,3-dipropyl xanthines as described in this paper, constitutes a new series of selective adenosine ligands displaying bronchospasmolytic activity. The effect of substitution at 3_{rd}- and 4_{th}-position of 8-phenyl xanthine has also been taken into consideration. The synthesized compounds showed varying binding affinities at different adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) and also good *in vivo* bronchospasmolytic activity against histamine aerosol induced asthma in guinea pigs. Most of the compounds showed maximum affinity towards the A_{2A} receptor subtype. The monosubstituted 3-aminoalkoxyl 8-phenyl xanthine with a diethylmoiety (compound **12e**) was found to be most potent A_{2A}adenosine receptor ligand (K_i = 0.036 μM) followed by disubstituted 4-aminoalkoxyl-3-methoxy-8-phenyl xanthine (K_i= 0.050μM) (compound **10a**).

KEYWORDS: Xanthines, Adenosine, Radioligand binding, Bronchospasmolytic, Antiasthmatics.

1. Introduction

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The therapeutic ability of adenosine was first evaluated in 1930's (Kaiser, 1999). Adenosine is a universal signaling nucleoside molecule containing an adenine group attached to ribose sugar. Adenosine and its receptors are used clinically as targets for drug development since 1940's (Trevor, 2014). There are four subtypes of adenosine receptors designated as A_1, A_{2A}, A_{2B}, A_3 and all belong to the GPCRs (G Protein-coupled receptors). All the adenosine receptors play therapeutic roles in treating various cardiovascular diseases (Fredholm, 2001), nervous and renal system disorders (Linden, 2005; Ascherio, 2001), pulmonary diseases (Fozard, 2002), inflammatory disorders(Erdmann, 2005; Antonioli, 2013), endocrine disorders, and visual disorders and also as targets in treating cancer (Neary, 1998; Gessi, 2011; Sousa, 2018). Adenosine receptors were categorized as A_1 and A_2 intially by Brunstock in 1978 depending on their ability to inhibit or stimulate adenyl cyclase (Polosa, 2002). The A_2 receptors were further classified as A_{2A} and A_{2B} based on their high affinity and low affinity to stimulate adenyl cyclase (Aherne, 2011). The activation of the A_{2A} AR produces broad-spectrum anti-inflammatory activity in various allergic models of asthma thus making them useful alternatives to glucocorticosteroids in the treatment of asthma (Fozard, 2002). The elucidation of a A_{2B} low-affinity subtype of the A_2 adenosine receptorsled to the possibility that there might be a physiological receptor to counter balance the high-affinity A_{2A} AR models but lack of pharmacologically specific targets reduced their physiological relevance (Aherne, 2011).

The high affinity adenosine A_{2A} receptors are expressed on mast cells and A_{2A} agonists increase intracellular levels of cAMP which in turn inhibits the release of histamine and other inflammatory stimuli from mast cells thus proven effective in asthmatic patients (Spicuzza, 2003; Baraldi, 2008). The low affinity A_{2B} receptor antagonists are under investigation based on their inhibiting action of adenosine on mast cells in addition to pro-inflammatory effects (Brown, 2008).

Theophylline is a xanthine derivative that shows a low affinity (in the micromolar range) for adenosine receptor, A_{2B} as an antagonist and does not discriminate between the A_1 and A_2 receptor subtypes while at relatively high concentrations, it inhibits proliferation of human peripheral blood mononuclear cells showing anti-proliferative action. (Landells, 2000). The binding affinity of theophylline is more towards A_{2A} receptors in low concentrations as compared to A_1 and A_{2B} (Yasui, 2000) and some of the Antiasthmatic effect observed with theophylline is also due to A_{2B} receptor

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antagonism. The anti-proliferative effect of theophylline is seen only at higher concentrations then required to antagonize A_{2B} receptors while A_{2A} agonist action can be seen in micromolar range. (Landells, 2000).

The affinity for adenosine receptors is increased when various substitutions in the xanthine moiety are introduced. Most of the A_{2A} agonists are directly related to adenosine itself so various synthetic modifications were done to the naturally occurring xanthines which are structural analogs of adenosine, to produce selective A_{2A} receptor ligands (Müller, 2011).

The various substituted xanthines retain almost 10,000 times higher affinity towards adenosine receptors as compared to theophylline and caffeine which are present naturally. In spite of broader pharmaceutical applications and similarity to purine family, xanthine-based research has not taken up full measure thus limiting synthesis of xanthine-based molecules (Monteiro, 2016). The most noticeable reason for this is adverse synthetic procedures such as ring closure and condensation method for the production of new products (Allwood, 2007; Hayallah, 2002; Sakai, 1992; Kim, 2010; Bandyopadhyay, 2012; Chen, 2014; Lee, 2016). In terms of xanthine-based drug development, large scale synthesis is not supported thus there is crucial need for the development of feasible synthetic methods so that new xanthine-based drug candidates can be introduced. Xanthine provides maximum possible sites of substitutions. There are five sites for mono substitution [1-, 3-, 7-, 8- and 9-], for di-substitutions there are eight sites [1,3-, 1,7-, 1,8-, 1,9-, 3,7-, 3,8-, 3,9- and 7, 8-] and there are three sites for tri- substitutions [1,3,7-,1,3,8-, 1,3,9-] (Miyamoto, 1993; Muller, 1998; Bansal, 2010). The structure of xanthine gives extreme possibility for variation in derivatization because of the presence of N^1, N^3, N^7 and C^8 positions for substitution. It was also observed that the increase in the length of carbon chain in 1- and 3-position from methyl to propyl increases the affinity of xanthine derivatives towards adenosine receptors.

Theophylline and other substituted xanthine derivatives possess inhibitory effect at adenosine receptors especially A_1 and A_2 where A_1 is inhibitory and A_2 is stimulatory to adenylate cyclase (Persson, 1981). Enprofylline (**A**), a 3-propyl xanthine proved more potent than theophylline in regulating adenosine induced contraction of airways (Belardinelli, 1981).

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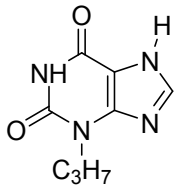

(**A**)

Considering all the literature reports into account, 1,3-dipropyl substituted xanthine derivatives were synthesized by substituting the 3rd- and 4th-position of the 8-phenyl ring introduced into the xanthine scaffold. Studies indicated that suitable substitution and position of substituents results in high affinity compounds (Bansal, 2009). The *para* and *meta* shifting of substituent play an important role towards binding affinity at adenosine receptors. The synthesized compounds were evaluated biologically using *in vitro* radioligand binding and *in vivo* histamine aerosol induced asthma in guinea pigs (Zabeer, 2006).

2. Results and discussion

2.1 Chemistry

The key intermediate 5,6-Diamino-1,3-dipropyluracil (**3**) was synthesized by the condensation of *N,N*'-dipropyluracil (**1**) and cyanoacetic acid (**2**)followed by nitrosation and reduction as depicted in **Scheme 1** (Papesch, 1951; Blicke, 1954). The synthesis of substituted aldehydes **4-6 (a-f)**, Schiff bases **7-9 (a-f)** and substituted xannthines**10-12 (a-f)** is depicted in **Scheme 2**. The starting aldehydes vanillin (**4**), isovanillin (**5**), and 3-hydroxybenzaldehyde (**6**) possesses a hydroxy group at *para* and *meta* positions where different amines (morpholine, piperidine, dimethylamine, pyridine, diethylamine and phthalimide derivatives of 2-chloroethyl aminohydrochlorides) were substituted to obtain substituted aldehydes**4-6**(**a-f**).

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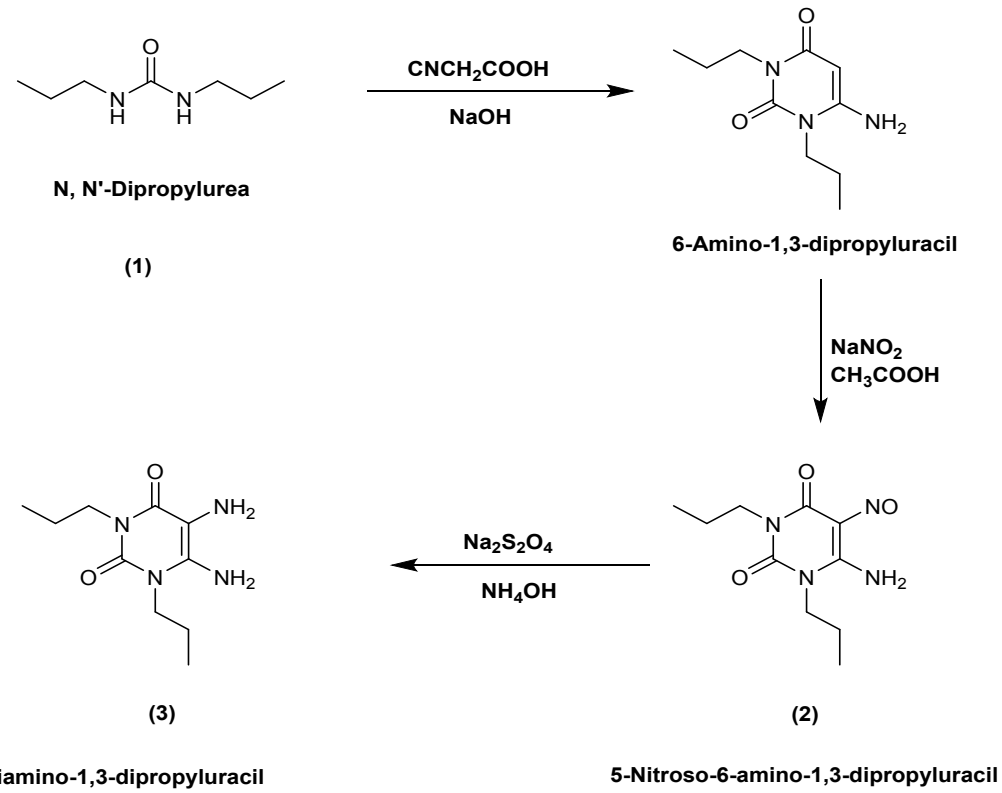

Scheme 1

These aldehydes **4-6 (a-f)** were condensed with key intermediate dipropyluracil **(3)** at reflux in the presence of ethanol and acetic acid to synthesize Schiff bases **7-9 (a-f)** which in turn were cyclized in refluxing thionyl chloride to synthesize resultant xanthine derivatives **10-12 (a-f)** as depicted in **Scheme 2**. The structures of the synthesized compounds were characterized by various spectral and elemental analyses. A singlet for one proton at ~11.3 ppm for N=CH was observed in all theSchiff bases **7-9 (a-f)** while disappearance of this peak on treatment with thionyl chloride confirmed cyclization of xanthine derivatives **10-12(a-f)**.

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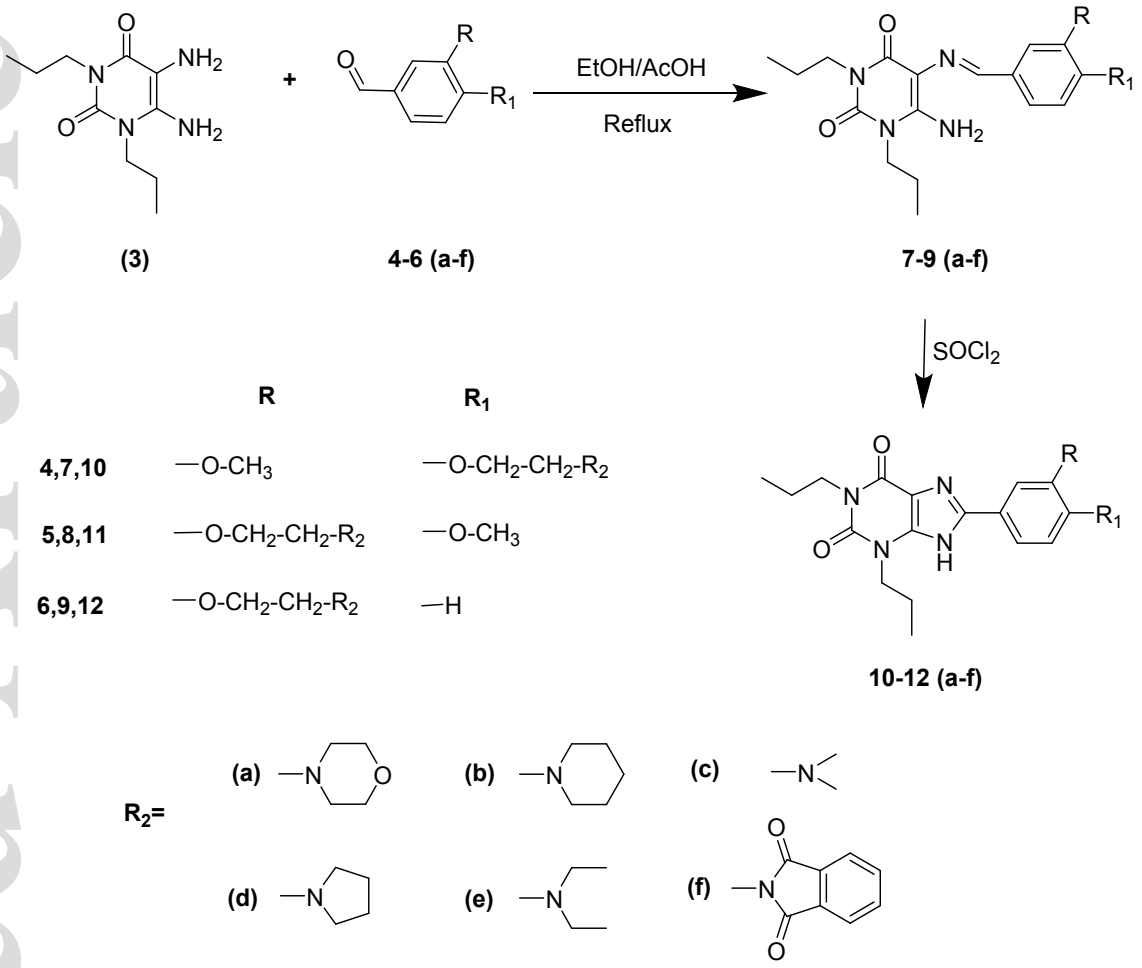

Scheme 2: Synthetic route for the synthesis of various 8-phenyl substituted xanthines, 10 a-f; 11a-f and 12 a-f.

2.2 Radioligand binding assays

Adenosine receptor binding affinity of various xanthine derivatives at adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) was also determined and summarized in **Table 1**. The substituted xanthines **10-12 (a-f)** showed potent binding affinities towards various adenosine receptor subtypes whereas they showed high selectivity towards A_{2A} subtype.Monosubstituted8-phenyl xanthines **12b, 12d and 12e** with a polar side chain at *meta* position of the 8-phenyl ring showed higher affinity and selectivity towards A_{2A} receptor subtypes followed by**10a-f and 11 a-f** derivatives with an additional methoxy group at *ortho* position to polar side chain.. Among all the series the monosubstituted derivatives, *8-[3-{2-(phthalimido)-ethoxy}-phenyl]-1,3-dipropylxanthine,* **12e**

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with K_i=*0.036 µm* found as the compound with the highest affinity of the series. On comparing disubstituted derivatives, the derivatives with polar side chain at *para* position of 3,4-disubstituted 8-phenyl xanthines (compounds **10a**) was found to have higher affinity than the derivatives with polar side chain at *meta* position (compounds**11f**). Thus, the newly synthesized derivatives displayed higher affinity and selectivity towards A_{2A} receptor subtypes then the previously reported xanthine derivatives.

Table 1: Binding Affinities of Various Xanthine derivatives at adenosine receptors (A_1, A_{2A}, A_{2B} and A_3)

S. No.	Comp. (Code)	A_1 (K_i µm)	A_{2A} (K_i µm)	A_{2B} (K_i µm)	A_3 (K_i µm)
1	**10a (RY-67)**	0.248 (0.209-0.296)	0.050 (0.037-0.068)	> 100	0.593 (0.503-0.698)
2	**10b (RY-69)**	1.50 (1.39-1.63)	0.622 (0.465-0.833)	> 100	1.12 (0.941-1.34)
3	**10c (RY-71)**	1.20 (1.01-1.42)	0.481 (0.338-0.685)	> 100	1.20 (0.882-1.62)
4	**10d (RY-73)**	1.19 (0.933-1.51)	0.364 (0.323-0.409)	> 100	0.861 (0.584-1.27)
5	**10e (RY-75)**	0.813 (0.620-1.07)	0.222 (0.127-0.390)	> 100	0.622 (0.484-0.799)
6	**10f (RY-77)**	2.05 (1.76-2.40)	0.543 (0.293-1.04)	> 100	4.62 (3.71-5.74)
7	**11a (RY-79)**	0.757 (0.720-0.796)	0.268 (0.156-0.461)	> 100	1.05 (0.713-1.54)
8	**11b (RY-81)**	0.896 (0.700-1.15)	0.818 (0.551-1.210)	> 100	1.780 (1.250-2.530)
9	**11c (RY-83)**	2.96 (2.64-3.33)	1.71 (1.55-1.89)	> 100	2.47 (1.81-3.36)
10	**11d (RY-85)**	0.891 (0.662-1.20)	0.655 (0.578-0.743)	> 100	1.33 (1.01-1.77)
11	**11e (RY-87)**	0.749 (0.436-1.29)	0.870 (0.832-0.909)	> 100	1.39 (1.05-1.83)
12	**11f (RY-89)**	0.189 (0.168-0.214)	0.055 (0.046-0.065)	> 100	0.255 (0.158-0.412)
13	**12a (RY-103)**	2.59 (2.16-3.10)	1.27 (1.18-1.37)	> 10	2.43 (2.03-2.92)
14	**12b (RY-105)**	0.110 (0.062-0.195)	0.054 (0.049-0.058)	1.26 (1.13-1.40)	0.639 (0.411-0.993)
15	**12c (RY-107)**	3.26 (2.26-4.69)	2.12 (1.58-2.86)	> 10	2.86 (2.02-4.06)
16	**12d (RY-109)**	0.196 (0.116-0.334)	0.116 (0.096-0.141)	3.430 (2.730-4.320)	2.020 (1.89-2.16)
17	**12e (RY-111)**	0.107 (0.080-0.143)	0.036 (0.032-0.041)	1.88 (1.33-2.67)	0.542 (0.408-0.721)
18	**12f (RY-113)**	0.119 (0.101-0.141)	0.058 (0.044-0.077)	2.86 (1.87-4.39)	0.088 (0.080-0.098)

Shown values are geometric means from 3 to 5 experiments in µM with 95% confidence intervals in parentheses, where:

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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. Antagonist affinities were determined by inhibition of NECA-stimulated adenylyl cyclase activity in membrane preparations.
d. Displacement of specific (^{3}H)HEMADO binding in CHO cells, stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (μM).

2.3 Bronchospasmolytic activity

The newly synthesized 1,3-dipropyl-8-substituted xanthines **10a, 10b, 10c, 10f, 11a, 11b and 12a-f**were evaluated against histamine aerosol-induced asthma in guinea pigs. The compounds showed a significant protection profile against the standard drug theophylline as evident from increased time for the onset of bronchospasm as compared to standard drug. In the vanillin substituted series, among10a, 10b, 10c and 10f, derivative **10f** with a phthalimide substitution was found to be most effective as it took more time for onset of spasm as compared to standard drug with 100% survival rate. The isovanillin substituted series i.e. 11a and 11bshowed lower values for onset of spasm as compared to standard but with 100% survival rate. The 3-hydroxybenzaldehyde substituted xanthine derivatives series, **12 a-f** proved to be most effective among all as their onset of spasm time is much higher in comparison to standard drug. Among this series, derivatives **12e** and **12f** with a diethyl and phthalimide substitution showed maximum time for the onset of bronchospasm followed by no jerks and 100% survival rate, thus, considered as most effective *in vivo* (Table 2).

All the newly synthesized derivatives exhibited prominent binding affinity towards A_{2A} receptors and also exhibited prominent bronchospasmolytic activity. The compound **12e** from the 3-monosubstituted series found as most potent towards A_{2A} receptor ligand and also took maximum time for the onset of bronchospasm followed by **12f** compound of this series. Among the other two series, compound **10f** of vanillin series exhibited higher activity then the standard drug.

Table 2: Protection by xanthine derivatives against bronchospasm induced by histamine aerosol (5 ml of 1% w/v aerosoled in 1 min) in guinea pigs

Compound No.	Mean time in seconds for onset of bronchospasm Mean ± S.E.M	Duration of jerks in seconds Mean ± S.E.M	Severity of Bronchospasm	Survival (%)

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Compound No.	Mean time in seconds for onset of bronchospasm Mean ± S.E.M	Duration of jerks in seconds Mean ± S.E.M	Severity of Bronchospasm	Survival (%)
Control	51.8 ± 3.49	183.4 ± 10.59	+++	0
Theophylline	105.6 ± 7.73*	94.8 ± 6.72*#	+	100
10a**(RY-067)**	98.26 ± 9,54*	32.6 ± 4.11*#	++	100
10b**(RY-069)**	86.33 ± 5.43*	42.8 ± 4.02*#	++	100
10c**(RY-071)**	97.87 ± 3,11*	56.2 ± 7.57#	+++	100
10f**(RY-077)**	115.34 ± 2.16*	36.2 ± 3.56*#	+	100
11a**(RY-079)**	101.18 ± 6.23*	39.6 ± 4.19*#	+	100
11b**(RY-081)**	50.19 ± 8.19*#	50.2 ± 1.93*	+++	100
12a**(RY-103)**	125.65 ± 5.76*	52.6 ± 7.58*#	++	100
12b**(RY-105)**	120.13 ± 3.53*	58.8 ± 8.28*	+++	100
12c**(RY-107)**	172.19 ± 4.31*#	60 ± 4.45*	++	100
12d**(RY-109)**	126.21 ± 4.37*	36.6 ± 6.57*#	++	100
12e**(RY-111)**	480 ± 0*#	-	-	100
12f**(RY-113)**	480 ± 0*#	-	-	100

Number of animals in each group (N) = 5

(-) means= not observed

**Newman-Keuls Multiple Comparison Test; $p<0.05$ as compare to normal control*

#*Newman-Keuls Multiple Comparison Test; $p<0.05$ as compare to theophylline*

3. Conclusion

The newly synthesized 1,3-dipropyl-8-substituted xanthines showed prominent binding affinity for adenosine receptors subtypes except the A_{2B} subtype with maximum affinity towards A_{2A} receptors. Thus, a conclusion can be drawn regarding A_{2A} receptor agonist action and A_{2B} receptor antagonist action of xanthines as synthesized derivatives showed maximum binding affinity at A_{2A} and minimum at A_{2B}. The suitable substitution on *para* and *meta* position of 8-phenyl ring resulted in high affinity compounds with potent bronchospasmolytic activity. The monosubstituted xanthine derivative **12e** found as highly potent bronchospasmolytic agent along with highest affinity towards A_{2A} receptors followed by *para*-substituted derivative **10a** and then *meta*-substituted **11a.** Thus, the number of substitutions and their position plays an important role in defining the chemistry of xanthines as

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bronchospasmolytic agents and also provides specific binding affinities towards various adenosine receptors.

4. Experimental section

4.1 Chemistry

All melting points were obtained using glass capillary tubes on Veego melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using precoated plates with silica gel G (Merck 60 $F_{254)}$ and the spots were visualized in iodine chamber. Infrared (IR) spectra were recorded on Agilent Technology cary 600 series Fourier Transform-Infrared spectrophotometer using potassium bromide pellets (υ_{max} in cm^{-1}). ^{13}C and ^{1}H-NMR spectroscopy were performed using a Bruker model 400 MHz spectrometer in deuterated dimethylsulfoxide (DMSO-d_6) and are reported in parts per million (ppm) downfield from tetramethylsilane (Me_4Si) as internal standard. The spin multiplicities are indicated as symbols, s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and the coupling constants (*J*) are given in Hertz (Hz). Anhydrous sodium sulfate was used as drying agent and all the solvents were freshly distilled off and dried prior to use according to the standard procedures. Elemental Analysis was carried out on Perkin Elmer series II-2400 analyzer. The results so observed were within the limits.

*4.1.1 General method for the synthesis of compound **3***

A mixture of *N, N'*-dipropylurea (1.0 g, 6.94 mmol), cyanoacetic acid (1.5 g, 17.64 mmol) and acetic anhydride (2.5 ml) was refluxed at 70-80 °C (3 h) excluding moisture. The excess anhydride and acetic acid formed during the reaction were removed under vacuum. The residue was then cooled (0-5°C) and a solution of 5 % sodium hydroxide (40 ml) was added with stirring resulting in precipitation of 6-amino-1, 3-dipropyluracil. A sodium nitrite solution (1.0 g, 14.49 mmol in 8 ml of water) was added to cool, stirred mixture and acidified with acetic acid dropwise (2 ml) over a period of one hour resulting in red-violet precipitates. The mixture was then kept on stirring at room temperature for a period of overnight. The mixture so obtained was then cooled, filtered off, washing

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was done with water and diethyl ether followed by drying to get 6-amino-1, 3-dipropyl-5-nitrosouracil **2** (1.33 g, 63.63 %), mp 215-220 °C.

The sodium dithionite (2.74 g, 15.73 mmol) was then added slowly with stirring. The salt dissolved and underwent a series of color changes. The solution was kept on stirring for further two hours at room temperature followed by cooling in an ice bath. The precipitates so obtained were filtered off, washing was done with few drops of cool water followed by drying to get 5, 6-diamino-1, 3-dipropyluracil (**3**, 0.76 g, 80.85 %), mp 128-132 °C.

4.1.2. General method for the synthesis of substituted aldehydes ***4-6 (a-f)***

Various amines such as 4-(2-chloroethyl)-amino hydrochlorides (1 g), [4-(2-chloroethyl)-morpholine hydrochloride (**a**, 5.36 mmol), 4-(2-chloroethyl)-piperidine hydrochloride (**b**, 5.42 mmol), 2-chloro-*N,N*'-dimethyl ethanamine (**c**, 6.93 mmol), 1-(2-chloroethyl)-pyrrolidine (**d**, 5.88 mmol), 2-chloro-*N,N*'-diethyl ethanamine (**e**, 5.82 mmol) and 1-(2-chloroethyl)-phthalimide (**f**, 4.77 mmol)] were added to the refluxing suspension of 4-Hydroxy-3-methoxy-benzaldehyde (**4**, 1 g, 6.58 mmol), 3-Hydroxy-4-methoxy-benzaldehyde (**5**, 1 g, 6.56 mmol) and 3-Hydroxybenzaldehyde (**6**, 1g, 6.80 mmol) in ethyl methyl ketone (20 ml). Dried potassium carbonate (3.0 g) was added to avoid incorporation of any moisture. After completion, suspension was filtered and reduced on rotary evaporator to afford the targeted compounds **4-6 (a-f)**.

4.1.3. General procedure for the synthesis of compounds ***7-9 (a-f)***

To the stirred solution of **4-6 (a-f)** in ethanol (5 ml), added 5, 6-diamino-1,3-dipropyluracil (**3**, 1 g, 4.42 mmol) with ethanol and acetic acid (8:2, 10 ml) and refluxed. After completion, excess amount of the solvent was evaporated at rotary evaporator. Added ice cold water to the residue and filtered to afford **7-9 (a-f)**.

4.1.4 General procedure for the synthesis of compounds ***10-12 (a-f)***

The Schiff bases **7-9 (a-f)** (1 g) were refluxed in thionyl chloride (15 ml) for 1 h at 70-80°C. Excess solvent was reduced at rotary evaporator and residue so obtained was neutralized to result in precipitation. The reaction mixture was cooled, filtered off and washed with cold water to obtain desired derivatives **10-12 (a-f)**.

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*4.1.4.1. 8-[4-(2-Morpholinoethoxy]-3-methoxyphenyl)-1,3-dipropyl xanthine (**RY-067, 10a**)*1.46 g, 69.56 %, mp 234-238 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.06 (s, 1H, -N*H*), 7.75 (s, 1H, Ar-*H*), 7.26 (s, 1H, Ar-*H*), 6.96 (s, 1H, Ar-*H*), 4.31 (s, 3H, -OCH_3), 3.95 (s, 4H, 2 x -OCH_2-), 3.75 (s, 2H, -OCH_2-), 2.89 (s, 4H, 2 x -NCH_2-), 2.63 (s, 6H, 3 x -NCH_2-),1.47 (s, 4H, 2 x -CH_2-) and 0.99 ppm (s, 6H,2 x -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 10.08-11.10 (2 **C**H_3), 20.01 (2 **C**H_2), 44.72 (2 N-**C**H_2), 53.37-54.01 (3 N-**C**H_2), 56.20-62.01 (3 O-**C**H_2), 65.57 (O-**C**H_3), 112.51 (Ar-**C**H), 114.41-115.56 (2 Ar-**C**), 119.54 (2 Ar-**C**H), 123.47 (Ar-**C**), 146.98-151.08 (3 Ar-**C**), 149.94 (**C**=O) and 152.61 ppm (**C**=O); FT-IR ν_{max}(KBr)cm^{-1}: 3410, 3202, 2959, 1691, 1647, 1556, 1478, 1271, 1168; Anal. Cal. for $C_{24}H_{33}N_5O_5$: C, 61.13%; H, 7.06%; N, 14.85% Found: C,63.15%; H, 6.37%; N, 16.38%.

*4.1.4.2 8-[4-(2-Piperidinoethoxy]-3-methoxyphenyl)-1,3-dipropyl-xanthine (**RY-069, 10b**)* 0.97 g, 99 %, mp 137-141 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1H, Ar-*H*), 7.22 (s, 1H, Ar-*H*), 6.93 (s, 1H, Ar-*H*), 4.07 (s, 1H, -N*H*), 3.90 (s, 4H, 2 x -NCH_2-), 3.73 (s, 6H, 3 x -NCH_2-), 3.01 (s, 2H, -OCH_2-), 2.75 (s, 3H, -OCH_3), 1.68 (s, 10H, 5 x -CH_2-) and 1.18 ppm (s, 6H,2 x -CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ10.08-11.10 (2 **C**H_3), 20.01 (2 **C**H_2), 24.73 (3 **C**H_2), 43.72 (2 N-**C**H_2), 54.01 (3 N-**C**H_2), 56.07 (O-**C**H_2), 67.57 (O-**C**H_3), 112.51 (Ar-**C**H), 115.41 (Ar-**C**), 119.54-120.02 (2 Ar-**C**H), 123.47 (Ar-**C**), 146.98 (Ar-**C**), 150.08 (Ar-**C**), 151.08 (Ar**C**), 151.94 (**C**=O), 153.61 (**C**=O) and 154.08 ppm (Ar**C**); FT-IRν_{max}(KBr) cm^{-1}:3565, 2920, 1698, 1650, 1516, 1460, 1258, 1209; Anal. Cal. for $C_{25}H_{35}N_5O_4$:C,63.94%; H,7.51%; N,14.91%**,** Found: C,64.18%; H, 6.28%;N, 15.34%.

*4.1.4.3.8-[4-{2-(Dimethylamino)-ethoxy}-3-methoxyphenyl]-1,3-dipropyl-xanthine (**RY-071,10c**)* 0.71 g, 71.42 %, mp 169-171 °C; ^{1}H-NMR (400MHz, $CDCl_3$):δ 7.69 (s, 1H,-N*H*), 6.94 (s, 3H, Ar-*H*), 3.91 (s, 6H, -3 x -NCH_2-), 3.00 (s, 6H, 2 x -NCH_3-), 2.49 (s, 3H, -OCH_3), 2.46 (s, 2H, -OCH_2-), 1.62 (s, 4H, 2 x -CH_2-) and 0.85 ppm (s, 6H, 2 x -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 10.08 (**C**H_3), 11.10 (**C**H_3), 21.07(**C**H_2), 23.01 (**C**H_2), 43.72 (N-**C**H_2), 45.05 (N-**C**H_2), 47.09 (2 N-**C**H_3), 54.01 (N-**C**H_2), 56.07 (O-**C**H_2), 66.57 (O-**C**H_3), 107.51-153.85 (Ar-**C**), 154.94 (**C**=O) and 157.61 ppm (**C**=O); FT-IR ν_{max}(KBr) cm^{-1}:3565, 2920, 1699, 1650, 1517, 1460, 1259, 1179; Anal. Cal. for $C_{22}H_{31}N_5O_4$:C, 61.52%; H, 7.27%; N, 16.31%; O, 14.90%; Found: C, 61.54%; H, 6.75%; N, 16.65%.

*4.1.4.4. 8-[4-{2-(Pyrrolidin-1-yl)-ethoxy}-3-methoxyphenyl]-1,3-dipropyl-xanthine(**RY-073, 10d**)*0.95 g, 99 %, mp 139-141 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 7.70 (s, 1H, Ar-*H*), 6.94 (s, 2H, Ar-*H*), 4.13 (s, 1H, -N*H*), 3.70 (s, 10H, 5 x -NCH_2-), 3.38 (s, 2H, -OCH_2), 3.14 (s, 3H, -OCH_3-), 1.67

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(s, 8H, 4 x -CH_2-) and 0.96 ppm (s, 6H, 2x-CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 11.01-12.08 (2 **C**H_3), 21.06 (2 **C**H_2), 23.97 (2 **C**H_2), 44.78-55.67 (5 N-**C**H_2), 56.62 (O-**C**H_3), 62.06 (O-**C**H_2), 107.86-154.45 (Ar-**C**) and 157.98 ppm (**C**=O); FT-IR ν_{max}(KBr) cm^{-1}:3565, 2918, 1698, 1649, 1516, 1460, 1259, 1038; Anal. Cal. for $C_{24}H_{33}N_5O_4$:C, 63.29%; H,7.31%; N,15.38%; O,14.05%;Found:C, 64.16%; H, 7.50%; N, 14.32%.

*4.1.4.5. 8-[4-{2-(Diethylamino)-ethoxy}-3-methoxyphenyl]-1,3-dipropyl xanthine (**RY-075, 10e**)*0.90 g, 90.45 %, mp 117-120 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 8.04 (s, 1H, -N*H*), 7.31 (s, 3H, Ar-*H*), 4.34 (s, 4H, -NCH_2-), 4.06 (t, 2H, -NCH_2-), 3.92 (t, 2H, -OCH_2), 3.88 (s, 3H, -OCH_3-), 3.47 (s, 2H, -CH_2-), 1.82-1.77 (q, 2H, -NCH_2-), 1.67-1.61 (q, 2H, -NCH_2-) and 0.97-0.91 ppm (q, 10H, 2 x -CH_2-, 2 x -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 10.91 (**C**H_3), 11.04 (**C**H_3), 14.04 (2 **C**H_3), 20.80 (**C**H_2), 20.85 (**C**H_2), 42.13 (2 N-**C**H_2), 44.41-54.08 (3 N-**C**H_2), 47.40 (O-**C**H_2), 55.80 (O-**C**H_3), 113.81-151.74 (**C**=O) and 154.22 ppm (**C**=O); FTIR ν_{max}(KBr) cm^{-1}:3565, 2920, 1698, 1649, 1516, 1460, 1259, 1021; Anal. Cal. for $C_{24}H_{35}N_5O_4$:C, 63.00%; H, 7.71%; N, 15.31%; O, 13.99%;Found:C, 64.52%; H, 6.67%; N, 13.32%.

*4.1.4.6. 8-[4-{2-(Phthalimido)-ethoxy}-3-methoxyphenyl]-1,3-dipropyl-xanthine (**RY-077, 10f**)*0.60 g, 60.24 %, mp 86-90 °C; ^{1}H-NMR (400 MHz, $CDCl_3$):δ 7.79 (s, 4H, Ar-*H*), 7.67 (s, 3H, Ar-*H*, 1H, -N*H*), 3.97 (s, 6H, 3 x -NCH_2-), 3.70 (s, 5H, -OCH_2-, -OCH_3), 1.62 (s, 4H, 2 x -CH_2-) and 0.92 ppm (s, 6H, 2x-CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 10.08 (**C**H_3), 11.01 (**C**H_3), 42.21 (**C**H_2), 39.45 (N-**C**H_2), 40.85 (N-**C**H_2), 56.62 (O-**C**H_3), 66.45 (O-**C**H_2), 107.07-156.62 (Ar-**C**), 158.04 (**C**=O) and 167.99 ppm (**C**=O); FT-IR ν_{max}(KBr) cm^{-1}:3028, 2961, 1768, 1704, 1648, 1542, 1462, 1260, 1088; Anal. Cal. for $C_{28}H_{29}N_5O_6$:C, 63.28%; H, 5.51%; N, 13.19%; O, 18.06%;Found:C, 64.46%; H, 6.03%; N, 14.51%.

*4.1.4.7. 8-[3-(2-Morpholinoethoxy)-4-methoxyphenyl]-1,3-dipropyl-xanthine (**RY-079, 11a**)*0.99 g, 99 %, mp192-195 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.43 (s, 1H, -N*H*), 7.59 (s, 1H, Ar-*H*), 6.84 (s, 2H, Ar-*H*), 4.28 (s, 3H, -OCH_3), 4.04 (s, 6H, 3 x -OCH_2-), 3.81 (s, 4H, 2 x -NCH_2-), 3.03 (m, 6H, 3 x -NCH_2-),1.76 (s, 2H, -CH_2-), 1.60 (s, 2H, -CH_2-) and 0.89 ppm (s, 6H, 2 x CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 10.08-11.10 (2 **C**H_3), 20.01 (2 **C**H_2), 44.72 (2 N-**C**H_2), 53.37-54.01 (3 N-**C**H_2), 56.20-62.01 (3 O-**C**H_2), 65.57 (O-**C**H_3), 112.51 (Ar-**C**H), 114.41-115.56 (2 Ar-**C**), 119.54 (2 Ar-**C**H), 123.47 (Ar-**C**), 146.98-151.08 (3 Ar-**C**), 149.94 (**C**=O) and 152.61 ppm (**C**=O); FT-IR ν_{max}(KBr)cm-

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[1]: 3564, 3179, 2961, 1699, 1654, 1543, 1488, 1262, 1210; Anal. Cal. for $C_{24}H_{33}N_5O_5$: C, 61.13%; H, 7.05%; N, 14.85%; O, 16.96%; Found: C, 32.95%; H, 4.58%; N, 7.66%.

*4.1.4.8. 8-[3-(2-Piperidinoethoxy)-4-methoxyphenyl]-1,3-dipropyl xanthine (**RY-081; 11b**)* 0.99 g, 99.2 %, mp 199-202 °C (decomp); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 7.64 (s, 1H, Ar-*H*), 7.19 (s, 1H, Ar-*H*), 6.83 (s, 1H, Ar-*H*), 4.20 (s, 1H, -N*H*), 4.07 (s, 4H, 2 x -NCH_2-), 3.83 (s, 6H, 3 x -NCH_2-), 2.83 (s, 2H, -OCH_2-), 2.54 (s, 3H, -OCH_3), 1.58 (s, 10H, 5 x -CH_2-) and 0.88 ppm (s, 6H, 2 x -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 11.25 (2 **C**H_3), 21.30 (2 **C**H_2), 23.97 (3 **C**H_2), 43.16 (2 N-**C**H_2), 45.20-55.03 (3 N-**C**H_2), 56.31 (O-**C**H_3), 66.80 (O-**C**H_2), 107.86 - 149.99 (Ar-**C**),150.90 (**C**=O), and 157.98 ppm (**C**=O); FT-IRν_{max}(KBr)cm^{-1}:3447, 3123, 2959, 1697, 1652, 1541, 1486, 1260, 1210; Anal. Cal. for $C_{25}H_{35}N_5O_4$:C, 63.95%; H, 7.52%; N 14.81%; O 13.63% Found: C, 64.42%; H, 8.11%; N, 13.76%.

*4.1.4.9. 8-[3-{2-(Dimethylamino)-ethoxy}-4-methoxyphenyl]-1, 3-dipropyl xanthine (**RY-083; 11c**)*0.40 g, 40.81 %, mp 205-208 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 6.94 (s, 3H, Ar-*H*), 3.98 (m, 13H, -N*H*, 3 x -NCH_2-, 2 x -NCH_3), 1.81 (m, 3H, -OCH_3), 1.67 (m, 2H, -OCH_2-) and 0.96 ppm (m, 10H, 2 x -CH_2, 2x-CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 10.08 (**C**H_3), 11.10 (**C**H_3), 21.07(**C**H_2), 23.01 (**C**H_2), 43.72 (N-**C**H_2), 45.05 (N-**C**H_2), 47.09 (2 N-**C**H_3), 54.01 (N-**C**H_2), 56.07 (O-**C**H_2), 66.57 (O-**C**H_3), 107.51-153.85 (Ar-**C**), 154.94 (**C**=O) and 157.61 ppm (**C**=O); FT-IRν_{max}(KBr)cm^{-1}:3421, 3199, 2964, 1663, 1650, 1542, 1462, 1259, 1179; Anal.Cal. for $C_{22}H_{31}N_5O_4$: C, 61.52%; H, 7.27%; N, 16.31%; O, 14.90%; Found: C, 61.54%; H, 6.75%; N, 16.65%.

*4.1.4.10. 8-[3-{2-(Pyrrolidin-1-yl)-ethoxy}-4-methoxyphenyl]-1, 3-dipropyl xanthine (**RY-085; 11d**)*0.84 g, 84.03 %, mp 145-148 °C (decomp.); ^{1}H-NMR (400MHz, $CDCl_3$):δ 7.62 (s, 1H, Ar-*H*), 6.84 (s, 2H, Ar-*H*), 4.36 (s, 1H, -N*H*), 3.88 (s, 4H, 2 x -NCH_2-), 3.82 (s, 6H, 3 x -NCH_2-), 3.32 (s, 2H, -OCH_2-), 3.17 (s, 3H, -OCH_3), 1.76 (s, 4H, 2 x -CH_2-), 1.60 (s, 4H, 2 x -CH_2-) and 0.88 ppm (s, 6H, 2xCH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 11.01-12.08 (**C**H_3), 21.06 (**C**H_2), 23.97 (**C**H_2), 44.78-55.67 (N-**C**H_2), 56.62 (O-**C**H_3), 62.06 (O-**C**H_2), 107.86 -154.45 (Ar-**C**) and 157.98 ppm (**C**=O);FT-IR ν_{max}(KBr) cm^{-1}:3744, 2961, 1698, 1655, 1552, 1519, 1258, 1181; Anal. Cal. for $C_{24}H_{33}N_5O_4$:C, 63.29%; H, 7.31%; N, 15.38%; O, 14.05%, Found: C, 64.02%; H, 6.15%; N, 14.67%.

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*4.1.4.11. 8-(3-(2-(Diethylamino)-ethoxy)-4-methoxyphenyl)-1,3-dipropyl xanthine (**RY-087, 11e**)* 0.99 g, 99 %, mp 185-188 °C (decomp.);^{1}H-NMR (400 MHz, $CDCl_3$):δ 11.26 (s, 1H, -N*H*), 6.87 (s, 3H, Ar-*H*), 3.87 (m, 10H, 5 x -NCH_2-), 1.70 (s, 2H, -OCH_2-), 1.48 (s, 3H, -OCH_3), 1.09 (s, 4H, 2 x -CH_2-) and 0.88 ppm (s, 12H, 4x-CH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 10.91 (**C**H_3), 11.04 (**C**H_3), 14.04 (**C**H_3), 20.80 (**C**H_2), 20.85 (**C**H_2), 42.13 (N-**C**H_2), 44.41-54.08 (3 N-**C**H_2), 47.40 (O-**C**H_2), 55.80 (O-**C**H_3), 113.81-151.55 (Ar-**C**), 151.74 (**C**=O) and 154.22 ppm (**C**=O); FT-IR ν_{max}(KBr)cm^{-1}:3615, 2959, 1699, 1651, 1547, 1463, 1257, 1175;Anal. Cal. for $C_{24}H_{35}N_5O_4$: C, 63.00%; H, 7.71%; N, 15.31%; O, 13.99%, Found: C, 64.13%; H, 7.20%; N, 16.93%.

*4.1.4.12. 8-[3-{2-(Phthalimido)-ethoxy}-4-methoxyphenyl]-1, 3-dipropyl xanthine (**RY-089; 11f**)*0.80 g, 80.54 %, mp 266-270 °C (decomp.)*;* ^{1}H-NMR (400MHz, $CDCl_3$):δ 13.25 (s, 1H, -N*H*), 8.99 (s, 1H, Ar-*H*), 7.92 (s, 1H, Ar-*H)*, 7.55 (d, 2H, Ar-*H, J_{ortho}*= 7.48 Hz), 6.88 (d, 2H, Ar-*H, J_{ortho}*= 8.32 Hz), 4.01 (t, 2H, -NCH_2-*,*J_1*=7.00, J_2*=6.92), 3.87 (s, 2H, -NCH_2-), 3.85 (s, 2H, -NCH_2-), 3.82 (s, 3H, -OCH_3), 1.74 (m, 2H, -OCH_2-),1.58 (m, 2H, -CH_2-) and 0.88 ppm (m, 8H, -CH_2-, 2 x -CH_3);^{13}C-NMR (100 MHz, $CDCl_3$):δ 10.92 (**C**H_3), 11.03 (**C**H_3), 42.98 (2 **C**H_2), 39.45 (N-**C**H_2), 44.33 (2 N-**C**H_2), 55.45 (O-**C**H_3), 64.37 (O-**C**H_2), 107.07-119.91 (3 Ar-**C**H), 114.41 (Ar-**C**), 123.55 (Ar-**C**), 127.27 (2 Ar-**C**H), 132.33 (2 Ar**C**H, 2 Ar**C**), 146.63 (Ar-**C**), 149.98 (2 Ar-**C**), 151.02 (**C**=O), 156.62 (Ar-**C**), 158.04 (**C**=O) and 167.99 ppm (2 **C**=O); FT-IR ν_{max}(KBr)cm^{-1}: 3186, 2960, 1742, 1694, 1651, 1525, 1490, 1264, 1145; Anal.Cal. for $C_{28}H_{29}N_5O_6$: C, 63.27%; H, 5.50%; N, 13.18%; O, 18.06%, Found: C, 62.88%; H, 5.33%; N, 14.63%.

*4.1.4.13. 8-[3-(2-Morpholinoethoxy)-phenyl]-1,3-dipropylxanthine (**RY-103, 12a**)*0.50 g, 50.42 %, mp 150-155 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 7.45 (s, 2H, Ar-*H*), 7.03 (m, 2H, Ar-*H*), 4.06 (m, 4H, -2OCH_2-), 2.09 (m, 1H, N*H*), 1.76 (m, 2H, -OCH_2-), 1.69 (m,4H, 2 x -NCH_2-), 1.41 (m, 2H, -NH_2), 1.25 (m, 2H, -CH_2-), 0.95 (m, 6H, 3 x -CH_2), 0.07 (m, 6H, -2 x -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 10.08-11.01 (2 **C**H_3), 20.01 (2 **C**H_2), 44.39 (2 N-**C**H_2), 54.02 (3 N-**C**H_2), 65.64 (2 O-**C**H_2), 66.08 (O-**C**H_2), 111.10 -156.41 (Ar**C**) and 162.75 (C=O) and 168.05 ppm (**C**=O); FT-IR $\nu_{max.}$ (KBr) cm^{-1}:2962, 1701, 1655, 1548, 1258; Anal.Cal. for $C_{23}H_{31}N_5O_4$: C, 62.57%; H, 7.08%; N, 15.86%; O, 14.49%, Found:C, 60.57%; H, 6.18%; N, 14.16%.

*4.1.4.14. 8-[3-(2-Piperidinoethoxy)-phenyl]-1,3-dipropylxanthine (**RY-105, 12b**)*0.40 g, 40.27 %, mp 180-185 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 7.83 (d, 2H, Ar-*H*), 7.37 (s, 1H, Ar-*H*), 7,00 (s,

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1H, Ar-*H*), 4.17 (m, 6H, 3 x -NCH_2-), 2.92 (s, 2H, -OCH_2-), 2.65 (s, 4H, 2 x -NCH_2-), 1.86 (s, 2H, -CH_2), 1.68 (s, 6H, 3 x -CH_2-), 1.49 (s,2H, -CH_2), 1.01 (m, 6H,2xCH_3); ^{13}C-NMR (100MHz, $CDCl_3$):δ 11.37-11.53 (2 **C**H_3), 21.24-25.56 (5 **C**H_2), 47.28-57.70 (5 N-**C**H_2), 65.64 (O-**C**H_2), 108.23-151.41 (5 Ar-**C**), 119.10 (2 Ar-**C**H), 129.90 (2 Ar-**C**H), 155.75 (**C**=O) and 159.05 ppm (**C**=O); FT-IR ν_{max}(KBr) cm^{-1}:3196 (-NH), 2933 (Ar-CH), 2787 (Ali-CH), 1698 (carbonyl), 1655 (CN), 1553 (H-C=C-H), 1259 (CO), 1186 (C-N); Anal. Cal. for $C_{24}H_{33}N_5O_3$:C, 65.58%; H, 7.57%; N, 15.93%; O, 10.92%, Found:C, 64.98%; H, 7.01%; N, 14.97%.

*4.1.4.15. 8-[3-{2-(Dimethylamino)-ethoxy}-phenyl]-1,3-dipropylxanthine (**RY-107, 12c**)*0.86 g, 86.33 %, mp>280 °C (decomp.); ^{1}H-NMR (400 MHz, DMSO-d_6):δ 7.30-6.91 (m, 4H, Ar-*H*), 4.01-3.83 (m, 4H, -OCH_2, -NCH_2), 1.93-1.55 (m, 4H, 2 x -NCH_2-), 1.80 (s, 4H, 2 x -CH_2-), 0.85 (s, 12H, 2 x -NCH_3, 2 x -CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6):δ 10.37-11.53 (2 **C**H_3), 21.24 (2 **C**H_2), 43.66 (2 N-**C**H_2), 45.80 (2 N-**C**H_3), 57.70 (N-**C**H_2), 65.64 (O-**C**H_2), 115.23-152.41 (5 Ar-**C**), 119.10-132.90 (4 Ar-**C**H) and 151.75-156.05 ppm (2 **C**=O); FT-IR $\nu_{max.}$ (KBr) cm^{-1}:3177 (-NH), 2960 (Ar-CH), 1695 (C=O), 1653 (C=N), 1550 (Ar-C=C), 1256 (C-O), 1175 (C-N); Anal. Cal. for $C_{21}H_{29}N_5O_3$:C, 63.14%; H, 7.32%; N, 17.53%; O, 12.01%, Found:C, 60.14%; H, 6.82%; N, 16.83%.

*4.1.4.16. 8-[3-(2-Pyrrolidinoethoxy)-phenyl]-1,3-dipropylxanthine (**RY-109, 12d**)*0.99 g, 99 %, mp230-235 °C (decomp.); ^{1}H-NMR (400MHz, DMSO-d_6):δ 13.76 (s, 1H, -N*H*), 7.31 (m, 4H, Ar-*H*), 4.42 (s, 2H, -OCH_2-), 3.96 (s, 6H, 3 x -NCH_2-), 3.89 (s, 4H, 2 x NCH_2-), 2.08 (d, 4H, 2 x -CH_2), 1.77 (d, 4H, 2 x -CH_2-) and 0.91 ppm (s, 6H, 2 x -CH_3);^{13}C-NMR (100 MHz, DMSO-d_6):δ 10.08-11.01 (2 **C**H_3), 20.08-25.56 (4 **C**H_2), 44.05 (2 N-**C**H_2), 54.08-56.67 (3 N-**C**H_2), 66.07 (O-**C**H_2), 114.67-128.89 (4 Ar-**C**H, Ar-**C**), 121.45 (Ar-**C**), 150.04 (Ar-**C**), 152.87 (**C**=O), 156.08 (2 Ar-**C**) and 156.78 ppm (**C**=O); FTIR ν_{max}(KBr) cm^{-1}:3223 (-NH), 2962 (Ar-CH), 1694 (C=O), 1648 (C=N), 1451 (Ar-C=C), 1254 (C-N); Anal. Cal. for $C_{23}H_{31}N_5O_3$:C, 64.93%; H, 7.35%; N, 16.47%; O, 11.28%; Found: C, 65.34%; H, 8.31%; N, 15.12%.

*4.1.4.17. 8-[3-{2-(Diethylamino)-ethoxy}-phenyl]-1,3-dipropylxanthine (**RY-111, 12e**)*0.76 g,76.92 %, mp 100-105 °C (decomp.); ^{1}H-NMR (400MHz, DMSO-d_6):δ 13.72 (s, 1H, -NH), 7.8(m, 1H, Ar-H), 7.40 (m, 2H, Ar-H), 7.05 (m, 1H, Ar-*H*), 4.40 (m, 2H, -OCH_2-), 4.07 (t, 2H, 2 x -NCH_2-), 3.92 (m, 4H, 2 x -NCH_2-), 1.79 (q, 4H, 2 x -N-CH_2), 1.63 (q, 2H, -CH_2-), 1.30 (m, 6H, 2 x -CH_3) and 0.93 ppm (m, 8H, 2xCH_3, -CH_2-); ^{13}C-NMR (100MHz, DMSO-d_6):δ 10.08-11.01 (2 **C**H_3), 13.08 (2 **C**H_3), 20.08

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(2 **C**H_2), 44.03-53.78 (5 N-**C**H_2), 66.07 (O-**C**H_2), 114.67-150.04 (Ar-**C**), and 156.78 ppm (**C**=O); FTIR ν_{max}(KBr) cm^{-1}:3181 (NH), 2961 (CH), 1743 (carbonyl), 1696 (C=O), 1651 (cyano), 1549 (H-C=C-H), 1257 (C-N); Anal. Cal. for $C_{23}H_{33}N_5O_3$:C, 64.61%; H, 7.78%; N, 16.38%; O, 11.23%, Found:C, 62.61%; H, 8.08%; N, 16.38%.

*4.1.4.18. 8-[3-{2-(Phthalimido)-ethoxy}-phenyl]-1,3-dipropylxanthine (**RY-113, 12f**)* 0.80 g, 80.69 %, mp 274-280 °C (decomp.); ^{1}H-NMR (400 MHz, $CDCl_3$):δ 13.66 (s, 1H, -N*H*), 7.56(m, 2H, Ar-H), 7.25 (t, 3H, Ar-*H,* J_{ortho}= 7.88Hz, J_{ortho}= 7.84Hz*)*,6.87 (d, 2H, Ar-*H,* J_{para}= 1.96Hz), 6.85 (d, 1H, Ar-*H,* J_{para}= 1.59Hz), 4.05 (t, 2H, -OCH_2-, J_1=7.00, J_2=7.48), 3.90 (t, 2H, -NCH_2-, J_1=7.32, J_2=7.52), 1.77 (m, 4H, 2 x NH_2-), 1.61 (m, 4H, 2 x -CH_2-) and 0.93 ppm (m, 6H, 2x-CH_3);^{13}C-NMR (100MHz, $CDCl_3$): δ 10.8-11.01 (2 **C**H_3), 20.02 (2 **C**H_2), 39.04-44.89 (3 N-**C**H_2), 66.04 (O-**C**H_2), 114.32 (2 Ar-**C**H, Ar-**C**), 123.76 (Ar-**C**), 128.05 (4 Ar-**C**H), 132.44 (2 Ar-**C**H, 2 Ar-**C**), 150.78 (Ar-**C**), 152.04 (**C**=O), 156.04 (2 Ar-**C**), 156.87 (**C**=O) and 167.09 ppm (2 **C**=O); FT-IR $\nu_{max.}$ (KBr) cm^{-1}: 3192 (-NH-), 2963 (Ar-CH-), 2876 (Ali-CH-), 1695 (C=O), 1644 (C=N), 1464 (Ar-C=C), 1262 (C-N), 1186 (C-O); Anal. Cal. for $C_{27}H_{27}N_5O_5$:C, 64.66%; H, 5.43%; N, 13.96%; O, 15.95%,Found:C, 62.15%; H, 6.13%; N, 14.16%.

4.2 Biological methods

4.2.1*Radioligand binding assays*

The binding affinities at A_1, A_{2A} and A_3 receptors (*Ki*-values) were determined in radioligand competition experiments. The binding affinities were tested using a 96 well microplate filtration system. The radioligands used were the agonists [^{3}H] CCPA (1 nM), [^{3}H]NECA (10 nM) and [^{3}H]HEMADO (1 nM) for A_1, A_{2A} and A_3, respectively. The samples were first incubated with membrane protein at 25ºC for 3 hours and then filtered followed by washing with ice cold binding buffer. After addition of scintillator to the dried filter plates, sample were counted. The binding data was calculated by nonlinear curve fitting using the program Prism [GraphPad Software](Klotz, 1998; Klotz, 2007).

4.2.2 *Bronchospasmolytic activity*

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The newly synthesized derivatives **10a, 10b, 10c, 10f, 11a, 11b, 12(a-f)**were evaluated for their in vivo bronchospasmolytic activity against the standard drug theophylline by histamine aerosol induced asthma in guinea pigs according to the method of Zabeer (Zabeer, 2006).

4.2.2.1 Experimental animals Guinea-pigs (Male; Dunkin Hartley) of 220-280 g, were obtained from LLRUAS, Hisar after the approval of IAEC, Banasthali University (BV/IAEC/2016/I dated 08.10.2016, Ref. No. BV/3421/16-17). The experimental animals so procured were kept in standard conditions as prescribed with proper food and water. They were monitored for 12 h day and night cycle for experimentation.

4.2.2.2 Drugs used. Histamine hydrochloride (bronchospasm agent), theophylline (standard drug), carboxymethyl cellulose (suspending agents) and test compounds (synthesized compound).

4.2.2.3. Experimental protocol. Guinea pigs (n =5) were designated as I for control animals (fed with carboxymethyl cellulose and water); II for positive animals (carboxymethyl cellulose, theophylline and water) and III for test animals (carboxymethyl cellulose, test drug and water). The assigned animal groups were allowed for fasting before treatment. The experimental animals were exposed in histamine chamber with histamine aerosol for 5 min after dosing of 1 h. Prior to exposure; animals received test drug (50 mg/kg), theophylline (50 mg/kg) and carboxymethyl cellulose orally, respectively. Different *in vivo* pharmacological behaviors of each animal was observed such as bronchospasm, jerks, death or survival. The onset of bronchospasm, duration of jerks, severity of bronchospasm and death or survival of the animal was recorded for each group. The results were expressed as Mean ± SEM. (**Table 2**). The animals were allowed to remain in the chamber for 15 min, if survived, animals were removed from the chamber and placed in fresh atmosphere with proper diet (Zabeer, 2006).

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Conflict of interest

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The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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