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BRONCHOSPASMOLYTIC ACTIVITY OF 8-PHENYL SUBSTITUTED (SULPHONAMIDE) XANTHINE DERIVATIVES

Divya Gumber and Rakesh Yadav*

Department of Pharmacy, Banasthali University, Banasthali-304022, Rajasthan, India.

***Corresponding Author: Dr. Rakesh Yadav**
Department of Pharmacy, Banasthali University, Banasthali-304022, Rajasthan, India.

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ABSTRACT
Background: A series of suplhonamide substituted xanthine derivatives were synthesized. The introduction of the heterocyclic spacers in the xanthine skeleton at C-8 improves therapeutic profile. The incorporation of polar substituents such as sulfonates was done in order to improve the limited water solubility of xanthines. Further incorporation of spacers like heteroatoms (nitrogen and sulphur) provided additional sites of interaction thus giving the desired selectivity with increased potency. **Findings:** The structures of the newly synthesized compounds were characterized by TLC, FT-IR, ^{1}H & ^{13}C-NMR and elemental analyses. The synthesized derivatives were also evaluated *in vivo* for bronchospasmolytic activity. Among the derivatives *8a-f*, the animals treated with compound **8f** showed more protective action in the histamine induced bronchospasm. Increased bulkiness on the *p*-position of the aromatic ring increases the bronchospasmolytic activity. **Conclusion:** The sulfonamide derivatives of 8-phenylsubstituted xanthines were synthesized and evaluated for their bronchospasmolytic activity.

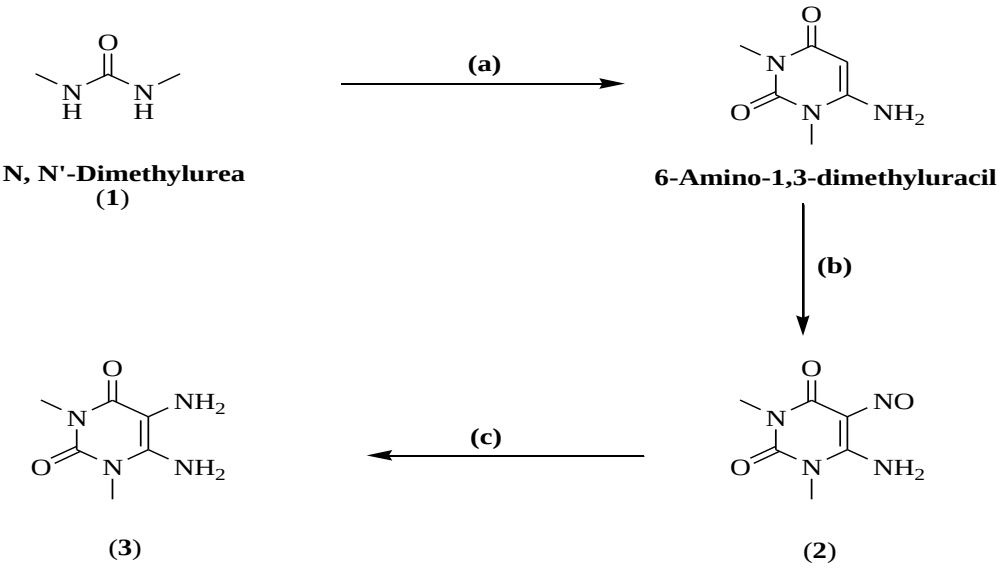
KEYWORDS: Xanthines, Sulphonamide, Bronchospasmolytic, Anti-asthmatics.

INTRODUCTION
Asthma is a common medical condition which affects approx. 300 million people worldwide and the number is expected to increase exponentially in the coming years.[1] It is a chronic obstructive pulmonary disease which affects the bronchi airways and results in the inflammation of bronchioles along with the hyper-responsiveness to some direct or indirect stimuli which leads to bronchoconstriction.[2] The implementation of the treatment given in various guidelines is found to be effective in both children and adults. But the complete remission of the disease by pharmacotherapy is very difficult as on date.[3]

Xanthine derivatives are found as most effective class of antiasthmatics till date. 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.[4-6] 8-Phenyltheophylline is the parent member of a variety of potent adenosine receptor antagonists.[6,7] 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.[8] The incorporation of polar substituents such as sulfonates improves the otherwise extremely limited water solubility of 8-phenylxanthines and consequently increases their effectiveness as potential therapeutic agents but the sulfonate group generally led to a decrease in adenosine receptor affinity so sulfonamide derivatives of 1,3-dialkylxanthines were synthesized by various researchers which were soluble across wide pH range and also can be absorbed perorally because of their amphoteric nature.[9]

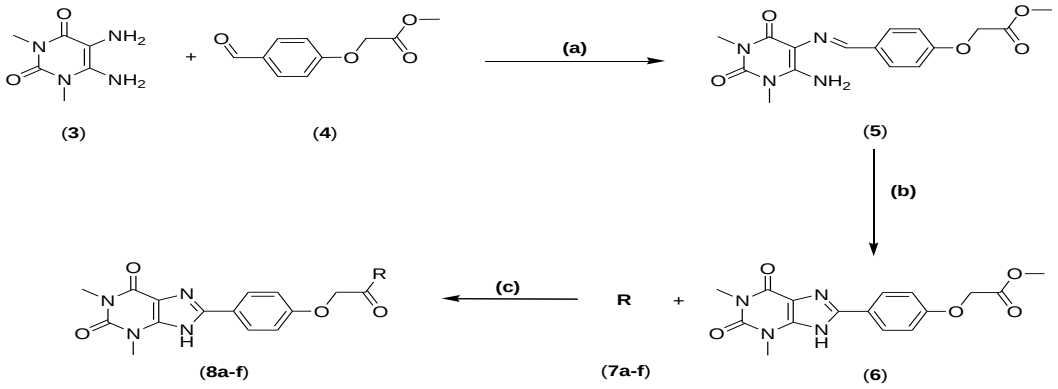
In the light of these observations, we decided to study the impact of polar sulphaminophenoxyacetate substituents at *para* position of the 8-phenyl substituted xanthines on the bronchospasmolytic activity. In order to examine specific structural features, it was also appealing to introduce certain heteroatoms in sulphamino ring. Keeping all the observations under consideration, we had synthesized a series of sulfonamide derivatives of 1, 3-Dialkylxanthine (**8a-f**) by coupling carboxylate ester with a series of sulfonamides through carboxamide linkage. The carboxamide linkage is also reported to decrease the hydrophobicity of reported xanthines.

MATERIALS AND METHOD
Chemistry
The synthetic route followed for the synthesis of various sulphonamide derivatives of 1, 3-dimethyl xanthines is depicted in **Scheme 1** and **2**.

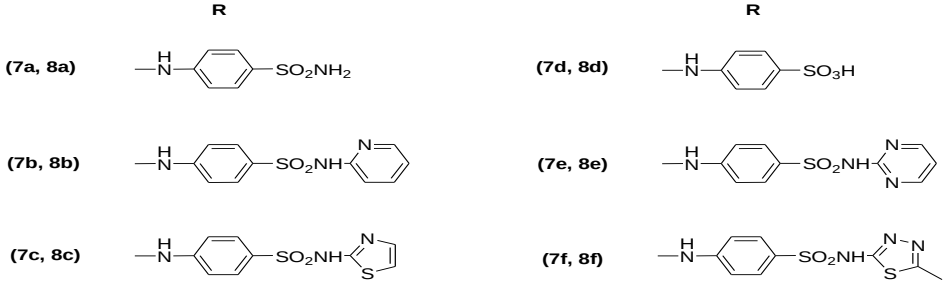


Scheme-1: Reagents and conditions: (a) $CNCH_2COOH$, Acetic Anhydride, 5% NaOH, (b) $NaNO_2$, CH_3COOH, (c) $Na_2S_2O_4$, NH_4OH

The current methodology involves the synthesis of a key compound i.e. 5, 6-diamino-1, 3-dimethyluracil (**3**) which is an important intermediate for the synthesis of all 8-substituted derivatives.[10,11] The condensation of *N, N'*-dimethyl urea (**1**) and cyanoacetic acid in the presence of acetic anhydride followed by nitrosation gave 5-nitroso-6-amino-1, 3-dimethyluracil (**2**) which on further reduction with sodium dithionite in concentrated ammonium hydroxide solution gave the desired key intermediate which is an unstable diaminouracil. This diaminouracil was then condensed with *p*-substituted benzaldehyde (**4**) to afford Schiff base (**5**) followed by its cyclization using thionyl chloride resulted in the synthesis of targeted xanthine carboxylate. The carboxylate ester (**6**) was then coupled with various sulfonamides (**7a-f**) in refluxing dimethylformamide to afford targeted compounds (**8a-f**). The structures of the synthesized compounds were characterized by various spectral and elemental analyses.



Scheme-2: Reagents and conditions : (a) MeOH/AcOH, room temperature, (b) $SOCl_2$, Reflux, (c) DMF, Reflux



Pharmacological evaluation

The synthesized derivatives **8a-f** were evaluated *in vivo* for bronchospasmolytic activity against the standard drug theophylline.[12] The results obtained are summarized in **Table 1**.

RESULTS AND DISCUSSION

Chemistry

The synthesis of sulphonamide derivatives of 1, 3-dimethylxanthines **8a-f** was performed as depicted in **Scheme 1** and **2**. The dimethyl urea and cyanoacetic acid were condensed followed by nitrosation and reduction to give the starting material 5,6-diamino-1,3-dimethyluracil (**3**). *p*-hydroxybenzaldehyde was treated with methyl chloroacetate under reflux to give *p*-substituted benzaldehyde (**4**). The substituted benzaldehyde (**4**) was then fused with compound 3 to obtain Schiff base **5** which was further cyclized by thionyl chloride to give carboxylate ester of 1, 3-dimethylxanthine (**6**). The condensation of **6** with various sulphonamides **7a-f** gave the desired targeted compounds **8a-f**. The structures of the synthesized compounds were confirmed by various spectral analyses such as IR, ^{1}H-NMR, ^{13}C-NMR and by elemental analyses. As representative example, the ^{1}H-NMR and ^{13}C-NMR spectra of 8-(4-(2-oxosulphanilamidethoxy)-phenyl)-1,3-dimethylxanthine (**8a**) are characterized as follows: the doublet at 7.99 ppm with two proton integration corresponds to –N*H*-. The doublet signal at 6.52 ppm, singlet at 6.81 ppm and doublet at 7.34 ppm with two, four and two proton integration corresponds to aromatic protons respectively. The singlet at 4.80 ppm corresponds to two proton integration of –OCH_2 while the singlets at 2.64 and 2.80 ppm corresponds to three proton integration of –NCH_3. The aliphatic methyl carbons (N-**C**H$_3$) appear at 27.65 and 29.75 ppm, methoxy carbons (-O**C**H$_3$) appear at 64.53 ppm and the aromatic carbons showed peaks between 112.44-151.87 ppm. The carboxyl carbons (**C**=O) appeared at 166.87 ppm. The elemental analysis of the synthesized compounds showed in agreement with their molecular formula. The IR data showed specific peaks for functional groups as an evidence for the formation of proposed structures.

Pharmacological Evaluation

Table 1 summarizes the *in vivo* bronchospasmolytic activity of the newly synthesized sulphonamide derivatives of 1, 3-dimethylxanthine against the standard drug theophylline. All the synthesized compounds showed remarkable activity against histamine aerosol induced asthma along with 100% survival against the standard drug theophylline. Out of all the derivatives, compound **8f** having sulphamethiazole substitution at *para* position of 8-phenylxanthine showed maximum time for the onset of bronchospasm and thus found to be most active. All other derivatives showed increased activity as compared to standard drug while the animals treated with **8b** showed no jerks.

It can be concluded that the presence of ring at *para* position of 8-phenylxanthine increases activity as compared to linear chain. Also, as the number of heteroatom's in the ring increases, the time taken for the onset of bronchospasm also increases.

Experimental

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.

General procedure for the synthesis of 5-Nitroso-6-amino-1, 3-dimethyluracil (2)

A mixture of *N,N'*-dimethylurea (**1**) (1.0 g, 11.36 mmol), cyanoacetic acid (1.0 g, 11.76 mmol) and acetic anhydride (2 ml) were heated for 3 hours under anhydrous conditions. The excess anhydride and acetic acid formed during the reaction were removed under reduced pressure. The residue was cooled (0-5°C) and a solution of 5% sodium hydroxide (20 ml) was added with stirring resulting in precipitation. A solution of sodium nitrite (1.0 g, 14.49 mmol in 8 ml of water) was added to cool, stirred mixture and acidified by the drop wise addition of acetic acid (2 ml) over a period of one hour resulting in red-violet precipitates. The mixture was further stirred overnight at room temperature. The mixture was then cooled, filtered, washed with water, ethanol finally dried with diethyl ether to obtain **2** (1.8 g, 84.7%), mp 235-240°C.

General procedure for the synthesis of 5, 6-Diamino-1, 3-dimethyluracil (3)

The 6-amino-1, 3-dimethyl-5-nitrosourail (**2**) (1.0 g, 5.43 mmol) was dissolved in concentrated ammonium hydroxide (8 ml) with slight warming. The sodium dithionite (2.74 g, 15.73 mmol) was then added slowly with stirring and warming. The salt dissolved and underwent a series of color changes. The solution was stirred further at room temperature for two hours and then cooled in an ice bath. The precipitate so obtained

was filtered, washed with few drops of cool water and dried to obtain **3** (0.76 g, 82.16%), mp 208-211°C.

General procedure for the synthesis of 8-[4-(Methyl-2-phenoxyacetate)]-1, 3-dimethyl xan- thine (4)

The methylchloroacetate (1ml) was added to refluxing suspension of 4-Hydroxybenzaldehyde (0.5 g, 4.09 mmol) and anhydrous potassium carbonate (3.0 g) in ethyl methyl ketone (20 ml) at 70-80°C with the exclusion of moisture. The suspension was further refluxed for 8 hours with continuous stirring. The progress of the reaction was monitored by thin layer chromatography (TLC) using precoated plates with silica gel G (Merck 60 F_{254}). The reaction mixture was then cooled, filtered, concentrated under vacuum to obtain oily residue of methyl-2-(4-formylphenoxy) acetate (**4**) which was used as such for next reaction.

General procedure for the synthesis of Schiff base (5)

To a stirred solution of 5, 6-diamino-1, 3-dimethyluracil (**3**) (0.5 g, 2.94 mmol) in methanol-acetic acid (MeOH-AcOH) (4:1, 10ml), the solution of above obtained oily residue (**4**) in methanol (5ml) was added. The reaction mixture was stirred further overnight at room temperature resulting in precipitation and the completion of reaction was monitored by thin layer chromatography. The precipitates so obtained were filtered off, washed with ice cold water and dried to obtain schiff base (**5**, 0.79g, mp 238-246°C), which was used as such for next step.

General procedure for the synthesis of Carboxylate ester of 1, 3-Dimethylxanthine (6)

The Schiff base **5** (0.5g, 1.44 mmol) was refluxed in thionyl chloride (15 ml) at 70-80°C for one hour. The excess acid was distilled off and crushed ice was added to the reaction mixture. The reaction mixture was neutralized by drop wise addition of concentrated ammonium hydroxide, resulting in precipitation. The reaction mixture was cooled, filtered and washed with cold water. It was then dried to obtain the precipitates of cyclised ester **6** (0.35g, 70.42%), mp > 280°C. **FT-IR ν_{max} (KBr) cm^{-1}:** 3167 (-NH), 3033 (Ar-CH-stretch), 2954 (Ali-CH-stretch), 1747 (C=O), 1689 (C=O), 1648 (C=N).

General procedure for the synthesis of various sulphonamide derivatives of 8-phenyl substituted 1, 3-dimethyl xanthine (8a-f)

The cyclised ester **6** (0.5 g, 1.45 mmol) was dissolved in hot DMF (10 ml) with stirring at reflux set up resulting in a clear solution. Various Sulfonamides **(7a-f)** (1 g, 5.81 mmol) were then added to the clear solution and refluxed till thin layer chromatography (TLC) confirms the completion of reaction. The excess solvent was removed under vacuum resulting in oily residue. The addition of diethyl ether to the above oily residue resulted in precipitation. The precipitates were filtered, washed with diethyl ether and dried to obtain **(8a-f).**

8-(4-(2-Oxosulphanilamidethoxy)-phenyl)-1, 3-dimethylxanthine (8a; RY-060)

Yield 1.13 g, 75.83%; mp 172-174°C (decomp.); **FT-IR ν_{max} (KBr) cm^{-1}:** 3477 (-NH_2 asym.), 3383 (-NH_2 sym.), 3318 (-NH), 3242 (Ar-CH-stretch), 1688 (C=O-NH), 1648 (C=O), 1626 (C=N), 1150 (-SO_2-); **^{1}H-NMR (400 MHz, DMSO-d_6):** δ 7.99 (d, 2H, -N*H*, J_{ortho} = 8.52 Hz), 7.34 (d, 2H, Ar-*H*, J_{ortho} = 8.56 Hz), 6.81 (s, 4H, Ar-*H*), 6.52 (d, 2H, Ar-*H*, J_{ortho} = 8.50 Hz), 5.71 (s, 2H, -NH_2), 4.80 (s, 2H, -OCH_2), 2.80 (s,3H, -NCH_3), 2.64 (s,3H, -NCH_3); **^{13}C-NMR (400 MHz, DMSO-d_6):** δ 27.65 (N-**C**H_3), 29.75 (N-**C**H_3), 64.53 (O-**C**H_2), 112.44 (5 Ar**C**H), 114.92 (Ar**C**), 127.40 (2 ArCH, ArC), 129.97 (2 ArCH), 149.90 (Ar**C**), 151.26 (Ar**C**), 151.87 (Ar**C**), 151.87 (2 **C**=O), 162.36 (Ar**C**), 166.87 (**C**=O); **Calcd. for $C_{21}H_{20}N_6O_6S$:** C, 52.06%; H, 4.16%; N, 17.35%,. **Found:** C 49.16%, H 4.75%, N 15.83%.

8-(4-(2-Oxosulpyridinethoxy)-phenyl)-1, 3-dimethylxanthine (8b; RY-061)

Yield 0.64 g, 91%; mp 230-234°C (decomp.); **FT-IR ν_{max} (KBr) cm^{-1}:** 3416 (-NH), 3363 (-NH), 3313 (-NH), 3242 (Ar-CH-stretch), 1748 (C=O-NH), 1687 (C=N), 1648 (C=O), 1368 (-SO_2-), 1263 (C-O), 1185 (C-N); **^{1}H-NMR (400 MHz, DMSO-d_6):** δ 13.70 (s, 1H, -N*H*),10.93 (s, 1H, -N*H*), 8.09 (d, 2H, Ar-*H*, J_{ortho} = 8.48 Hz), 7.95 (s, 1H, Ar-*H*),7.65 (t, 1H, Ar-*H*, J_{meta} = 1.76 Hz, J_{ortho} = 6.88 Hz), 7.58 (d, 2H, Ar-*H*, J_{ortho} = 8.8 Hz), 7.08 (d, 2H, Ar-*H*, J_{ortho} = 8.64 Hz), 6.90 (t, 1H, Ar-*H*, J_{meta} = 1.0 Hz, J_{ortho} = 5.6 Hz), 6.71 (s, 1H, Ar-*H*), 6.57 (d, 2H, Ar-*H*, J_{ortho} = 8.68 Hz), 5.94 (s, 2H, -OCH_2), 4.90 (s, 1H, -N*H*), 2.73 (s, 3H, -NCH_3), 2.51 (s, 3H, -NCH_3); **^{13}C-NMR (400 MHz, DMSO-d_6):** δ 27.75 (N-**C**H_3), 29.73 (N-**C**H_3), 64.54 (O-**C**H_2), 112.11 (Ar**C**H), 112.39 (3 Ar**C**H), 114.93 (2 Ar**C**H), 117.05 (Ar**C**H), 121.89 (Ar**C**), 125.65 (Ar**C**H), 128.02 (Ar**C**H), 128.85 (2 Ar**C**H), 138.70 (Ar**C**H), 146.27 (Ar**C**), 151.18 (Ar**C**), 152.27 (Ar**C**), 152.70 (2 Ar**C**H), 154.10 (**C**=O), 159.09 (**C**=O), 168.95 (**C**=O); **Calcd. for $C_{26}H_{23}N_7O_6S$:** C, 55.61%; H, 4.13%; N, 17.46%. **Found:** C, 54.74%; H, 4.61%; N, 15.8%.

8-(4-(2-Oxosulphathiazolethoxy)-phenyl)1, 3-dimethylxanthine (8c; RY-062)

Yield 1.15 g, 99%; mp 193-196 °C (decomp.). **FT-IR ν_{max} (KBr) cm^{-1}:** 3467 (-NH), 3363 (-NH), 3141 (Ar-CH-stretch), 1746 (C=O-NH), 1688 (C=N), 1648 (C=O), 1239 (C-O), 1142 (-SO_2-); **^{1}H-NMR (400 MHz, DMSO-d_6):** δ 13.70 (s, 1H, -N*H*), 12.45 (s, 1H, -N*H*), 8.09 (s, 2H, Ar-*H*), 7.44 (s, 1H, Ar-*H*), 7.18 (s, 1H, Ar-*H*), 7.09 (s, 1H, Ar-*H*), 6.94 (s, 1H, Ar-*H*), 6.74 (s, 1H, Ar-*H*), 6.57 (s, 1H, Ar-*H*), 6.25 (s, 1H, Ar-*H*), 6.15 (s, 1H, Ar-*H*), 5.84 (s, 2H, -OCH_2), 4.89 (s, 1H, -N*H*), 2.73 (s, 3H, -NCH_3), 2.51 (s, 3H, -NCH_3); **Calcd. for $C_{24}H_{21}N_7O_6S_2$:** C, 50.79%; H, 3.73%; N, 17.27%; **Found:** C, 48.74%; H, 3.74%; N, 14.67%.

8-(4-(2-(Carboxyaminobenzenesulfonicacid)-ethoxy)-phenyl)-1, 3-dimethylxanthine (8d; RY-063)

Yield 0.31 g, 26.72%; mp >270°C. **FT-IR ν_{max} (KBr) cm^{-1}:** 3522 (-NH), 3169 (Ar-CH-stretch), 1742 (C=O-NH), 1701 (C=N), 1656 (C=O), 1243 (C-O), 1187 (-SO_3H); **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ 13.61 (s, 1H, -O*H*), 8.12 (d, 2H, Ar-*H*, J_{ortho} = 8.96 Hz), 8.09 (s, 1H, Ar-*H*), 7.14 (d, 2H, Ar-*H*, J_{ortho} = 8.8 Hz), 7.07 (d, 2H, Ar-*H*, J_{ortho} = 8.52 Hz), 4.95 (s, 2H, -OCH_2), 3.74 (s, 2H, -N*H*), 2.95 (s, 3H, -NCH_3), 2.79 (s, 3H, -NCH_3); **^{13}C-NMR (400 MHz, DMSO-d$_6$):** δ 27.74 (N-**C**H$_3$), 29.72 (N-**C**H$_3$), 64.55 (O-**C**H$_2$), 114.92 (3 Ar**C**H), 121.91 (2 Ar**C**H), 125.21 (Ar**C**), 126.42 (Ar**C**), 127.90 (Ar**C**), 128.01 (2 Ar**C**H), 147.23 (Ar**C**), 148.51 (Ar**C**), 149.70 (Ar**C**), 151.17 (2 Ar**C**), 154.10 (**C**=O), 159.09 (**C**=O), 168.94 (**C**=O); **Calcd. for $C_{21}H_{19}N_5O_7S$:** C, 51.95%; H, 3.94%; N, 14.43%. **Found:** C, 53.02%; H, 4.75%; N, 13.96%.

8-(4-(2-Oxosulphadiazinethoxy)-phenyl)-1, 3-dimethylxanthine (8e; RY-064)

Yield 0.17 g, 72.03%; mp 245-248°C (decomp.). **FT-IR ν_{max} (KBr) cm^{-1}:** 3434 (-NH-), 1653 (C=O), 1596 (C=N), 1577 (C=C), 1475 (NH-bend), 1319 (-SO_2-), 1185 (C-N), 1092 (C-O),**^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ 13.62 (s, 1H, -N*H*), 11.29 (s, 1H, -N*H*), 8.46 (d, 2H, Ar-*H*, J_{ortho} = 4.28 Hz), 8.10 (d, 1H, Ar-*H*, J_{ortho} = 8.24 Hz), 7.69 (d, 2H, Ar-*H*, J_{ortho} = 8.2 Hz), 7.05 (d, 1H, Ar-*H*, J_{ortho} = 8.2 Hz), 6.97 (s, 2H, Ar-*H*), 6.70 (s, 1H, Ar-*H*), 6.58 (d, 2H, Ar-*H*, J_{ortho} = 8.2 Hz), 5.92 (s, 2H, -OCH_2), 4.86 (s, 1H, -N*H*), 2.91 (s, 3H, -NCH_3), 2.76 (s, 3H, -NCH_3); **^{13}C-NMR (400 MHz, CDCl$_3$-DMSO-d$_6$):** δ 27.69 (N-**C**H$_3$), 29.75 (N-**C**H$_3$), 64.62 (O-**C**H$_2$), 112.02 (Ar**C**,2 Ar**C**H), 114.72 (Ar**C**H, Ar**C**), 115.25 (2 Ar**C**H), 124.81 (Ar**C**), 127.99 (2 Ar**C**H), 129.72 (2 Ar**C**H, Ar**C**), 152.90 (2 Ar**C**), 157.21 (Ar**C**, 2 Ar**C**H, **C**=O), 158.98 (**C**=O), 168.78 (Ar**C**, **C**=O); **Calcd. for $C_{25}H_{22}N_8O_6S$:** C, 53.38%; H, 3.94%; N, 19.92%. **Found:** C, 50.28%; H, 4.18%; N, 19.18%.

8-(4-(2-Oxosulphamethizolethoxy)-phenyl)-1, 3-dimethylxanthine (8f; RY-065)

Yield 0.19 g, 82.25%; mp 172-174°C (decomp.). **FT-IR ν_{max} (KBr) cm^{-1}:** 3463 (-NH), 3216 (Ar-H-stretch), 2923 (Ali-CH-stretch), 1671 (C=O), 1597 (C=N), 1297 (-SO_2), 1142 (C-S), 1086 (C-O); **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ 13.69 (s, 1H, -N*H*), 8.23 (d, 2H, Ar-*H*, J_{ortho} = 5.64 Hz), 7.42 (s, 3H, Ar-*H*), 7.22 (s, 1H, Ar-*H*), 6.80 (s, 2H, Ar-*H*), 6.00 (s, 2H, -OCH_2), 5.38 (s, 1H, -N*H*), 4.85 (s, 1H, -N*H*), 2.83 (s, 3H, -NCH_3), 2.45 (s, 3H, -NCH_3), 1.25 (s, 3H, –CH_3); **^{13}C-NMR (400 MHz, CDCl$_3$-DMSO-d$_6$):** δ 16.02 (**C**H$_3$), 27.72 (N-**C**H$_3$), 29.53 (N-**C**H$_3$), 64.50 (O-**C**H$_2$), 112.51 (4 Ar**C**H), 114.54 (2 Ar**C**), 127.47 (4 Ar**C**H), 127.98 (2 Ar**C**), 152.19 (2 Ar**C**, **C**=O), 158.94 (Ar**C**, **C**=O), 168.61 (**C**=O). **Calcd. for $C_{24}H_{22}N_8O_6S_2$:** C, 49.48%; H, 3.81%; N, 19.23%; **Found:** C, 48.23%; H, 4.33%; N, 17.70%.

Pharmacological Evaluation

The synthesized derivatives were evaluated for their *in vivo* bronchospasmolytic activity against the standard drug theophylline. The animals were housed under standard laboratory conditions, maintained on a 12 hour light and dark cycle and having free access to food (green leafy vegetables, cucumber, carrots) and water. The experimental protocols were approved by Institutional Animal Ethics Committee of Banasthali University, Banasthali (BV/IAEC/2016/I dated 08.10.2016, Ref. No. BV/3421/16-17) and conducted according to the guidelines for the use and care of experimental animals. Male guinea-pigs of 220±30 g, bred in disease free small animal house of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) were obtained. The animals were divided into three groups and each group contains five animals: 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 pretreated with test drug (50 mg/kg), theophylline (50 mg/kg) and CMC (control) per oral 1h before exposal to aerosol. Each group of the animal 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 animal remained in the chamber for 8 min after which they were removed in fresh air and fed with proper water and food. The onset of bronchospasm, duration of jerks, severity of bronchospasm and death or survival of the animal was recorded for each group. The results are expressed as Mean ± SEM. (**Table 1**).

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

Comp. No. (Code)	Onset of bronchospasm (Seconds) Mean ± S.E.M	Duration of Jerks (Seconds) Mean ± S.E.M	Severity of bronchospasm	Survival (%)
Control	51.80 ± 3.49	183.4 ± 10.59	+++	0
Theophylline	105.60 ± 7.73*	94.8 ± 6.72*$^{\#}$	+	100
8a (RY 060)	117.56 ± 8.18*	71.8 ± 11.78*	+++	100
8b (RY 061)	193.37 ± 13.21*$^{\#}$	-	+	100
8c (RY 062)	196.76 ± 9.23*$^{\#}$	57.0 ± 7.62*	+++	100
8e (RY 064)	203.28 ± 9.20*$^{\#}$	41.2 ± 3.70*$^{\#}$	+++	100
8f (RY 065)	292.32 ± 11.24*$^{\#}$	59.2 ± 7.19*	+++	100

Number of animals in each group (N) = 5

Dose of standard and test drug = 50 mg/kg
(-) 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

CONCLUSION

The newly synthesized sulphonamide derivatives of 8-phenylsubstituted 1, 3-dialkylxanthines **8a-f** were prepared by coupling of various commercially available sulphonamides with carboxylate ester of 1, 3-dialkylxanthine. The synthesized derivatives were evaluated for *in vivo* bronchospasmolytic activity against histamine aerosol induced asthma. The compound **8f** of this series was found to be most active in comparison to theophylline while no jerks were observed in case of animals treated with **8b**. All other derivatives showed similar activity as compared to the drug.

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