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Potent bronchospasmolytic *in vivo* activity of three new hybrids of xanthines

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ABSTRACT

We present in this article syntheses of three new hybrids compounds (7, 10, 13) that were efficiently prepared in two or three steps from our previous prototype 5,6-diaminouracil with the NSAIAs (Diclofenac acid and Indomethacin) and 5-nitro-2-furaldehyde. The histamine chamber-aerosol induced method (5 ml of 1% w/v aerosoled in 1 min) is able to determine that all investigated new hybrids showed stronger bronchospasmolytic properties than their precursors.

Keywords: Xanthines, Bronchospasmolytic, Hybrid, Histamine.

INTRODUCTION

Due to continuous increase in the prevalence and morbidity of asthma, there is an urgent need to improve present therapy with new modes of actions. Asthma is characterized by hyper-responsiveness of tracheobronchial smooth muscle to a variety of stimuli, resulting in narrowing of air tubes, often accompanied by increased secretion, mucosal edema and mucus plugging. [1-2]

Histamine plays an important role in bronchoconstriction mediated by histamine receptors which provoke bronchial asthma attack. Drugs used in the treatment of asthma are sympathomimetics, β_2-adrenoceptor agonists, methylxanthines, anti-cholinergic, mast cell stabilizers and corticosteroids. [3]

Xanthine is a purine base which has been found in the human tissues and fluids, assumed to act through a number of mechanisms such as non-selective inhibition of phosphodiesterase, adenosine receptor antagonists (A_1, A_{2A}, A_{2B}-receptors), by inhibiting nuclear factor, through inhibition of phosphoinositide-3 kinase, increasing secretion of interleukin-10, apoptosis of inflammatory cells, and histone deacetylase activity. [4-7]

Xanthines are frequently used for the management of asthma and chronic obstructive pulmonary disease. The first marketed xanthine was theophylline which was found to be adenosine receptor antagonist. The expert panel reports of the National Asthma Education and Prevention Program in US and reports from different countries have proved xanthine based compounds as beneficial for the management of asthma. [8-10]

One of the significant developments in the medicinal chemistry over the last few years is the design and synthesis of new hybrid compounds. Pharmacophores from well established known entities encompassed in a single scaffold are nowadays effective to treat multifactorial diseases.
8-substituted theophylline is the parent member of a variety of antiasthmatic agents [11-13]. It has been established from the literature that appropriate substituents on the phenyl ring at C_8 affects the potency and selectivity towards their pharmacological effects.

Based on these studies, the investigators thought worthwhile to synthesize such hybrid prototypes which would be effective against asthma. In line of this antiinflammatory drug such as diclofenac acid and indomethacin,

heterocyclic moiety such as 5-nitro-furfuraldehyde was selected for substitution at 8th-position of the xanthine scaffold.

MATERIALS AND METHODS

Chemistry

The chemicals were purchased from Merck India, Central drug house (CDH), Sigma-Aldrich, MP Biomedical etc. and were of LR grade. Melting points were determined using digital melting point apparatus (Veego) and are uncorrected. FT-IR spectra were recorded on Agilent Technology carx 600 series (υ max in cm^{-1}) spectrophotometer by KBr pellet method. ^{1}H- and ^{13}C-nuclear magnetic resonance spectra were obtained using Bruker 400 MHz instrument using deuterated solvents. Chemical shifts are reported as δ parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis was performed using CHNS/O Analyser of Perkin Elmer series II-2400. All the solvents used were freshly distilled and dried prior to use. Anhydrous sodium sulfate was used as a drying agent. The purity of the compounds was confirmed by thin layer chromatography (TLC silica gel F_{254}) by Merck, Germany. The spot were visualized under ultraviolet lamp or in iodine chamber.

6-Amino-1, 3-dimethyl-5-nitrosouracil (3)

A mixture of *N,N'*-dimethylurea (**1**) (1.0 g, 11.35 mmol), cyanoacetic acid (1.0 g, 11.75 mmol) and acetic anhydride (2 ml) was heated with exclusion of moisture, at 70-80 °C for 3h. The excess anhydride and the acetic acid formed during the reaction was removed under reduced pressure. Sodium hydroxide solution (5%, 6 ml) was added slowly to the cooled, stirred residue where upon the 6-amino-1,3-dimethyluracil (**2**) precipitated. A solution of sodium nitrite (1.0 g, 14.49 mmol in 8 ml H_2O) was added to the cooled, stirred mixture and it was acidified by the drop wise addition of acetic acid (2 ml) over a period of 1 h. The stirring was continued for an additional 2 h at room temperature. The mixture was thoroughly cooled, the red-violet precipitate was filtered, washed with water, 95% ethanol and finally with ether to afford (**3**) (85.09 %, mp 232-235° C, Lit[21] 233°C).[14]

5, 6-Diamino-1, 3-dimethyluracil (4)

The crude 5-nitrosouracil (**3**) (1.0 g, 5.43 mmol) was dissolved in concentrated ammonium hydroxide (8 ml) and sodium dithionite (2.74 g, 15.73 mmol) was added slowly with stirring and warming. The salt was dissolved and the solution underwent a series of color changes which was further stirred for 2 h. The resulting precipitate was cooled on ice bath, filtered and dried to obtain (**4**) (72.85%, mp: 200 °C, Lit[21] 209°C). The product so obtained was suitable for use without further purification. [15]

1, 3-Dimethyl-8-{3-[(2, 6-dichlorophenyl)amino]benzyl}-xanthine (7)

Diclofenac (**5**) (1.0 g) was coupled with 5, 6-diaminouracil (**4**) (1.0 g, 5.87 mmol) in the presence of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1.0 g, 5.21 mmol) and methanol (40 ml). The reaction mixture becomes turbid after 10 min and was stirred for 24 h for completion. The precipitated product obtained was collected by filtration, washed with methanol and dried to afford (**6**). (62.59%, mp: 100-102 °C)

The amide coupled product **6** (1.0 g) thus obtained was refluxed in thionyl chloride (20 ml) for 30-40 min to afford cyclization. The excess thionyl chloride was removed under vacuum to obtain the solid product. Ice cold water was added to it and resulted suspension was neutralized by ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried to obtain desired product **7**. (57.04%, m.p: 168-170 °C), FT-IRυ_{max} (KBr): 3433 (NH), 2921 (CH_2), 1670 (C=O), 1564 (C=C), 791 (C-Cl) cm^{-1}, ^{1}H-NMR ($CDCl_3$-d_6): 1.62 (s, 2H, -CH_2), 0.83-1.45 (s, 6H, -CH_3), 2.96 (s, 2H, -NH), 6.32-6.90 (m, 7H, arom.), ^{13}C-NMR ($CDCl_3$-d_6): 129.01 (Ar-C), 29.99-29.81 (C-Cl), CH_3 (29.04). Analysis calculated for $C_{20}H_{17}Cl_2N_5O_2$: C, 55.04; H, 3.89; N, 16.05. Found: C, 55.12; H, 3.78; N, 16.11

1, 3-Dimethyl-8-{2-[1-(4-chlorophenyl)carbonyl)]-5-methoxy-2-methyl-1H-indol-3-yl}-xanthine (10)

Indomethacin (**8**) (1.0 g) was coupled with 5, 6-diaminouracil (**4**) (1.0 g, 5.87 mmol) in the presence of EDCI (1.0 g, 5.21 mmol) and methanol (40 ml). The reaction mixture becomes turbid after 10 min and was stirred for 24 h for completion. The precipitate obtained was collected by filtration, washed with methanol and dried to afford (**9**) (61.09%, m.p: 212-214 °C).The amide coupled product (**9**) (1.0 g) thus obtained was refluxed in thionyl chloride (20 ml) for 30-40 min to afford cyclization. The excess thionyl chloride was removed under reduced pressure to obtain a solid product. Ice cold water was added to it and resulted suspension was neutralized by ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried to obtain desired product (**10**) (64.01%, m.p: 255-258 °C), FT-IRυ_{max} (KBr): 3431 (NH), 2922(CH_2), 1673 (C=O), 1561 (C=C), 748 (C-Cl) cm^{-1}, ^{1}H-NMR($CDCl_3$-d_6): 1.11-1.63 (s, 6H, -CH_3), 1.65 (s, 2H, -CH_2), 2.04 (s, 1H, -NH), 3.1 (s, 3H, -CH_3), 3.58 (s, 3H, -OCH_3), 6.22-6.80 (m, 3H, arom.), 7.39 (d, J=8.1 Hz, 2H, arom.), 7.93 ppm (d, J=8.4 Hz, 2H, arom.) ^{13}C-NMR ($CDCl_3$-d_6): 150.28 (CH), 129.21 (CH), 130.88 (CH), 128.34 (CH). Analysis calculated for $C_{25}H_{22}ClN_5O_4$: C, 61.03; H, 4.47; N, 14.24 Found: C, 61.08; H, 4.48; N, 14.82

1, 3-Dimethyl-8-[(5-nitrofuran-2yl) methyl]-xanthine (13)

To the stirred solution of 5, 6-diamino-1, 3-dimethyluracil (**4**) (1.0 g, 5.87 mmol) in methanol-acetic acid (MeOH-AcOH) (4:1, 40 ml) , a solution of 5-nitro-2-furaldehyde (**11**) was added. After stirring at room temperature for 1 h the precipitation occurs. The reaction mixture was future stirred overnight at room temperature and the completion of the reaction was monitored by TLC. The precipitate obtained was filtered off, washed with methanol and dried to afford (**12**). (85.71%, m.p: 185-188 °C). The Schiff base (**12**) (1.0 g) thus obtained was refluxed in thionyl chloride (20 ml) for 30-40 min to afford cyclization. The excess thionyl chloride was removed under reduced pressure to obtain a solid product. Ice cold water was added to it and resulted suspension was neutralized by ammonium hydroxide solution. The precipitate so obtained was collected by filtration, dried to obtain desired product (**13**). (48.19%, m.p: 248°C), FT-IRυ_{max} (KBr): 3471(NH), 1658 (C=O), 1347 (NO_2), 2920 (CH), 1524 (C=C), 745 (CH) cm^{-1}, ^{1}H-NMR ($CDCl_3$-d_6): 1.3-1.6 (s, 6H, N-CH_3), 2.1 (s, 1H,-NH), 3.5 (d, 1H, -CH, J = 7.5 Hz) and 3.6 ppm (d, 1H, -CH, J = 7.5 Hz)), ^{13}C-NMR ($CDCl_3$-d_6): 151-150.10 (CN), 145.30 (CH), 109.18 (CH), 29.99-29.81 (CH_3), 27.65 (CH_3), 157.54 (CH), 155.08 (CO), 118.47 (Ar-C). Analysis calculated for $C_{11}H_9N_5O_5$: C, 45.36; H, 3.09; N, 24.05. Found: C, 45.34; H, 3.10; N, 24.10

Biological Activity

The newly synthesized xanthine derivatives **7**, **10** and **13** were evaluated for *in vivo* bronchospasmolytic activity against histamine aerosol induced bronchospasm in guinea pig according to the method of Zabeer et al. [16].

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 Banasthali University, Banasthali and conducted according to the guidelines for the use and care of experimental animals.

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

Experimental protocol: Three groups of guinea pig was 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 overnight fasting and were pretreated with 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 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 animal was recorded for each group. The animal remained in the chamber for 8 min after which they were removed in fresh air and fed with proper water and food.

RESULTS AND DISCUSSION

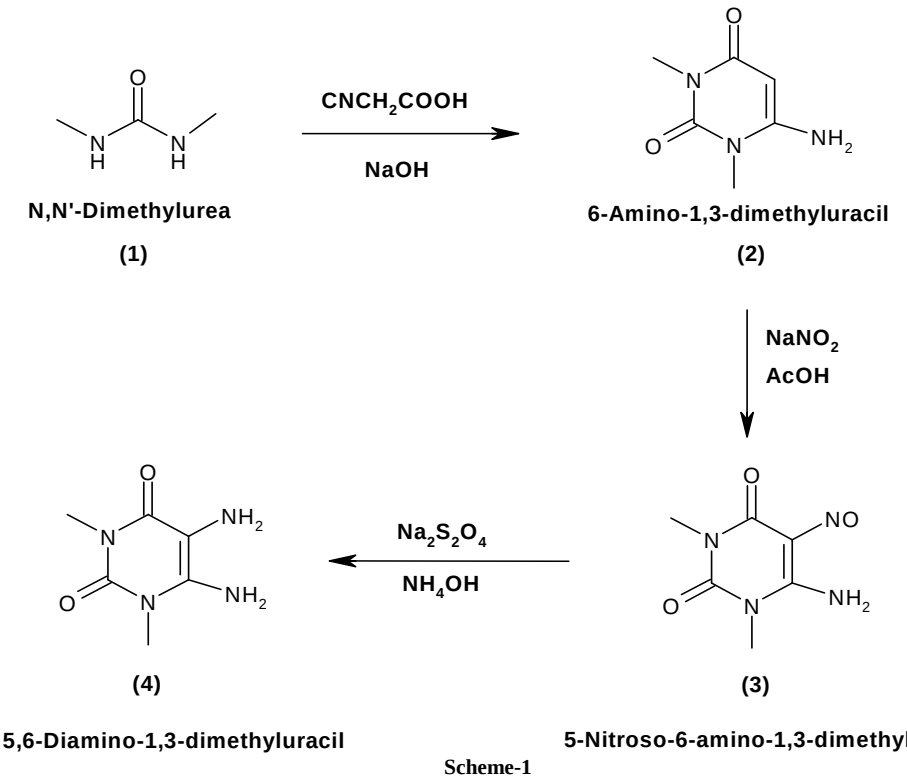
Chemical synthesis

Synthesis of 5,6-diamino-1,3-dimethyluracil (**4**), a key intermediate to the synthesis of all the 8-substituted derivatives, was performed according to the general method summarized in *scheme 1*. [14,15]

1,3-Dimethyl-5-nitrosouracil (**3**) was prepared by condensing *N,N'*-dimethylurea and cyanoacetic acid in the presence of acetic anhydride to obtain 6-aminouracil and subsequent nitrosation with sodium nitrite. Reduction of nitrosouracil **3** with sodium dithionite in concentrated ammonium hydroxide afforded quite an unstable diaminouracil **4**.[20]

Various heterocyclic compounds were condensed with diaminouracil to afford corresponding carboxamide derivatives/Schiff bases. Subsequent cyclization using thionyl chloride yielded the desired 8-substituted-1,3-dimethylxanthines in accordance with the earlier literature reports.[21]

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



Scheme-1

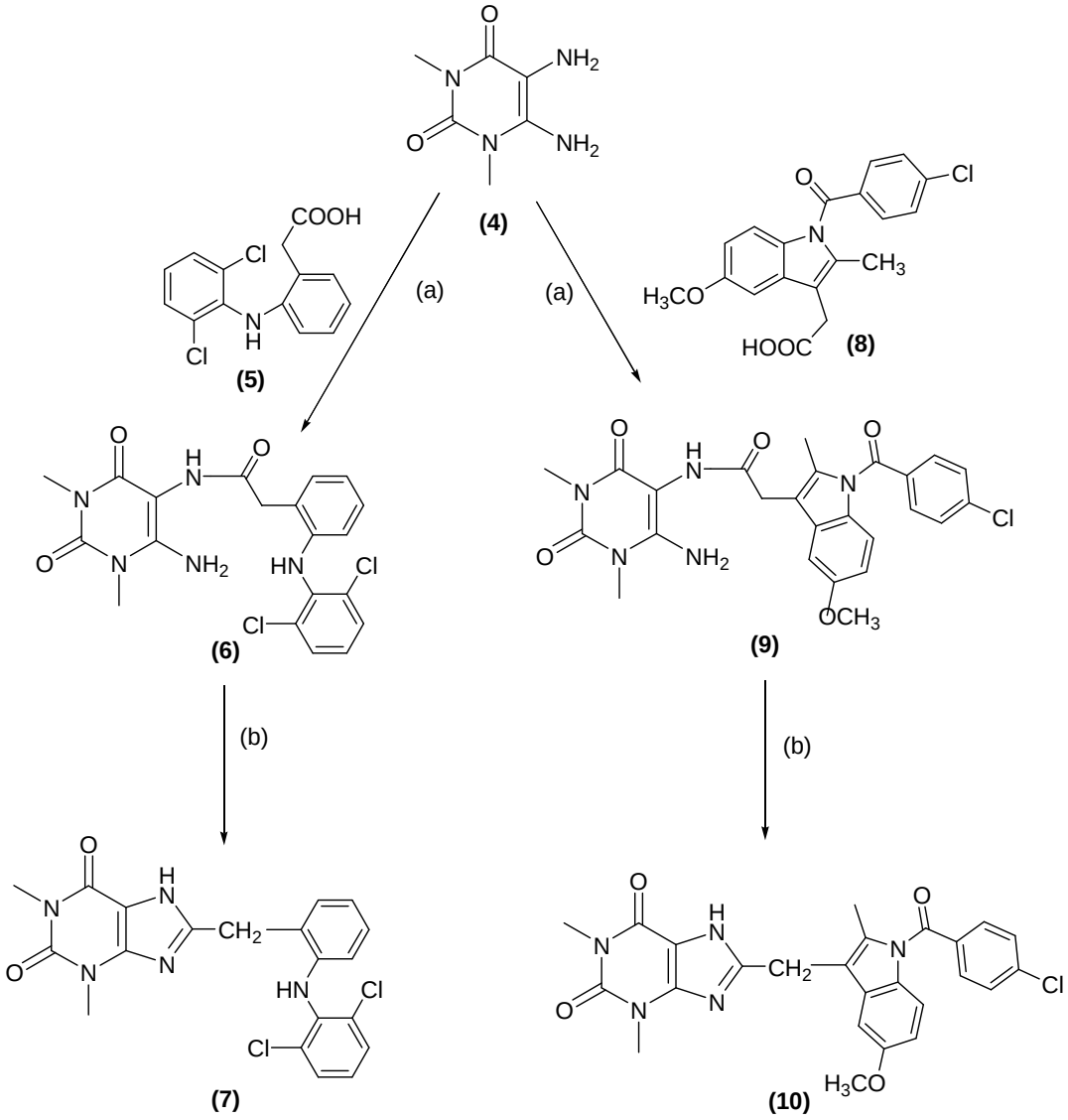
Cyclization of **6** was affected on refluxing with thionyl chloride to obtain the desired compound **7** with a methylene spacer between C-8 of xanthine and phenyl ring. The disappearance of absorption band of free -N*H*$_2$ in the IR spectrum at 3321 cm^{-1} supported the formation of 1,3-dimethyl-8-{3-[(2,6-dichlorophenyl) amino]benzyl}-xanthine (**7**). The protons of the methylene spacer resonated as a multiplet together with the four protons of -OC*H*$_2$- group at δ 4.1 ppm. Elemental analyses further supported the structure of compound.

Similarly, indomethacin (**8**) was condensed with 5,6-diamino-1,3-dimethyluracil (**4**) in the presence of 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDCI) in methanol at room temperature to **9** as shown in *scheme 2*. ^{1}H-NMR spectrum of carboxamide derivative **9** displayed a characteristically downfield singlet integrating for two protons of NH-CO-C*H*$_2$-Ar group at δ 3.67 ppm. Two protons of the free –N*H*$_2$ group resonated as a singlet at 5.87 ppm in the NMR spectrum. Symmetric and asymmetric stretching bands of free -NH$_2$ group appeared at 3321 and 3200 cm^{-1} in the IR spectrum of the compound **9**.

Cyclization of **9** was affected on refluxing with thionyl chloride to obtain the desired compound **10** with a methylene spacer between C-8 of xanthine and phenyl ring. The disappearance of absorption band of free -N*H*$_2$ in the IR spectrum at 3321 cm^{-1} supported the formation of 1, 3-dimethyl-8-{2-[1-(4-chlorophenyl) carbonyl)]-5-methoxy-2-methyl-1H-indol-3-yl}-xanthine (**10**). The protons of the methylene spacer resonated as a multiplet together with the four protons of -OC*H*$_2$- group at δ 4.1 ppm. Elemental analyses further supported the structure of compound.

Treatment of 5-nitro-furaldehyde **11** with 5,6-diamino-1,3-dimethyluracil (**4**) in MeOH-AcOH (4:1) at room temperature resulted in the formation of corresponding benzylidene adducts **12**, which was used as such for further cyclization.

Subsequent ring closure of this intermediate by refluxing in thionyl chloride for 30-40 min afforded the desired target compound **13**. The structure of this 8-phenyl derivative was confirmed using various spectral analyses.



Scheme 2: Reagents and conditions: (a) MeOH, EDCI, 24 hr., (b) $SOCl_2$, 1hr. Reflux

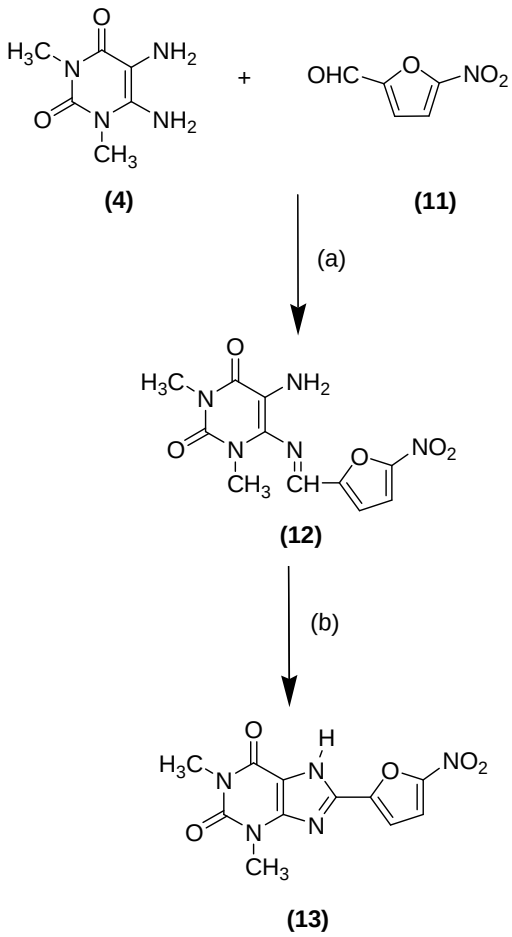
Biological evaluation

The newly synthesized xanthine derivatives were evaluated for their bronchospasmolytic effects against histamine aerosol induced bronchospasm in guinea pigs according to the method of *Zabeer et al*. [22] All the newly synthesized xanthine derivatives displayed noteworthy protection against histamine aerosol induced bronchospasm in guinea pigs. The compounds produced potent bronchospasmolytic activity in comparison to standard drug theophylline as given in Table 1.

Table 1: 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 (%)
Control	67±2	172±4	+++	0
Theophylline	92±3	89±7	+	100
7 (RY-1)	180±3	40±5	+	100
10 (RY-2)	150±5	*	+	100
13 (RY-3)	96±2	*	+	100

Number of animals in each group (N) = 4
Dose of standard and tested compounds = 50 mg/kg
**Animals do not show any jerks after treatment with test compounds*



Scheme 3: Reagents and conditions: (a) MeOH, CH_3COOH, RT stirring, 24hrs., (b) $SOCl_2$, Reflux

CONCLUSION

The newly synthesized 8-substituted xanthine derivatives were characterized and evaluated *in vivo* for their antiasthmatic activity in guinea pigs using histamine chamber method. The synthesized compounds exhibited highly potent bronchospasmolytic activity in comparison to the standard drug theophylline.

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