European Journal of Medicinal Chemistry 75 (2014) 327–335

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel 8-(*p*-substituted-phenyl/benzyl)xanthines with selectivity for the A_{2A} adenosine receptor possess bronchospasmolytic activity

Rakesh Yadav [a], Ranju Bansal [a,*], Sonja Kachler [b], K.N. Klotz [b]

[a] University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India
[b] Institut für Pharmakologie und Toxikologie, Universität Würzburg, Germany

ARTICLE INFO

Article history:
Received 15 October 2013
Received in revised form
13 January 2014
Accepted 19 January 2014
Available online 30 January 2014

Keywords:
8-Phenylxanthines
Bronchospasmolytic activity
Adenosine receptors

ABSTRACT

A new series of 8-(*p*-substituted-phenyl/benzyl)xanthines has been synthesized and evaluated *in vitro* for adenosine receptor binding affinity and *in vivo* for bronchospasmolytic effects. It was observed that the nature of substituent at *para*-position of 8-phenyl/benzyl group on the xanthine scaffold remarkably affects the binding affinity and selectivity of xanthine derivatives for various adenosine receptor subtypes and also their bronchospasmolytic effects. Newly synthesized 8-phenylxanthines displayed potent binding affinity and significant selectivity for A_{2A} receptors and also produced potent bronchospasmolytic effects. Replacement of phenyl ring with benzyl moiety at C_8 of xanthine skeleton resulted in notable reduction in adenosine receptor affinity and broncholytic effects.

1. Introduction

Asthma remains one of the most common respiratory diseases with unmet medical needs. Enough evidence has accumulated in support of the role of adenosine in pathogenesis of asthma [1,2]. Selective agonists or antagonists of adenosine receptors are being explored by various research groups and pharmaceutical industry in an attempt to generate novel antiasthmatic agents. All four adenosine receptor (AR) subtypes (A_1, A_{2A}, A_{2B} and A_3) are expressed in lungs and in inflammatory cells involved in asthma. This has led to investigations into all AR subtypes as potential therapeutic targets for the treatment of asthma [3–5]. As binding studies in human lungs have indicated higher abundance of A_2 than other subtypes, therefore these receptors seem to play a more prominent role in mediating the bronchoconstriction and proinflammatory effects of adenosine in lungs [6].

Substituted xanthines constitute one of the most persuasive categories of adenosine receptor antagonists reported to date and are known for variable potency and selectivity for adenosine receptor subtypes [7–9]. 8-Phenyltheophylline is the parent member of a variety of potent adenosine receptor antagonists, e.g., MRS-1754 (**1**) (Fig. 1) [9,10]. Although 8-phenyl substitution exhibits maximum adenosine receptor antagonistic activity, it confers extremely limited water solubility to xanthines, which restricts their usefulness as *in vivo* research tools and their possible use as therapeutic agents [11]. The incorporation of polar substituents improves the otherwise extremely limited water solubility of 8-phenylxanthines and consequently increases their effectiveness as potential therapeutic agents. Furthermore, appropriate substitutions on the 8-phenyl ring greatly affects the potency and selectivity of xanthines towards AR subtypes and thus their pharmacological effects [10,12,13].

In the light of these observations, we decided to study the impact of substituting polar dialkylaminoethoxy substituents at *para* position of the 8-phenyl ring of xanthines on adenosine receptor binding affinity and selectivity. In order to examine specific structural features, which may be important for adenosine receptor binding, it was also appealing to introduce a methylene spacer between the aromatic unit and the C_8 of the xanthine nucleus and study the resulting effects on biological activity. As a continuation of our earlier xanthine based research [10,14,15], a new series of 8-(substituted-phenyl/benzyl)xanthines has been synthesized and evaluated for their binding affinity for various adenosine receptor subtypes and also for bronchospasmolytic effects.

2. Chemistry

The synthesis of various 8-(substituted-phenyl)xanthines has been depicted in Schemes 1 and 2.

Substituted aromatic aldehydes **3**–**9** [16] were prepared by treating 4-hydroxybenzaldehyde with hydrochloride of desired

* Corresponding author. Tel.: +91 172 2541142; fax: +91 172 2543101.
E-mail address: ranju29in@yahoo.co.in (R. Bansal).

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2014.01.045

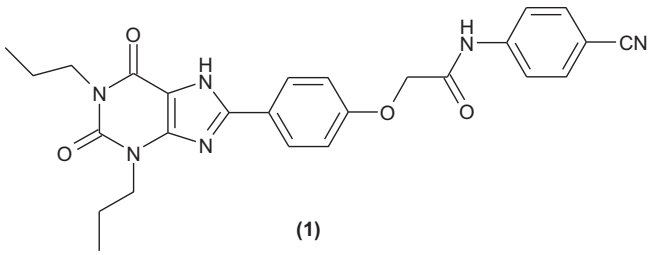


Fig. 1. Structure of xanthine based adenosine receptor antagonist MRS-1754 (**1**).

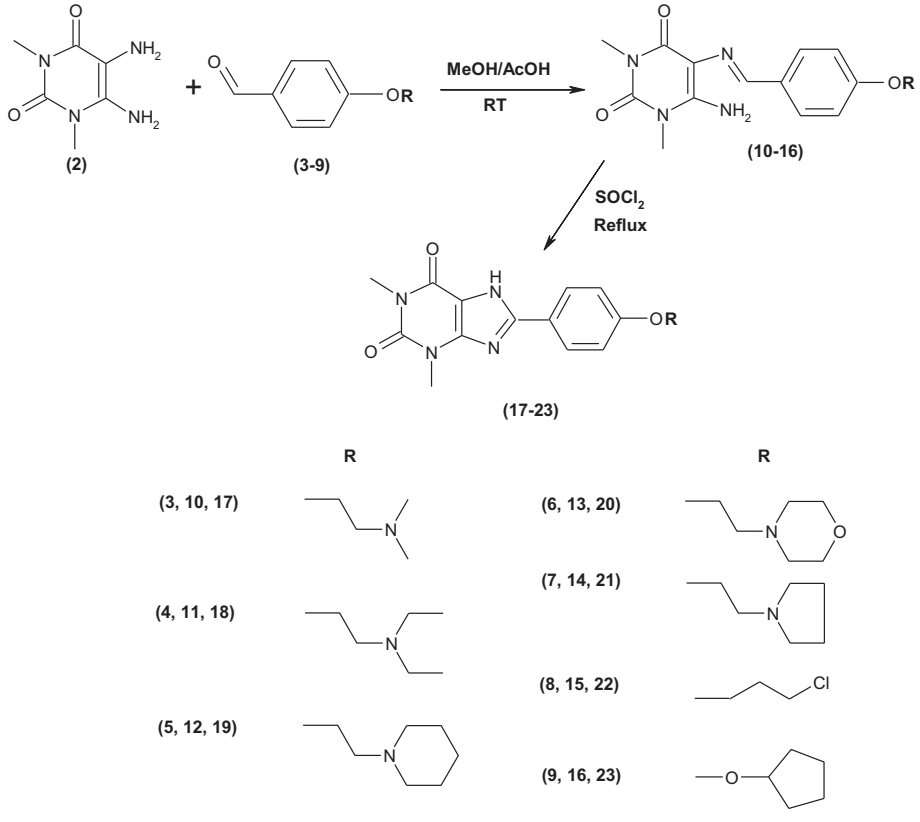
dialkylamino ethyl chloride, 1-bromo-3-chloropropane and cyclopentyl bromide, respectively, in refluxing ethyl methyl ketone in the presence of anhydrous potassium carbonate (Scheme-1).

Treatment of obtained aldehydes **3**–**9** with 5,6-diamino-1,3-dimethyluracil (**2**) [17,18] in MeOH-AcOH (4:1) at room temperature resulted in the formation of corresponding benzylidene adducts **10**–**16**, subsequent ring closure of which in refluxing thionyl chloride afforded the target compounds **17**–**23** as shown in Scheme-1. Characteristic NMR signals appeared at δ 2.93 [s, 6H, $-N(CH_3)_2$] for **17**, 1.32 [s, 6H, $-N(CH_2CH_3)_2$] for **18**, 2.98–3.06 (m, 4H, $-N(CH_2)_2$, piperidine) for **19**, 3.70 [t, 4H, O-$(CH_2)_2$, morpholine] for **20**, 2.88 [s, 4H, $-N(CH_2)_2$, pyrrolidine] for **21**, 3.77 ppm (t, 2H, $-CH_2Cl$) for **22** and a multiplet at 4.80–4.91 ppm for $-OCH$ of cyclopentyloxy ring substituted xanthine **23**. Further, two separate singlets for both methyl groups present at 1- and 3-position of purine nucleus were found at $\delta \sim$ 3.4 and 3.6, and $-OCH_2-$ protons resonated as a triplet around 4.3 ppm for all the compounds.

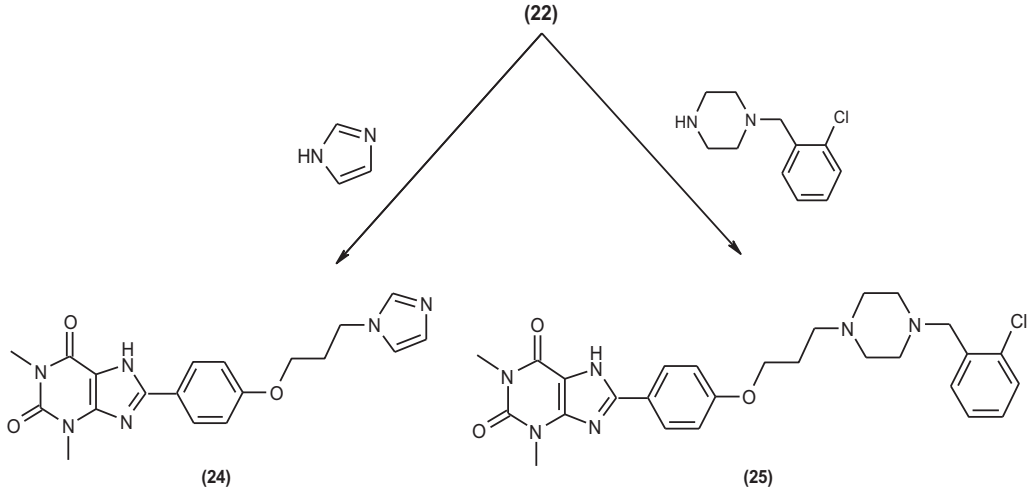
Keeping in mind the reported adenosine binding affinity of imidazole [15] derived xanthines and antihistaminic properties of aryl piperazine substituted 8-phenylxanthines, chloroalkoxy derivative **22** was thermally fused separately with powdered imidazole and 1-(2-chlorobenzyl)piperazine at 160 °C for 2 h to afford the corresponding xanthine congeners **24** and **25** (Scheme-2). Imidazolyl protons appeared at δ 6.97, 7.03 and 7.52 and *N*-methylene protons of $-CH_2-N<$ resonated downfield as a triplet at 3.99 for compound **24**, while an 8-proton multiplet of piperazinyl ring was seen at 2.56–2.60 ppm in the NMR spectrum of **25**.

For the preparation of target 8-[4-(aminopropoxy)benzyl]xanthines, attempts to synthesize the starting substituted acidic compound **29** by direct alkylation of 4-hydroxyphenylacetic acid with 1-bromo-3-chloropropane remained unsuccessful. Therefore an alternative synthetic route was adopted as shown in Scheme 3. 4-Hydroxyphenylacetic acid was esterified by heating under reflux in methanol in presence of a catalytic amount of sulfuric acid to afford an oily residue of methyl-4-hydroxyphenyl acetate (**27**). Alkylation of the compound **27** was carried out by treating with 1-bromo-3-chloropropane in ethyl methyl ketone using anhydrous potassium carbonate to give the oily residue methyl-[4-(3-chloropropoxy)phenyl]acetate (**28**). Alkaline hydrolysis of ester **28** led to the formation of the desired starting compound **29** as shown in Scheme 3.

4-Chloropropoxyphenylacetic acid (**29**) was condensed with 5,6-diamino-1,3-dimethyluracil (**2**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) in methanol at room temperature to yield 6-amino-5-[{4-(3-chloropropoxy)phenyl} carboxacetamido]-1,3-dimethyluracil (**30**) as shown in Scheme 3. ^{1}H NMR spectrum of carboxamide derivative **30** displayed a characteristically downfield singlet integrating for two protons of NH–CO–CH_2–Ar group at δ 3.67, while protons of the free $-NH_2$ group resonated as a singlet at 5.87 ppm. Symmetric and asymmetric stretching bands of free $-NH_2$ group appeared at 3321 and 3200 cm^{-1} in the IR spectrum of the compound **30**.



Scheme 1. Synthetic route to 8-(*p*-substituted phenyl)xanthine derivatives **17**–**23**.



Scheme 2. Synthesis of xanthine derivatives **24** and **25**.

Cyclization of **30** on refluxing with a mixture of sodium hydroxide and dioxane resulted in the formation of desired xanthine derivative **31** with a methylene spacer between C_8 of the xanthine nucleus and phenyl ring. The disappearance of absorption band of free $-NH_2$ in the IR spectrum at 3321 cm^{-1} supported the formation of 8-[4-(3-chloropropoxy)benzyl]-1,3-dimethylxanthine (**31**). The protons of the methylene linker and of $-OCH_2-$ group resonated together as a multiplet at δ 4.1 ppm. To introduce various polar functionalities, chloropropoxy substituted 8-benzylxanthine was further fused with various heterocyclic amines like morpholine, imidazole and 1,4-dioxa-8-azaspiro[4.5]decane at 80–100 °C to obtain target 8-aminoalkoxybenzylxanthines **32**–**34**.

8-[4-(3-Morpholinopropoxy)benzyl]-1,3-dimethylxanthine (**32**) exhibited a broad singlet integrating for four protons of the $-N-(CH_2)_2$ and a triplet of $-O-(CH_2)_2$ protons at δ 2.46 and 3.71 ppm, respectively. Singlets of imidazolyl protons were found at δ 6.93, 7.04 and 7.45 for compound **33**, four protons of *N*-methylene groups of 1,4-dioxa-8-azaspiro[4.5]decane and two protons of $-OCH_2CH_2CH_2-N<$ appeared together as a multiplet at 2.52–2.55 ppm in the ^{1}H NMR spectrum of compound **34.** Protons of the methylene linker resonated as a singlet at $\sim \delta$ 4.1 ppm for 8-benzyl substituted xanthines.

3. Biological evaluation

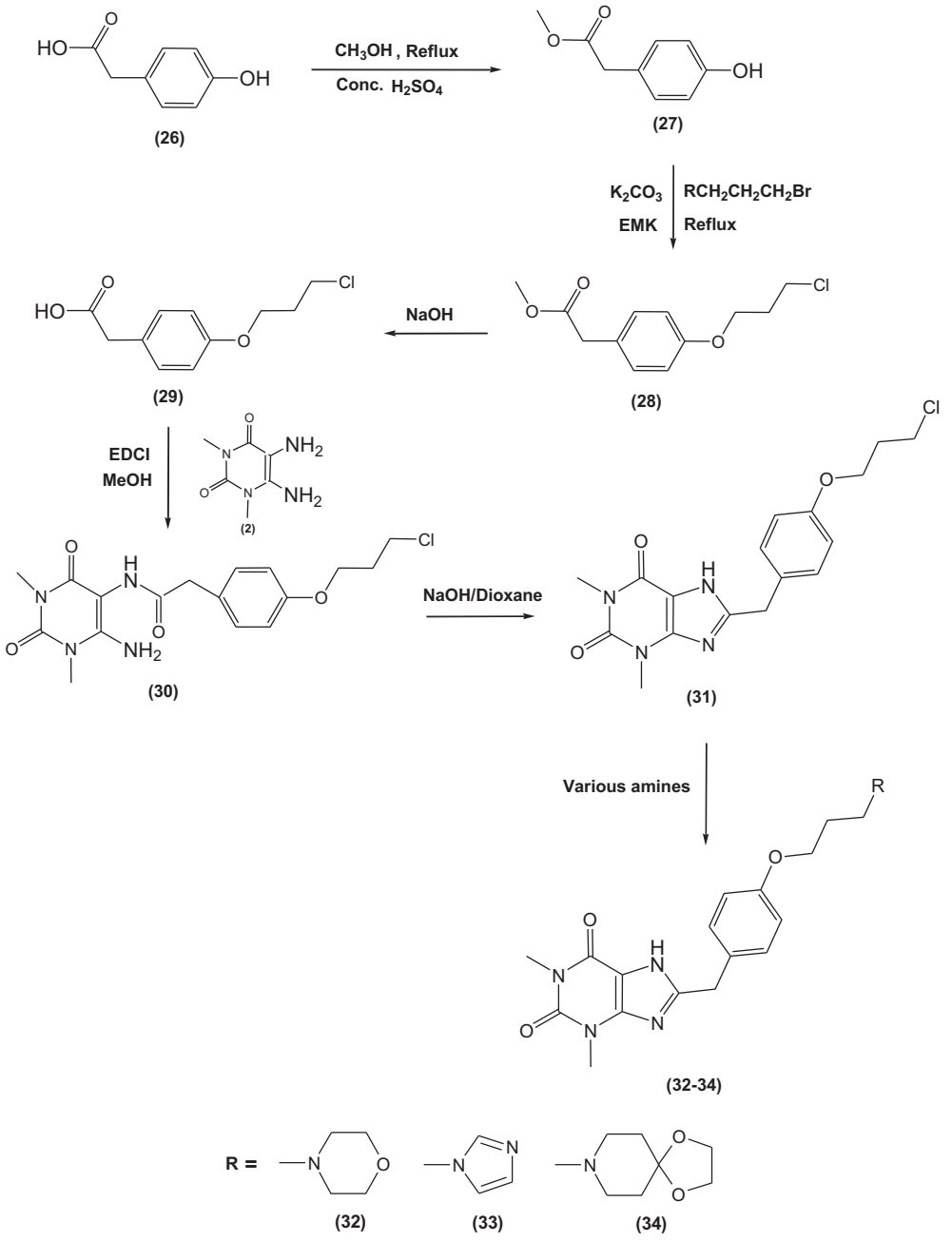
The 8-substituted xanthine derivatives **17**–**25** and **31**–**34** were evaluated using *in vitro* radioligand binding studies at cloned adenosine receptors [19,20]. In this study all human subtypes were stably transfected into Chinese Hamster Ovary (CHO) cells to study their pharmacological profile in an identical cellular background utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}). Affinities of various newly synthesized xanthine derivatives for adenosine A_1, A_{2A}, A_{2B} and A_3 receptors have been summarized in Table 1. The *in vivo* bronchospasmolytic activity of compounds **17**–**25** and **31**–**34** was studied using a histamine chamber model [21]. The data obtained has been arranged in Table 2. The results have been compared with standard drug theophylline.

4. Results and discussion

Table 1 summarizes the observed binding affinities of newly synthesized 8-phenyl/benzylxanthine derivatives towards various human adenosine receptor (A_1, A_{2A}, A_{2B} and A_3) subtypes. In general, 8-phenyl substituted xanthines **17**–**25** displayed high binding affinity for all the adenosine receptors with maximum affinity, and thus resulting in selectivity for the A_{2A} subtype. The binding selectivity for A_{2A} is somewhat more pronounced *versus* A_3 receptors compared to the A_1 and A_{2B} subtypes in most of the cases. No significant difference was observed in binding affinity of 8-alkylaminoethoxyphenyl substituted xanthines possessing open (**17**, **18**) and cyclized ring systems (**19**–**21**) in side chain towards individual adenosine receptors. The maximum binding affinity of these compounds was observed for A_{2A} receptors, which is ~5, 10, and 20 times higher as compared to A_1, A_{2B}, and A_3 receptors, respectively. In comparison to amino substituted xanthines **17**–**21**, chloropropoxy phenyl substituted **22** (K_i = 45 nM) and cyclopentyloxy derivative **23** (K_i = 72 nM) exhibited remarkable affinity and selectivity for A_{2A} receptors. Both the compounds were found to possess ~200–600 times higher binding for A_{2A} in comparison to other AR subtypes, but xanthine **23** was only 14 times more selective for the A_{2A} *versus* A_{2B} subtype (Table 1). Imidazole (**24**) and chlorobenzyl piperazine (**25**) derived xanthines displayed some selectivity for A_{2A} against A_1 and A_{2B}, which is little more pronounced against A_3 receptor subtype. The imidazolyl substituted xanthine **24** was the most potent compound of the series with K_i = 42 nM for A_{2A} receptors.

Introduction of a methylene spacer between side chain and purine core structure resulted in loss of binding affinity of 8-benzylxanthine analogs **31** and **32** for all adenosine receptor subtypes. However, introduction of an imidazole or dioxaazaspirodecan group in the side chain (**33**, **34**) revived binding to A_{2A} with no measurable effect on binding to other subtypes (Table 1).

All the newly synthesized 8-phenylxanthines **17**–**25** displayed significant protection against histamine aerosol induced bronchospasm in guinea pigs; effects being more prominent in comparison to 8-benzyl congeners **31**–**34**. The compounds showed more potency in comparison to the standard drug theophylline as apparent from enhanced time of onset of bronchospasm in case of test compounds compared to theophylline except cyclopentyloxy substituted xanthine **23**. The chloroarylpiperazinyl substituted xanthine **25** was the most effective compound of the series with no jerks, least severity of bronchospasm and 100% survival of animals. The 8-benzyl substituted xanthines **31**–**34** displayed decreased bronchospasmolytic effects in comparison to theophylline and 8-phenylxanthine congeners. The compounds **31** and **32** although lack AR binding affinity but still produces bronchospasmolytic effects, with later displaying more prominence. Cyclopentyloxy



Scheme 3. Synthetic route to 8-(*p*-substituted benzyl)xanthine derivatives **31**–**34**.

substituted 8-phenylxanthine **23** shows high affinity for A_{2A} but bronchospasmolytic effects are missing. Still the possibility of a causal relationship between A_{2A} antagonism and broncholysis cannot be ruled out. Bronchospasmolytic effects of the compounds may be attributed to combined antiinflammatory effects (resulting from A_{2A} binding) and bronchorelaxant effects (due to inherent property of xanthine skeleton), therefore to some extent bronchospasmolytic effects are related to A_{2A} binding but not completely in this particular series of xanthine derivatives.

In conclusion, the newly synthesized 8-phenylxanthines displayed significant binding affinity for adenosine receptors with highest potency for A_{2A}, resulting in selectivity for A_{2A} *versus* other AR subtypes. These compounds also produced potent bronchospasmolytic effects, however replacement of phenyl ring with benzyl moiety resulted in notable reduction in adenosine affinity and broncholytic effects. The effect of varying substituents on the 8-phenyl ring on biological properties in case of both *in vitro* and *in vivo* assays is clearly visible. Suitable introduction of 8-phenyl/benzyl substituents on the xanthine scaffold results in potent and selective binding for A_{2A} adenosine receptors along with potent bronchospasmolytic effects. These dual action xanthine derivatives may act as useful candidates for asthma therapy.

5. Experimental protocols

5.1. Chemistry

All melting points were obtained using glass capillary tubes on a Veego melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin–Elmer RX1 Fourier Transfrom-

Table 1
Binding affinities of xanthine derivatives at various adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3).

Comp. No. (CODE)	K_i (μM)				A_{2A} selectivity		
	(A_1)[a]	(A_{2A})[b]	(A_{2B})[c]	(A_3)[d]	A_1/A_{2A}	A_{2B}/A_{2A}	A_3/A_{2A}
17 (RB-337)	1.82 (1.55–2.13)	0.40 (0.21–0.77)	4.95 (3.21–7.64)	9.38 (6.12–14.40)	4.6	12	24
18 (RB-396)	1.59 (1.19–2.13)	0.25 (0.16–0.38)	2.59 (1.81–3.72)	6.82 (5.14–9.05)	6.4	10	27
19 (RB-394)	1.33 (1.11–1.60)	0.29 (0.14–0.58)	2.9 (2.21–3.79)	6.51 (4.26–9.95)	4.6	10	22
20 (RB-393)	2.58 (2.48–2.68)	0.31 (0.21–0.46)	1.34 (1.04–1.72)	2.74 (1.51–4.97)	83	43	8.8
21 (RB-395)	1.20 (1.05–1.37)	0.20 (0.12–0.34)	1.97 (1.51–2.56)	4.60 (3.97–5.32)	6.0	9.9	23
22 (RB-338)	>10	0.045 (0.036–0.054)	>10	>30	>220	>220	>670
23 (RB-397)	>30	0.072 (0.05–0.12)	1.0	>10	>420	14	>420
24 (RB-339)	0.89 (0.68–1.18)	0.042 (0.03–0.07)	1.05 (0.79–1.41)	2.27 (1.91–2.7)	21	25	54
25 (RB-448)	1.85 (1.70–2.01)	0.12 (0.076–0.19)	0.17 (0.15–0.19)	6.89 (4.04–11.8)	15	1.4	57
31 (RB-452)	>30	>10	>10	>30	–	–	–
32 (RB-453)	>30	>10	>10	>30	–	–	–
33 (RB-454)	>30	3.17 (2.56–3.92)	>10	>30	>9.5	>3.2	>9.5
34 (RB-455)	>30	8.09 (4.39–14.9)	>10	>30	>3.7	>1.2	>3.7

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

Infrared spectrophotometer using potassium bromide pellets (υ_{max} in cm^{-1}). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectroscopy were performed using a Bruker AC-400F, 400 MHz spectrometer 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 standard. The spin multiplicities are indicated by the symbols, s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), br (broad) and the coupling constants (J) are given in Hertz (Hz). Elemental analyses were carried out on a Thermo-Flash EA-1112 CHNS–O analyzer. The results are within 0.4% of the theoretical values. Precoated plates with silica gel G (E. Merck 60 F_{254}, 0.25 mm) were used for thin layer chromatography (TLC). Chromatographic spots were visualized by ultra-violet light in the UV cabinet (Perfit, India). Anhydrous sodium sulfate was utilized as drying agent. All solvents were freshly distilled and dried prior to use according to standard procedures.

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.

Comp. no. (Code)	Mean time in seconds for onset of bronchospasm	Duration of jerks in seconds	Severity of bronchospasm	Survival (%)
	Mean ± S.E.M	Mean ± S.E.M		
Control	67 ± 2[a]	172 ± 4[a]	+++	0
Theophylline	92 ± 3	89 ± 7	+	100
17 (RB-337)	111 ± 5	39 ± 1[a]	+	100
18 (RB-396)	115 ± 3	49 ± 3[a]	++	75
19 (RB-394)	121 ± 4[a]	22 ± 2[a]	+	75
20 (RB-393)	104 ± 5	45 ± 2[a]	+	100
21 (RB-395)	111 ± 6	32 ± 1[a]	+	100
22 (RB-338)	114 ± 5	30 ± 2[a]	+	100
23 (RB-397)	72 ± 3	35 ± 2[a]	++	50
24 (RB-339)	108 ± 4	71 ± 4	++	75
25[b] (RB-448)	109 ± 5	–	+	100
31 (RB-452)	75 ± 4	44 ± 3[a]	++	50
32[b] (RB-453)	108 ± 4	–	+	100
33[b] (RB-454)	83 ± 3	–	++	50
34 (RB-455)	73 ± 3	23 ± 3[a]	++	50

Number of animals in each group (N) = 4.
Dose of standard and tested compounds = 50 mg/kg.
[a] Tukey's test; $p < 0.05$.
[b] No jerks were observed in animals after treatment with test compounds.

*5.1.1. General procedure for the synthesis of various aldehydes **3**–**9***

Requisite alkylaminoethyl chloride hydrochloride, chlorobromopropane and cyclopentyl bromide (6.0 mmol) were added to a stirred and refluxing slurry of 4-hydroxybenzaldehyde (1.0 g, 8.19 mmol) in ethyl methyl ketone (C_4H_8O) (40 ml) in the presence of anhydrous potassium carbonate (2.0 g, 14.47 mmol). The reaction mixture was further refluxed for 6 h with continuous stirring. The completion of the reaction was monitored by TLC. On completion, the reaction mixture was cooled, filtered and the solvent was removed under reduced pressure to obtain an oily residue of corresponding aldehyde **3**–**9**, which was used as such for further reaction.

*5.1.2. General procedure for the synthesis of various benzylidene derivatives **10**–**16***

To a stirred solution of 5,6-diamino-1,3-dimethyluracil (**2**) (1.0 g, 5.87 mmol) in MeOH-AcOH (4:1, 40 mL) was slowly added the solution of the above obtained oily residue of the relevant aldehyde **3**–**9** in methanol (24 ml). The reaction mixture was further stirred overnight at room temperature. The completion of the reaction was monitored by TLC. The residue obtained after removal of solvent under reduced pressure was dissolved in ice-cold water and made alkaline with sodium hydroxide. The resultant turbid solution was cooled in ice for complete precipitation. The precipitate obtained was filtered off, washed with ice cold water and dried to obtain the corresponding benzylidene derivatives **10**–**16**, which were used as such for further reaction.

*5.1.3. General procedure for the synthesis of various 8-(substituted-phenyl)xanthine derivatives **17**–**25***

Benzylidene derivatives **10**–**16** (2.5 mmol) thus obtained were refluxed separately in thionyl chloride (20 ml) for 30–40 min to affect cyclization. The excess thionyl chloride was removed under reduced pressure to obtain a solid product. Ice cold water was added to it and the resultant suspension was neutralized with ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried and recrystallized from a mixture of DMF and ethanol to afford the desired products **17**–**23**, respectively.

Further, the chloropropoxy derivative **22** was fused with imidazole and 1-(2-chlorobenzyl)piperazine at 160 °C for 2 h to afford the desired compounds **24** and **25**, respectively. Imidazolyl substituted xanthine **24** was crystallized from a mixture of DMF

and ethanol and compound **25** using a mixture of chloroform and methanol.

*5.1.3.1. 8-[4-(2-Dimethylaminoethoxy)phenyl]-1,3-dimethylxanthine (**17**).* Yield: 54.3%; m.p. >280 °C. FTIR: 3174, 1691, 1650, 1482, 1245, 1183; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.93 (s, 6H, −N−(CH_3)$_2$), 3.39 (s, 3H, N−CH_3), 3.56 (s(br), 2H, −CH_2N<), 3.62 (s, 3H, N−CH_3), 4.49 (t, 2H, −OCH_2−), 7.04 (d, 2H, 2-C*H* and 6-C*H*, aromatic, J_o = 8.92 Hz), 8.14 (d, 2H, 3-C*H* and 5-C*H*, aromatic, J_o = 8.92 Hz) and 13.44 ppm (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 27.62 (2× N−**C**H$_3$), 29.59 (2× N−**C**H$_3$), 44.65 (N−**C**H$_2$), 56.88 (O−**C**H$_2$), 107.1 (Ar**C**), 114.66 (2× Ar**C**H), 121.51 (Ar**C**)) 128.01 (2× Ar**C**H), 148.2 (Ar**C**), 149.95 (Ar**C**), 151.16 (Ar**C**), 154.23 (**C**═O) and 160 ppm (**C**═O). Anal. Calc. for $C_{17}H_{21}N_5O_3$: C, 59.46; H, 6.16; N, 20.39%. Found: C, 59.34; H, 6.27; N, 20.21%.

*5.1.3.2. 8-[4-(2-Diethylaminoethoxy)phenyl]-1,3-dimethylxanthine (**18**).* Yield: 89.6%; m.p. >280 °C. FTIR: 3168, 1696, 1650, 1478,1245; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 6H, −N(CH_2CH_3)$_2$), 3.16 (s, 4H, −N(CH_2CH_3)$_2$), 3.34 (s, 3H, N−CH_3), 3.48 (s, 2H, −CH_2N<), 3.57 (s, 3H, N−CH_3), 4.40 (s, 2H, −OCH_2−), 7.05 (d, 2H, 2-C*H* and 6-C*H*, arom, J_o = 8.84 Hz), 8.14 (d, 2H, 3-C*H* and 5-C*H*, arom, J_o = 8.76 Hz) and 13.54 (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 10.86 (2x**C**H$_3$), 27.74 (N−**C**H$_3$), 29.72 (N−**C**H$_3$), 47.01 (N−(**C**H$_2$)$_2$), 50.84 (N−**C**H$_2$), 65.33 (O−**C**H$_2$), 107.20 (Ar**C**), 114.84 (2× Ar**C**H), 121.30 (Ar**C**), 128.02 (2× Ar**C**H), 148.49 (Ar**C**), 149.81 (Ar**C**), 151.15 (Ar**C**), 154.04 (**C**═O) and 159.78 ppm (**C**═O). Anal. Calc. for $C_{19}H_{25}N_5O_3$: C, 61.48; H, 6.79; N, 18.87. Found: C, 61.38; H, 6.65; N, 18.82%.

*5.1.3.3. 1,3-Dimethyl-8-[4-(2-piperidin-4-ylethoxy)phenyl]xanthine (**19**).* Yield: 25.2%; m.p. 258–260 °C. FTIR: 3165, 2920, 1693, 1648, 1473, 1241, 1019; ^{1}H NMR (DMSO-d_6): δ 1.21–1.23 (m, 2H, −CH_2−, piperidine), 1.95–1.97 (m, 4H, 2× CH_2, piperidine), 2.98–3.06 (m, 4H, N−(CH_2)$_2$, piperidine), 3.51–3.53 (m, 5H, N−CH_3 and −CH_2N<), 3.59 (s, 3H, N−CH_3), 4.51 (t, 2H, −OCH_2−, J = 5.97 Hz), 7.02 (d, 2H, 2-C*H* and 6-C*H*, arom, J_o = 8.84 Hz), 8.13 (d, 2H, 3-C*H* and 5-C*H*, arom, J_o = 8.64 Hz) and 10.62 (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 21.19 (**C**H$_2$), 22.39 (2× **C**H$_2$), 27.74 (N−**C**H$_3$), 29.71 (N−**C**H$_3$), 52.67 (N−(**C**H$_2$)$_2$), 54.67 (N−**C**H$_2$), 62.49 (O−**C**H$_2$), 107.22 (Ar**C**), 115.01 (2× Ar**C**H), 121.85 (Ar**C**), 128.03 (2xAr**C**H), 148.47 (Ar**C**), 149.63 (Ar**C**), 151.11 (Ar**C**), 154.02 (**C**═O) and 159.01 ppm (**C**═O). Anal. Calc. for $C_{20}H_{25}N_5O_3$: C, 62.65; H, 6.57; N, 18.26. Found: C, 62.80; H, 6.80; N, 18.46%.

*5.1.3.4. 1,3-Dimethyl-8-[4-(2-morpholin-4-ylethoxy)phenyl]xanthine (**20**).* Yield: 52.3%; m.p. >280 °C. FTIR: 3174, 2951, 1691, 1650, 1482, 1250, 1114; ^{1}H NMR (DMSO-d_6): δ 2.59 (s(br), 4H, −N(CH_2)$_2$, morpholine), 2.82 (t, 2H, −CH_2N<, J = 5.64 Hz), 3.38 (s, 3H, N−CH_3), 3.62 (s, 3H, N−CH_3), 3.70 (t, 4H, O(CH_2)$_2$, morpholine), 4.17 (t, 2H, −OCH_2−, J = 5.62 Hz), 6.99 (d, 2H, 2-C*H* and 6-C*H*, arom, J_o = 8.88 Hz), 8.09 (d, 2H, 3-C*H* and 5-C*H*, arom, J_o = 8.88 Hz) and 13.32 (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 27.72 (N−**C**H$_3$), 29.70 (N−**C**H$_3$), 53.47 (N−(**C**H$_2$)$_2$), 55.67 (N−**C**H$_2$), 63.49 (O−**C**H$_2$), 65.13 (O-(**C**H$_2$)$_2$), 107.11 (Ar**C**), 114.01 (2xAr**C**H), 123.85 (Ar**C**), 129.03 (2xAr**C**H), 148.17 (Ar**C**), 149.61 (Ar**C**), 153.11 (Ar**C**), 154.12 (**C**═O) and 159.11 ppm (**C**═O). Anal. Calc. for $C_{19}H_{23}N_5O_4$: C, 59.21; H, 6.01; N, 18.17. Found: C, 59.44; H, 6.14; N, 18.04%.

*5.1.3.5. 1,3-Dimethyl-8-[4-(2-pyrrolidin-1-ylethoxy)phenyl]xanthine (**21**).* Yield: 35.7%; m.p. >220 °C. FTIR: 3165, 2949, 1694, 1650, 1477, 1244, 1053; ^{1}H NMR (DMSO-d_6): δ 1.86 (s, 4H, 2× CH_2, pyrrolidine), 2.88 (s, 4H, −N(CH_2)$_2$, pyrrolidine), 3.14 (s, 2H, −CH_2N<), 3.33 (s, 3H, N−CH_3), 3.61 (s, 3H, N−CH_3), 4.24 (s, 2H, −OCH_2−), 6.99 (d, 2H, 2-C*H* and 6-C*H*, arom, J_o = 8.72 Hz), 8.11 (d, 2H, 3-C*H* and 5-C*H* arom, J_o = 8.68 Hz) and 13.46 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): δ 22.79 (2× **C**H$_2$), 27.72 (N−**C**H$_3$), 29.70 (N−**C**H$_3$), 53.37 (N−**C**H$_2$), 53.86 (N−(**C**H$_2$)$_2$), 65.06 (O−**C**H$_2$), 107.23 (Ar−**C**), 114.90 (2× Ar**C**H), 121.53 (Ar**C**), 128.03 (2xAr**C**H), 148.47 (Ar**C**), 149.75 (Ar**C**), 151.13 (Ar**C**), 154.04 (**C**═O) and 159.52 ppm (**C**═O). Anal. Calc. for $C_{19}H_{23}N_5O_3$: C, 61.77; H, 6.27; N, 18.96. Found: C, 61.80; H, 6.27; N, 18.71%.

*5.1.3.6. 8-[4-(3-Chloropropoxy)phenyl]-1,3-dimethylxanthine (**22**).* Yield: 114.7%; m.p. >220 °C. FTIR: 3163, 2947, 1693, 1652, 1484, 1293, 1026; ^{1}H NMR (DMSO-d_6): δ 2.27 (p, 2H, −$CH_2CH_2CH_2$Cl), 3.43 (s, 3H, N−CH_3), 3.65 (s, 3H, N−CH_3), 3.77 (t, 2H, −CH_2Cl, J = 6.32 Hz), 4.18 (t, 2H, −OCH_2−, J = 5.84 Hz), 6.98 (d, 2H, 2-CH and 6-C*H*, aromatic, J_o = 8.84 Hz), 8.10 (d, 2H, 3-CH and 5-C*H*, aromatic, J_o = 8.84 Hz) and 13.26 ppm (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 27.74 (N−**C**H$_3$), 29.72 (N−**C**H$_3$), 31.57 (**C**H$_2$), 41.88 (**C**H$_2$Cl), 64.48 (O−**C**H$_2$), 107.27 (Ar**C**), 114.82 (2xAr**C**H), 121.39 (Ar**C**), 128.06 (2xAr**C**H), 148.54 (Ar**C**), 149.87 (Ar**C**), 151.17 (Ar**C**), 154.08 (**C**═O) and 159.9 ppm (**C**═O). Anal. Calc. for $C_{16}H_{17}N_4O_3Cl$: C, 55.15; H, 4.92; N, 16.08. Found: C, 55.33; H, 4.68; N, 16.36%.

*5.1.3.7. 8-[4-(Cyclopentyloxy)phenyl]-1,3-dimethylxanthine (**23**).* Yield: 60.4%; m.p. 208–210 °C. FTIR: 3148, 2939, 1704, 1647, 1606, 1474, 1367, 1051; ^{1}H NMR ($CDCl_3$): δ 1.78–2.01 (m, 8H, 4× −CH_2−, cyclopentyl), 3.42 (s, 3H, N−CH_3), 3.65 (s, 3H, N−CH_3), 4.80–4.91 (m, 1H, O−C*H*<, cyclopentyl), 6.93 (d, 2H, 2-C*H* and 6-C*H*, aromatic, J_o = 8.80 Hz) and 8.08 ppm (d, 2H, 3-C*H* and 5-C*H*, aromatic, J_o = 8.80 Hz). Anal. Calc. for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.42; H, 5.80; N, 16.56%.

*5.1.3.8. 1,3-Dimethyl-8-[4-(3-imidazol-1-ylpropoxy)phenyl]xanthine (**24**).* Yield: 84.6%; m.p. 255 °C (decomp.). FTIR: 3167, 2946, 1692, 1650, 1482, 1291, 1026; ^{1}H NMR (DMSO-d_6): δ 2.28 (p, 2H, −$CH_2CH_2CH_2$N<), 3.42 (s, 3H, N−CH_3), 3.65 (s, 3H, N−CH_3), 3.99 (t, 2H, −CH_2N<, J = 5.16 Hz), 4.23 (t, 2H, −OCH_2−, J = 6.64 Hz), 6.96–7.00 (m, 3H, 2-C*H* and 6-C*H*, aromatic and C*H*, imidazole), 7.03 (s, 1H, C*H*, imidazole), 7.52 (s, 1H, C*H*, imidazole), 8.09 (d, 2H, 3-C*H* and 5-C*H*, aromatic, J_o = 7.40 Hz) and 13.25 ppm (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 27.73 (N−**C**H$_3$), 29.71 (N−**C**H$_3$), 30.04 (**C**H$_2$), 42.97 (N−**C**H$_2$), 64.70 (O−**C**H$_2$), 107.20 (Ar**C**), 114.80 (2xAr**C**H), 119.45 (Ar**C**H, imid), 121.38 (Ar**C**), 122.67 (Ar**C**H, imid), 128.04 (2xAr**C**H), 137.27 (Ar**C**H, imid), 148.49 (Ar**C**), 149.83 (Ar**C**), 151.15 (Ar**C**), 154.04 (**C**═O) and 159.92 ppm (**C**═O). Anal. Calc. for $C_{19}H_{20}N_6O_3$: C, 59.99; H, 5.30; N, 22.09. Found: C, 59.68; H, 5.17; N, 22.29%.

*5.1.3.9. 8-[4-{3-(4-(2-Chlorobenzyl)piperazin-1-yl)propoxy}phenyl]-1,3-dimethylxanthine (**25**).* Yield: 33.5%; m.p. >220 °C. FTIR: 3169, 2942, 1694, 1650, 1478, 1291, 1048; ^{1}H NMR ($CDCl_3$): δ 2.02 (p, 2H, −$OCH_2CH_2CH_2$-), 2.17 (s, 2H, −CH_2−N<, J = 6.10 Hz), 2.56–2.60 (m, 8H, 4× −CH_2−, piperazine), 3.54 (s, 3H, N−CH_3), 3.70 (s, 3H, N−CH_3), 3.64 (s, 2H, −CH_2−), 4.09 (t, 2H, −OCH_2−, J = 6.30 Hz), 6.98 (d, 2H, 2-C*H* and 6-C*H*, aromatic, J_o = 8.76 Hz), 7.22–7.29 (m, 2H, 4′-C*H* and 5′-C*H*, aromatic), 7.35 (dd, 1H, 6′-C*H*, aromatic, J_m = 1.36 Hz; J_o = 7.72 Hz), 7.47 (dd, 1H, 3′-C*H*, aromatic, J_o = 7.04 Hz), 8.15 (d, 2H, 3-C*H* and 5-C*H*, aromatic, J_o = 8.76 Hz) and 13.38 ppm (s, 1H, N−*H*). ^{13}C NMR (DMSO-d_6): δ 27.58 (N−**C**H$_3$), 29.01 (**C**H$_2$), 29.55 (N−**C**H$_3$), 52.52 (N−(**C**H$_2$)$_2$), 52.70 (N−(**C**H$_2$)$_2$), 54.21 (N−**C**H$_2$), 58.53 (N−**C**H$_2$−Ar), 65.83 (O−**C**H$_2$), 107.13 (Ar**C**), 114.45 (2× Ar**C**H), 121.12 (Ar**C**)) 126.59 (Ar**C**H), 127.96 (2× Ar**C**H), 128.14 (Ar**C**H), 128.99 (Ar**C**H), 130.49 (Ar**C**H), 133.33 (Ar**C**), 135.48 (Ar**C**),148.45 (Ar**C**), 149.98 (Ar**C**), 151.12 (Ar**C**), 153.98 (**C**═O) and 160.11 ppm (**C**═O). Anal. Calc. for $C_{27}H_{31}N_6O_3Cl$: C, 62.00; H, 5.97; N, 16.07. Found: C, 59.92; H, 5.88; N, 16.14%.

*5.1.4. 6-Amino-5-[{4-(3-chloropropoxy)carboxacetamido}phenyl]-1,3-dimethyluracil (**30**)*

A solution of 4-hydroxyphenylacetic acid (**26**) (1.0 g, 6.02 mmol) in methanol (30 ml) was refluxed for 3 h in the presence of a

catalytic amount of sulfuric acid, the reaction being monitored by thin layer chromatography. On completion, the excess solvent was removed to afford an oily product of methyl-4-hydroxyphenyl acetate (**27**), which was used as such for further reaction.

The ester **27** so obtained was heated under reflux in ethyl methyl ketone (10 ml) with 1-bromo-3-chloropropane (1.0 ml) in the presence of anhydrous potassium carbonate (1.0 g). The reaction mixture was further refluxed with stirring for 8 h and the completion of the reaction was monitored by thin layer chromatography. After cooling, the reaction mixture was filtered off and the excess solvent was removed to dryness to give methyl-[4-(3-chloropropoxy)phenyl]acetate [22] (**28**), which was further treated with 1.0 N sodium hydroxide. The mixture was stirred at room temperature for 8 h for completion of the reaction. The reaction mixture was further acidified and on continuous stirring some semisolid product separated out. The precipitate so obtained was filtered, washed with water and dried to obtain 4-(3-chloropropoxy)phenylacetic acid [22] (**29**, 0.35 g) mp 83–87 °C.

The compound **29** (1.0 g, 4.38 mmol) was stirred with 5,6-diaminouracil (**2**) (1.0 g, 5.87 mmol) in methanol (40 ml) in presence of 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide (EDCI) [23] (1.0 g, 5.21 mmol) at room temperature. After 10 min the reaction mixture turned turbid and was further stirred for 24 h. The completion of reaction was monitored by thin layer chromatography. The precipitate obtained was collected by filtration, washed with methanol and dried to afford the desired product **30**.

Yield: 135.7%; m.p. 218–220 °C. FT-IR: 3321, 3200, 2955, 1705, 1644, 1599, 1508, 1304, 1238, 1043, 930 and 756 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 2.23 (p, 2H, $-OCH_2CH_2CH_2Cl$, $J = 6.04$ Hz), 3.27 (s, 3H, N–CH_3), 3.41 (s, 3H, N–CH_3), 3.67 (s, 2H, $-CH_2-$), 3.75 (t, 2H, $-CH_2Cl$, $J = 6.30$ Hz), 4.10 (t, 4H, $-OCH_2-$, $J = 5.8$ Hz), 5.87 (s, 2H, $-NH_2$), 6.88 (d, 2H, 2-C*H* and 6-C*H*, aromatic, $J_o = 8.52$ Hz), 7.31 (d, 2H, 3-C*H* and 5-C*H*, aromatic, $J_o = 8.48$ Hz) and 7.76 ppm (s, 1H, N–*H*). Anal. Calcd. for $C_{17}H_{21}N_4O_3Cl$: C, 55.66; H, 6.32; N, 15.27%. Found: C, 55.80; H, 6.27; N, 15.17%.

5.1.5. 8-[4-(3-Chloropropoxy)benzyl]-1,3-dimethylxanthine (**31**)

The compound **30** (1.0 g, 2.73 mmol) was refluxed in a mixture of sodium hydroxide:dioxane (1:1) for 3 h to affect cyclization. The mixture was cooled and acidified with dil. HCl (4 N). The precipitate obtained was collected by filtration, dried and crystallized from a mixture of chloroform and methanol to afford the desired product **31.** Yield: 69.2%; m.p. 228–230 °C. FT-IR: 3142, 3034, 1705, 1655, 1504, 1522, 1400, 1244, 938 and 750 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.22 (p, 2H, $-OCH_2CH_2CH_2Cl$, $J = 6.04$ Hz), 3.42 (s, 3H, N–CH_3), 3.61 (s, 3H, N–CH_3), 3.73 (t, 2H, $-CH_2Cl$, $J = 6.32$ Hz), 4.08–4.11 (m, 4H, $-OCH_2-$ and $-CH_2-$), 6.86 (d, 2H, 2-C*H* and 6-C*H*, aromatic, $J_o = 8.68$ Hz), 7.24 (d, 2H, 3-C*H* and 5-C*H*, aromatic, $J_o = 8.68$ Hz) and 11.94 ppm (s, 1H, N–*H*). ^{13}C NMR (DMSO-d_6): δ 27.62 (N–**C**H_3), 29.71 (N–**C**H_3), 31.60 (**C**H_2), 33.46 (**C**H_2), 41.96 (**C**H_2Cl), 64.13 (O-**C**H_2), 106.15 (Ar**C**), 114.50 (2× Ar**C**H), 129.04 (Ar**C**), 129.66 (2× Ar**C**H), 148.14 (Ar**C**), 151.11 (Ar**C**), 152.82 (Ar**C**), 154.03 (**C**=O) and 157.14 ppm (**C**=O). Anal. Calcd. for $C_{17}H_{19}N_4O_3Cl$: C, 56.28; H, 5.28; N, 15.44%. Found: C, 56.18; H, 5.37; N, 15.55%.

5.1.6. General procedure for the synthesis of various amine fused 8-(substituted-benzyl)xanthine derivatives **32–34**

A mixture of compound **31** (1.0 g, 2.76 mmol) and the desired amine (in excess) was heated for 1 h at 80–100 °C. The completion of reaction was monitored with thin layer chromatography. The excess amine was removed by washings with dry ether to obtain a solid product, which was collected by filtration, dried and crystallized from methanol to afford the corresponding products **32–34.**

5.1.6.1. 1,3-Dimethyl-8-[4-(3-morpholinopropoxy)benzyl]xanthine (**32**).

Yield: 44.1%; m.p. 198–200 °C. FT-IR: 3146, 3095, 3037, 2940, 2870, 2833, 1703, 1659, 1561, 1400, 1245, 1059, 984 and 798 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.95 (p, 2H, $-OCH_2CH_2CH_2-$, $J = 7.18$ Hz), 2.46 (s(br), 4H, N–(CH_2)$_2$, morpholine), 2.50 (t, 2H, $-OCH_2CH_2CH_2$-N, $J = 7.34$ Hz), 3.43 (s, 3H, N–CH_3), 3.62 (s, 3H, N–CH_3), 3.71 (t, 4H, O-(CH_2)$_2$, morpholine $J = 4.64$ Hz), 3.99 (t, 2H, $-OCH_2-$, $J = 6.32$ Hz), 4.13 (s, 2H, $-CH_2-$), 6.84 (d, 2H, 2-C*H* and 6-C*H*, aromatic, $J_o = 6.62$ Hz), 7.24 (d, 2H, aromatic, 3-C*H* and 5-C*H*, $J_o = 8.64$ Hz) and 11.84 ppm (s, 1H, N–*H*). ^{13}C NMR (DMSO-d_6): δ 26.26 (N–**C**H_3), 28.36 (N–**C**H_3), 30.28 (**C**H_2), 34.63 (**C**H_2), 53.67 (N–(**C**H_2)$_2$), 55.52 (N–**C**H_2), 66.07 (O-**C**H_2), 66.83 (O-(**C**H_2)$_2$), 106.79 (Ar**C**), 114.91 (2× Ar**C**H), 127.74 (Ar**C**), 129.85 (2xAr**C**H), 149.39 (Ar**C**), 151.54 (Ar**C**), 154.29 (Ar**C**), 155.63 (**C**=O) and 158.24 ppm (**C**=O). Anal. Calcd. for $C_{21}H_{27}N_5O_4$: C, 61.0; H, 6.58; N, 16.94%. Found: C, 61.08; H, 6.60; N, 17.0%.

5.1.6.2. 1,3-Dimethyl-8-[4-{3-(1H-imidazol-1-yl)propoxy}benzyl]xanthine (**33**).

Yield: 87.4%; m.p. 210–212 °C. FT-IR: 3382, 3143, 3100, 3035, 2987, 2941, 1706, 1662, 1560, 1401, 1241, 1060, 984 and 760 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 2.21 (p, 2H, $-OCH_2CH_2CH_2-$, $J = 8.12$ Hz), 3.38 (s, 3H, N–CH_3), 3.58 (s, 3H, N–CH_3), 3.86 (t, 2H, $-OCH_2CH_2CH_2-$, $J = 5.70$ Hz), 4.04 (s, 2H, $-CH_2-$), 4.19 (t, 2H, $-OCH_2-$, $J = 6.70$ Hz), 6.81 (d, 2H, 2-C*H* and 6-C*H*, aromatic, $J_o = 8.64$ Hz), 6.93 (s, 1H, C*H*, imidazole), 7.04 (s, 1H, C*H*, imidazole), 7.24 (d, 2H, 3-C*H* and 5-C*H*, aromatic, $J_o = 8.64$ Hz), 7.45 (s, 1H, C*H*, imidazole) and 12.99 ppm (s, 1H, N–*H*). ^{13}C NMR (DMSO-d_6): δ 27.61 (N–**C**H_3), 29.71 (N–**C**H_3), 30.13 (**C**H_2), 33.46 (**C**H_2), 42.87 (N–**C**H_2), 64.35 (O-**C**H_2), 106.20 (Ar**C**), 114.50 (2xAr**C**H), 119.32 (Ar**C**H, imid), 128.37 (Ar**C**H, imid), 129.05 (Ar**C**), 129.64 (2× Ar**C**H), 137.29 (Ar**C**H, imid), 148.10 (Ar**C**), 151.12 (Ar**C**), 152.78 (Ar**C**), 154.03 (**C**=O) and 157.16 ppm (**C**=O). Anal. Calcd. for $C_{20}H_{22}N_6O_3$: C, 60.90; H, 5.62; N, 21.31%. Found: C, 60.85; H, 5.72; N, 21.20%.

5.1.6.3. 1,3-Dimethyl-8-[4-{3-(1,4-dioxa-8-azaspiro[4.5]decan-1-yl)-propoxy}benzyl]-xanthine (**34**).

Yield: 38.8%; m.p. 194–196 °C. FT-IR: 3147, 3096, 3037, 2955, 2820, 1706, 1661, 1561, 1403, 1244 and 797 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.75 (t, 4H, 2× $-CH_2-$, $J = 5.70$ Hz), 1.95 (p, 2H, $-OCH_2CH_2CH_2-$, $J = 7.18$ Hz), 2.52–2.55 (m, 6H, $-OCH_2CH_2CH_2$-N and N(CH_2)$_2$), 3.41 (s, 3H, N–CH_3), 3.62 (s, 3H, N–CH_3), 3.95 (s, 4H, 2× $-OCH_2-$), 4.0 (t, 2H, $-OCH_2-$, $J = 6.30$ Hz), 4.12 (s, 2H, $-CH_2-$), 6.86 (d, 2H, 2-C*H* and 6-C*H*, aromatic, $J_o = 8.647$ Hz), 7.21 (d, 2H, 3-C*H* and 5-C*H*, aromatic, $J_o = 8.60$ Hz) and 11.84 ppm (s, 1H, N–*H*). ^{13}C NMR (DMSO-d_6): δ 26.48 (**C**H_2), 27.63 (N–**C**H_3), 29.72 (N–**C**H_3), 33.46 (**C**H_2), 34.41 (2× **C**H_2), 50.89 (N–(**C**H_2)$_2$), 53.98 (N–**C**H_2), 63.50 (O-(**C**H_2)$_2$), 65.75 (O-**C**H_2), 106.15 (Ar**C**), 106.41 (**C**), 114.46 (2× Ar**C**H), 128.73 (Ar**C**), 129.61 (2× Ar**C**H), 148.14 (Ar**C**), 151.12 (Ar**C**), 152.87 (Ar**C**), 154.04 (**C**=O) and 157.40 ppm (**C**=O). Anal. Calcd. for $C_{24}H_{31}N_5O_5$: C, 61.39; H, 6.65; N, 14.91%. Found: C, 61.29; H, 6.49; N, 15.02%.

5.2. Biological activity

5.2.1. Adenosine binding assays

The adenosine binding assays were performed as reported earlier [19,20].

5.2.1.1. Binding studies for A_1 and A_{2A} receptors

5.2.1.1.1. Materials. [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) was from PerkinElmer, Rodgau, Germany; [^{3}H]NECA (5′-*N*-ethylcarboxamidoadenosine) was obtained from Amersham, Braunschweig, Germany; [^{3}H]HEMADO (2-(hexyn-1-yl)-6-methyladenosine) was from Tocris, Bristol, UK and [α-^{32}P]ATP was from Hartmann Analytik, Braunschweig, Germany. 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.

5.2.1.1.2. 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.

5.2.1.1.3. 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.

5.2.1.1.4. 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) [19,20]. 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. 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 of the microplates and washed three times with 200 ml of ice-cold binding buffer. After addition of 20 ml of scintillator to the dried filter plates samples were counted in a Wallac Micro-Beta counter. Nonspecific binding was determined in the presence of 1 mM theophylline (A_1) or 100 mM R-PIA (N^6-phenylisopropyladenosine) (A_{2A}, A_3). All binding data were calculated by non-linear curve fitting with the program SCTFIT.

5.2.1.1.5. 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 [19,20]. Concentration-dependent inhibition of NECA-stimulated adenylyl cyclase caused by antagonists was measured in membranes from CHO cells stably transfected with the human A_{2B} adenosine receptor. Membranes were incubated with about 150,000 cpm of [α-^{32}P]ATP for 20 min as previously described [19]. From the measured IC_{50}-values K_i-values were then calculated with the Cheng and Prusoff equation [19].

5.2.2. Bronchospasmolytic activity

The newly synthesized xanthine derivatives **17**–**25** and **31**–**34** were evaluated for bronchoprotective effects against histamine aerosol induced bronchospasm in guinea pigs according to the method of Zabeer et al. [21].

5.2.2.1. Animals. Male guinea-pigs (Dunkin Hartley) of 250 ± 30 g, bred in the disease free small animal house of Chaudhary Charan Singh Haryana Agriculture University (Hisar, Haryana) were obtained. The animals were housed under standard laboratory conditions, maintained on a 12 h light and dark cycle and had free access to food (carrots, cucumbers, leafy vegetables etc) and water. The experimental protocols were approved by the Institutional Animal Ethics Committee of the Panjab University, Chandigarh (CAH/1476 dated 14.09.2009), and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

5.2.2.2. Drugs. Histamine hydrochloride (Himedia, India), theophylline, carboxymethyl cellulose (CMC) and test compounds.

5.2.2.3. Experimental protocol. Three groups of animals (4 in each) were made and designated as I, II and III for control (CMC + distilled water), standard (CMC + theophylline + distilled water) and test drug (CMC + test drug + distilled water), respectively. The grouped animals were kept for overnight fasting and were pretreated with the test drug (50 mg/kg), theophylline (50 mg/kg), and CMC (control) per oral application 1 h before exposure to aerosol. Each group of the animals was kept in the histamine chamber (M/s Inco, Ambala) separately and exposed to histamine aerosol. Five ml of 1% solution of histamine was aerosoled in 1 min to each animal of each group. The onset of bronchospasm, duration of jerks, severity of bronchospasm and death or survival of the animals was recorded for each group. The animals remained in the chamber for 8 min after which they were removed to fresh air and fed with proper water and food.

Acknowledgments

The financial support provided by the Indian Council of Medical Research, New Delhi, India is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.045.

References

[1] R. Bansal, R. Yadav, G. Kumar, ASTHMA – Molecular Basis and Treatment Approaches, Lambert Academic Publishing, Germany, 2011.
[2] G. Vass, I. Horvath, Curr. Med. Chem. 15 (2008) 917–922.
[3] K.A. Jacobson, Z.G. Gao, Nat. Rev. Drug Discovery 5 (2006) 247–264.
[4] K. Rao, J. Zablocki, Annu. Rep. Med. Chem. 44 (2009) 265–277.
[5] Y.C. Kim, X. Ji, N. Melman, J. Linden, K.A. Jacobson, J. Med. Chem. 43 (2000) 1165–1172.
[6] E. Elzein, R.V. Kalla, X. Li, T. Perry, A. Gimbel, D. Zeng, D. Lustig, K. Leung, J. Zablocki, J. Med. Chem. 51 (2008) 2267–2278.
[7] R.V. Kalla, E. Elzein, T. Perry, X. Li, V. Palle, V. Varkhedkar, A. Gimbel, T. Maa, D. Zeng, J. Zablocki, J. Med. Chem. 15 (2006) 3682–3692.
[8] Q. Li, K. Ye, C.C. Blad, H. den Dulk, J. Brouwer, A.P. Ijzerman, M.W. Beukers, J. Pharmacol. Exp. Ther. 2 (2007) 637–645.
[9] J. Zablocki, R. Kalla, T. Perry, V. Palle, V. Varkhedkar, D. Xiao, A. Piscopio, T. Maa, A. Gimbel, J. Hao, N. Chu, K. Leung, D. Zeng, Bioorg. Med. Chem. Lett. 15 (2005) 609–612.
[10] R. Bansal, G. Kumar, D. Gandhi, L.C. Young, A.L. Harvey, Eur. J. Med. Chem. 44 (2009) 2122–2127.
[11] K.N. Klotz, Naunyn Schmiedeberg's. Arch. Pharmacol. 362 (2000) 382–391.
[12] T. Borrmann, S. Hinz, D.C. Bertarelli, W. Li, N.C. Florin, A.B. Scheiff, C.E. Müller, J. Med. Chem. 52 (2009) 3994–4006.
[13] A. Carotti, M.I. Cadavid, N.B. Centeno, C. Esteve, M.I. Loza, A. Martinez, R. Nieto, E. Ravina, F. Sanz, V. Segarra, E. Sotelo, A. Stefanachi, B. Vidal, J. Med. Chem. 49 (2006) 282–299.
[14] R. Bansal, G. Kumar, D. Gandhi, R. Yadav, L.C. Young, A.L. Harvey, Arzneim. Forsch. 60 (3) (2010) 131–136.
[15] R. Bansal, G. Kumar, D. Gandhi, L.C. Young, A.L. Harvey, Chem. Biodiversity 8 (2011) 1290–1300.

[16] R. Bansal, G. Narang, C. Zimmer, R.W. Hartmann, Med. Chem. Res. 20 (2011) 661–669.
[17] V. Papesch, E.F. Schroeder, J. Org. Chem. 16 (1951) 1879–1890.
[18] F.F. Blicke, H.C. Godt, J. Am. Chem. Soc. 76 (1954) 2798–2800.
[19] K.-N. Klotz, J. Hessling, J. Hegler, J.C. Owman, B. Kull, B.B. Fredholm, M.J. Lohse, Naunyn Schmiedeberg's. Arch. Pharmacol. 357 (1998) 1–9.
[20] K.-N. Klotz, N. Falgner, S. Kachler, C. Lambertucci, S. Vittori, R. Volpini, G. Cristalli, Eur. J. Pharmacol. 556 (2007) 14–18.
[21] A. Zabeer, A. Bhagat, O.P. Gupta, G.D. Singh, M.S. Youssouf, K.L. Dhar, O.P. Suri, K.A. Suri, N.K. Satti, B.D. Gupta, G.N. Qazi, Eur. J. Med. Chem. 41 (2006) 429–434.
[22] G. Adembri, C. Anselmi, A. Camparini, M.G. Rosseti, M. Scotton, J. Heterocycl. Chem. 25 (1988) 1019–1022.
[23] G. Strappaghetti, S. Corsano, R. Barbaro, G. Giannaccini, L. Betti, Bioorg. Med. Chem. Lett. 9 (2001) 575–583.