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SYNTHESIS, ANTI-BRONCHOCONSTRICTIVE, AND ANTIBACTERIAL ACTIVITIES OF SOME NEW 8-SUBSTITUTED-1,3-DIMETHYLXANTHINE DERIVATIVES

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عرفت مجموعة ميثيل الزانثينات خاصة ثيوفيللين كموسعات قوية للشعب الهوائية لعلاج ضيق التنفس الحاد منذ أكثر من خمسين عاما، ووجد حديثا أن العدوى البكتيرية لها دور فى الإصابة بضيق التنفس. ومن هنا فإن هذه الدراسة تشتمل على تخليق سلاسل مختلفة من ٨ مستبدلات (اريل، أرالكيل، الكيل حلقى، أريل غير متجانس الحلقة) ٣،١ ثنائى ميثيل زانثين. تم التأكد من التراكيب البنائية لهذه المركبات بواسطة الأشعة تحت الحمراء و الرنين النووى المغناطيسى للهيدروجين والكربون المشع و التحاليل الدقية للعناصر المكونة ومطياف الكتلة لبعض من هذه المركبات. هذا وقد تم دراسة التأثير الموسع للشعب الهوائية بواسطة تحفيز ضيق التنفس بالأستيل كولين فى الخنازير الغينية، وقد وجد أن معظم المركبات لها تأثير فعال كمضادات لضيق الشعب الهوائية مقارنة بعقار الأمينوفيللين المستخدم علاجيا. ومن جانب اخر فقد تم دراسة التأثير المضاد للبكتيريا لكل المركبات المستهدفة ضد كلا من البكتيريا الموجبة والسالبة الجرام، وقد أوضحت النتائج أن لبعض المركبات تأثير فعال كمضادات للبكتيريا مقارنة بعقار الأمبسيللين المستخدم علاجيا. أيضا تم تصميم نموذج للفارماكوفور لإلقاء الضوء على الخواص التركيبية الجوهرية اللازمة للتأثير الموسع للشعب الهوائية.

Methylxanthines especially theophylline have been recognized as potent bronchodilators for the relief of acute asthma for over 50 years. Recently, it was found that bacterial infection has a role in asthma pathogenesis. Accordingly, the present work involves the synthesis of different series of 8-substituted (aryl, aralkyl, cycloalkyl, and heteroaryl)-1,3-dimethylxanthines. The chemical structures of these compounds were elucidated by IR, ^1H NMR, ^{13}C NMR, elemental analyses, and high resolution EI-MS or FAB-MS for some compounds. The bronchodilator activity was evaluated using acetylcholine induced bronchospasm in guinea pigs, and

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most of the compounds showed significant anti-bronchoconstrictive activity in comparison with aminophylline as a standard. Also, the antibacterial activity of all the target compounds was investigated in-vitro against Gram-positive and Gram-negative bacteria using ampicillin as a reference drug. Results showed that some of the tested compounds have potent antibacterial activity. A pharmacophore model was computed to get useful insight on the essential structural features of bronchodilator activity.

INTRODUCTION

Asthma is a chronic lung disease characterized by temporary obstruction of airflow that leads to breathing difficulty, inflammation of the airways, and increased sensitivity of the airways to a variety of triggers that cause breathing difficulty[1]. Bronchodilators are used to open air passages and facilitate breathing as well as diminish bronchospasms by relaxing the smooth muscles of the bronchioles. They provide respiratory relief from conditions such as asthma, bronchitis, emphysema, or bronchiectasis. The xanthine drugs, especially theophylline, are thought to be the most useful bronchodilators for moderate or severe reversible bronchospasm. Moreover, they also improve respiratory exchange by increasing diaphragmatic contractility. The mechanism for the therapeutic effect of theophylline on respiratory systems is not clear[2]. However, it may be due, in part, to increased cyclic adenosine monophosphate (cAMP) following competitive inhibition of phosphodiesterase, the enzyme that degrades cAMP. Other proposed mechanisms include mobilization of intracellular calcium in smooth muscles, inhibition of prostaglandin action, blockade of adenosine receptors, and inhibition of the release of histamine and leukotrienes from mast cells[3].

The role of infection in asthma is complex and still not fully understood. Although viral infections are now well established as being associated with acute asthma exacerbations (asthma attacks)[4&5], there is increasing evidence from controlled studies to support an association between atypical bacterial infection - particularly with *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* - and both chronic stable asthma and acute exacerbations of asthma[6]. Recent study stated that neonates colonized in the hypopharyngeal region with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* are at increased risk for recurrent wheeze and asthma early in life[7]. Moreover, it has recently been reported that asthma is a risk factor for invasive pneumococcal disease[8], suggesting that asthmatic patients might also have increased susceptibility to bacterial infections.

Several studies of both chronic and acute asthma using macrolide (roxithromycin, clarithro-mycin)[9&10], or ketolide (telithro-mycin)[11] suggested that these antibiotics do have a beneficial effect in asthma[12].

The adverse effects such as nausea, vomiting, epigastric pain, palpitation, sinus tachycardia, diuresis, insomnia, and headache of theophylline and its narrow thera-peutic index[13&14], also, adverse drug reactions and the promotion of the development of antibiotic resistance owing to the use of antibacterial agents[15-17], represent an important problem, and require searching for novel approaches to asthma therapy.

It was reported that the alkyl substitution at the 8-position of the methylxanthine series renders the compounds more active than theophylline on tracheal relaxation in guinea pigs[18]. Several 8-substituted theophylline derivatives were synthesized, and showed a potent bronchodilator activity[19-21]. Further-more, there are some reports studied the antibacterial effect of xanthines and xanthine derivatives on various microorganisms, and stated that some compounds showed significant degree of activity[22-26]. Other studies reported that methylxanthines had a synergistic effect on antibacterial agents[27&28].

In view of these data, the present work aimed at the synthesis of some new 8-thio-substituted theopylline derivatives as possible antibroncho-constrictive agents with potent antibacterial activity.

EXPERIMENTAL

Chemistry

Reagents used for synthesis were purchased from Sigma-Aldrich (Gillingham – Dorset, UK) and MERCK (Schuchardt, Germany). All solvents were obtained from commercial suppliers and used without further purification. Melting points (m.p.) were determined on an electrothermal Stuart Scientific SMP1 (UK) melting point apparatus and were uncorrected. Thin-layer chromatography (TLC, Rf values) was carried out using TLC aluminium sheets kieselgel 60 F_{254} (MERCK) and dichloromethane/methanol (9.5:0.5) as a mobile phase and visualization was effected with ultraviolet lamp Spectroline ENF-240C/F (USA) at short wavelength (λ= 254 nm). All chemical yields are unoptimized and generally represent the result of a single experiment. IR spectra were recorded on a Shimadzu spectrophotometer (IR-470) as potassium bromide discs. NMR spectra were recorded on either a Bruker DPX 300 MHz spectrometer or a Varian EM-360 60 MHz spectrometer. DMSO-d_6 was used as a solvent and the chemical shifts are given in δ (ppm), coupling constants (*J*) are in Hertz (Hz). Chemical shifts are expressed either relative to tetramethylsilane (TMS) as an internal standard or to the chemical shifts of the remaining protons of DMSO-d_6 : ^{1}H: δ 2.49 ppm, ^{13}C: 39.7 ppm. The EI-MS were determined using either EI-Finnigan MAT 95XL (Thermo Finnigan, Bremen) or JOEL JMS600 mass spectrometer, and FAB-MS were determined using Concept 1H (Kratos, Hofheim), with *m*-nitrobenzyl alcohol as a matrix. The microanalyses for C, H, N and S were performed on Perkin-Elmer 240 elemental analyzer.

1,3-Dimethyl-8-thioxo-3,7,8,9-tetra-hydro-1H-purine-2,6-dione (6)

To a stirred solution of compound **5** (3 g, 17.6 mmol) in anhydrous DMF (25 mL), CS_2 (1.5 mL, 26.4 mmol) was added. The reaction mixture was refluxed for 4 hrs, and then allowed to cool. Cold water (25 mL) was added to the reaction with stirring, the precipitate formed was filtered, washed successively with cold water, methanol, diethyl ether, and dried. Physical and micro-analytical data are given in Table 1.

IR cm^{-1}: 3455, 3330 (N-H); 2925, 2800 (C-H aliphatic); 1695, 1643 (C=O); 1618 (C=C); 1540 (N-H); 1226 (C=S). ^{1}H NMR (300 MHz): 3.16 (s, 3H, N1-CH_3), 3.35 (s, 3H, N3-CH_3), 12.97 (br s, 1H, N9-H), 13.39 (br s, 1H, N7-H). ^{13}C NMR (300 MHz): 28.21 (N1-CH_3), 31.51 (N3-CH_3), 103.99 (C5), 139.82 (C4), 150.46 (C2), 151.92 (C6), 164.25 (C8). EI-MS (*m/z*, % base): 212.05 (M^+, 64.5), 182.96 (11.1), 155.07 (25.4), 127.12 (39.7), 99.06 (100), 68.03 (85), 53.01 (74.2). FAB^+-MS (*m/z*, % base): 213 (M^++1, 100).

General method for synthesis of compounds 14-20

To a stirred solution of compound **6** (1 g, 4.7 mmol) in aqueous NaOH 1% (20 mL), the appropriate *p*-(un)-substituted phenacylbromide[32&33] (4.7 mmol) dissolved in the least amount of ethanol was added portion wise, a heavy precipitate was formed immediately. The reaction mixture was stirred at the ambient temperature for 4 hrs, and then cooled in refrigerator for 3 hrs. The product was filtered, washed with water, diethyl ether, dried, and crystallized from the appropriate solvent. Physical and microanalytical data are given in Table 1.

1,3-Dimethyl-8-[(2-oxo-2-phenyl-ethyl)thio]-3,7-dihydro-1H-purine-2,6-dione (14)

IR cm^{-1}: 3435 (N-H); 3050 (Ar-H); 2880 (C-H aliphatic); 1686, 1642 (C=O); 1536 (N-H); 739, 696 (Ar-H). ^{1}H NMR (60 MHz): 3.48 (s, 3H, N1-CH_3), 3.60 (s, 3H, N3-CH_3), 5.08 (s, 2H, SCH_2), 7.52-8.35 (m, 5H, Ar-H), 14.07 (br s, 1H, N7-H). FAB^+-MS (*m/z*, % base): 331.2 (M^++1, 37).

1,3-Dimethyl-8-{[2-(4-methyl-phenyl)-2-oxoethyl]thio}-3,7-dihydro-1H-purine-2,6-dione (15)

IR cm^{-1}: 3415 (N-H); 3045 (Ar-H); 2880 (C-H aliphatic); 1688, 1641 (C=O); 1537 (N-H); 802 (Ar-H). ^{1}H NMR (60 MHz): 2.65 (s, 3H, 4`-$CH_3$), 3.55 (s, 3H, N1-$CH_3$), 3.65 (s, 3H, N3-$CH_3$), 5.35 (s, 2H, $SCH_2$), 8.00 (d, *J* = 8.7 Hz, 2H, 3`,5`Ar-H), 8.65 (d, *J* = 8.7 Hz, 2H, 2`,6`Ar-H),

Table 1: Physical and microanalytical data of compounds **6**, **14-20**, and **56-90**.

No.	Yield %	m.p.°C	Crystal. solvent[e]	TLC R_f	Molecular formula	Microanalyses		
							Calcd. %	Found %
6	89	320-1[a]	1	0.28	$C_7H_8N_4O_2S$ $.H_2O$	C H N	36.52 4.38 24.33	36.26 3.79 24.34
14	80	225-6[b]	2	0.54	$C_{15}H_{14}N_4O_3S$	-	-	-
15	79	230-2	2	0.58	$C_{16}H_{16}N_4O_3S$	C H N	55.80 4.68 16.27	55.78 4.76 16.22
16	81	219-21	2	0.56	$C_{16}H_{16}N_4O_4S$	C H N S	53.32 4.47 15.55 8.90	52.98 4.85 15.55 9.11
17	80	238-40	3	0.51	$C_{15}H_{13}FN_4O_3S$ $.½H_2O$	C H N	50.41 3.95 15.68	50.65 4.27 15.34
18	82	231-3	3	0.53	$C_{15}H_{13}ClN_4O_3S$ $.½H_2O$	C H N S	48.20 3.77 14.99 8.58	48.17 3.77 15.31 8.20
19	82	229-30[c]	3	0.54	$C_{15}H_{13}BrN_4O_3S$	-	-	-
20	73	222-3[d]	4	0.54	$C_{15}H_{13}N_5O_5S$	-	-	-
56	81	238-41 decomp.	5	0.46	$C_{15}H_{15}N_5O_3S$ $.½H_2O$	C H N	50.84 4.55 19.76	51.07 4.86 19.81
57	81	246-8 decomp.	2	0.42	$C_{16}H_{17}N_5O_3S$	C H N	53.47 4.77 19.49	53.82 5.06 19.12
58	81	249-51 decomp.	5	0.35	$C_{16}H_{17}N_5O_4S$ $.½H_2O$	C H N	49.99 4.72 18.22	49.93 5.20 17.95
59	81	272-5 decomp.	2	0.36	$C_{15}H_{14}ClN_5O_3S$	C H N S	47.43 3.72 18.44 8.44	47.65 4.07 18.45 8.96
60	82	274-5	2	0.38	$C_{15}H_{14}BrN_5O_3S$	C H N	42.46 3.33 16.51	42.55 3.53 16.22
61	82	265-8 decomp.	5	0.37	$C_{15}H_{14}IN_5O_3S$ $.½H_2O$	C H N S	37.51 3.15 14.58 6.68	37.79 3.59 14.99 6.96
62	81	269-71	6	0.36	$C_{15}H_{14}N_6O_5S$	C H N	46.15 3.61 21.53	45.89 4.08 21.36
63	65	264-6	6	0.22	$C_{15}H_{15}N_5O_4S$ $.½H_2O$	C H N S	48.64 4.35 18.91 8.66	48 .55 4.41 19.36 9.07

Table 1: Continued

No.	Yield %	m.p.°C	Crystal. solvent[e]	TLC R_f	Molecular formula	Microanalyses		
							Calcd. %	Found %
64	76	273-6 decomp.	6	0.18	$C_{16}H_{15}N_5O_5S$.½H_2O	C H N	48.24 4.05 17.58	48.19 4.30 17.65
65	80	264-7 decomp.	2	0.4	$C_{17}H_{17}N_5O_4S$	C H N	52.70 4.42 18.08	52.33 4.60 18.49
66	74	244-7 decomp.	5	0.51	$C_{16}H_{17}N_5O_3S$ .H_2O	C H N S	50.92 5.07 18.56 8.50	50.75 5.50 19.00 8.79
67	73	233-5	2	0.27	$C_{16}H_{17}N_5O_4S$	C H N	51.19 4.56 18.66	50.85 4.64 18.55
68	83	226-8	5	0.67	$C_{17}H_{19}N_5O_4S$.½H_2O	C H N S	51.25 5.06 17.58 8.05	51.26 5.02 18.17 8.37
69	76	244-6	5	0.26	$C_{15}H_{14}ClN_5O_3S$ .H_2O	C H N S	45.29 4.05 17.60 8.06	45.23 4.49 17.14 7.88
70	79	228-30	6	0.31	$C_{15}H_{14}N_6O_5S$.½H_2O	C H N S	45.11 3.79 21.04 8.03	44.97 4.02 21.20 8.24
71	70	208-10	6	0.29	$C_{15}H_{15}N_5O_4S$.½H_2O	C H N S	48.64 4.35 18.91 8.66	48.45 4.56 18.38 8.54
72	69	252-4	6	0.14	$C_{16}H_{15}N_5O_5S$.½H_2O	C H N	48.24 4.05 17.58	47.80 4.38 17.62
73	80	248-52 decomp.	2	0.41	$C_{17}H_{17}N_5O_4S$	C H N	52.70 4.42 18.08	52.27 4.92 18.12
74	78	214-7 decomp.	2	0.37	$C_{16}H_{17}N_5O_3S$	C H N S	53.47 4.77 19.49 8.92	53.66 5.14 19.43 9.38
75	75	182-4	5	0.43	$C_{16}H_{17}N_5O_4S$ .H_2O	C H N S	48.85 4.87 17.80 8.15	48.49 4.71 17.47 7.88
76	78	237-40 decomp.	2	0.44	$C_{15}H_{14}ClN_5O_3S$	C H N S	47.43 3.72 18.44 8.44	46.98 4.02 18.01 8.70
77	81	251-3	6	0.43	$C_{15}H_{14}N_6O_5S$.½H_2O	C H N	45.11 3.79 21.04	44.67 4.26 21.04
78	72	250-2	6	0.16	$C_{15}H_{15}N_5O_4S$.½H_2O	C H N S	48.64 4.35 18.91 8.66	48.63 4.69 19.46 9.05

Table 1: Continued

No.	Yield %	m.p.°C	Crystal. solvent[e]	TLC R_f	Molecular formula	Microanalyses		
							Calcd. %	Found %
79	83	251-3 decomp.	5	0.49	$C_{17}H_{16}BrN_5O_4S$.½H_2O	C H N	42.96 3.60 14.73	42.86 3.97 14.75
80	79	215-8 decomp.	4	0.32	$C_{16}H_{17}N_5O_3S$.½H_2O	C H N	52.16 4.92 19.01	52.12 5.08 19.16
81	79	234-7 decomp.	4	0.35	$C_{17}H_{19}N_5O_3S$ .H_2O	C H N	52.16 5.41 17.89	52.46 5.56 17.72
82	80	219-21	4	0.37	$C_{17}H_{19}N_5O_3S$	C H N S	54.68 5.13 18.75 8.59	54.76 4.83 18.89 9.07
83	85	252-5 decomp.	4	0.33	$C_{15}H_{21}N_5O_3S$	C H N	51.27 6.02 19.93	50.92 6.36 20.01
84	88	252-4	2	0.38	$C_{19}H_{17}N_5O_3S$	C H N	57.71 4.33 17.71	58.08 4.14 18.05
85	68	252-5 decomp.	7	0.26	$C_{16}H_{15}N_7O_3S$	C H N S	49.86 3.92 25.44 8.32	49.40 4.29 25.59 8.31
86	82	244-6 decomp.	5	0.38	$C_{16}H_{14}N_6O_3S_2$.½H_2O	C H N	46.70 3.67 20.42	46.85 4.14 20.24
87	80	271-3	8	0.26	$C_{15}H_{17}N_7O_5S$ .H_2O	C H N S	42.35 4.50 23.05 7.54	42.73 4.95 22.74 7.52
88	70	258-60	9	0.37	$C_{14}H_{14}N_6O_3S$.½H_2O	C H N S	47.32 4.25 23.65 9.02	47.02 4.48 23.55 8.62
89	80	204-6	2	0.62	$C_{16}H_{17}N_5O_3S$	C H N S	53.47 4.77 19.49 8.92	53.41 4.91 19.90 9.35
90	84	219-21	2	0.39	$C_{19}H_{22}N_6O_3S$	C H N	55.06 5.35 20.28	55.26 5.81 20.67

(*a*): as reported[29-31]
(*b*): the reported mp 234-6°C[34] and 242-3°C[35]
(*c*): the reported mp 241-2°C[35]
(*d*): as reported[35]
(*e*): solvents of recrystallisation are water (1), ethanol (2), methanol/chloroform (3), methanol (4), aqueous ethanol (5), DMF/water (6), DMF (7), DMSO/water (8) and 1,4-dioxan/petroleum ether (9).

14.75 (br s, 1H, N7-H). High resolution EI-MS (*m/z*, % base): 344.0940 (M^+, 45) (calc. 344.0943), 326.1 (7), 225 (19), 211 (5), 119 (100), 99 (10), 91 (16).

8-{[2-(4-Methoxyphenyl)-2-oxo-ethyl]thio}-1,3-dimethyl-3,7-di-hydro-1H-purine-2,6-dione (16)

IR cm^{-1}: 3395 (N-H); 3045 (Ar-H); 2935 (C-H aliphatic); 1690, 1644 (C=O); 1538 (N-H); 1251, 1054 (C-O); 824 (Ar-H). ^1H NMR (300 MHz): 3.21 (s, 3H, N1-CH_3), 3.30 (s, 3H, N3-CH_3), 3.86 (s, 3H, 4\`-$OCH_3$), 4.93 (s, 2H, $SCH_2$), 7.08 (d, $J$ = 8.7 Hz, 2H, 3\`,5\`Ar-H), 8.02 (d, $J$ = 8.7 Hz, 2H, 2\`,6\`Ar-H).

8-{[2-(4-Fluorophenyl)-2-oxoethyl]-thio}-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (17)

IR cm^{-1}: 3445 (N-H); 3055 (Ar-H); 2875 (C-H aliphatic); 1687, 1636 (C=O); 1535 (N-H); 1224 (C-F); 824 (Ar-H). ^1H NMR (60 MHz): 3.44 (s, 3H, N1-CH_3), 3.55 (s, 3H, N3-CH_3), 5.24 (s, 2H, SCH_2), 7.65-8.10 (m, 2H, 3\`,5\`Ar-H), 8.45-8.90 (m, 2H, 2\`,6\`Ar-H), 14.48 (br s, 1H, N7-H).

8-{[2-(4-Chlorophenyl)-2-oxoethyl]-thio}-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (18)

IR cm^{-1}: 3455 (N-H); 3050 (Ar-H); 2945 (C-H aliphatic); 1686, 1643 (C=O); 1538 (N-H); 1083 (C-Cl); 815 (Ar-H). ^1H NMR (300 MHz): 3.20 (s, 3H, N1-CH_3), 3.29 (s, 3H, N3-CH_3), 4.96 (s, 2H, SCH_2), 7.64 (d, J = 8.7 Hz, 2H, 3\`,5\`Ar-H), 8.05 (d, J = 8.7 Hz, 2H, 2\`,6\`Ar-H), 13.54 (br s, 1H, N7-H).

8-{[2-(4-Bromophenyl)-2-oxoethyl]-thio}-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (19)

IR cm^{-1}: 3465 (N-H); 3050 (Ar-H); 2950 (C-H aliphatic); 1685, 1636 (C=O); 1535 (N-H); 803 (Ar-H). ^1H NMR (60 MHz): 3.48 (s, 3H, N1-CH_3), 3.58 (s, 3H, N3-CH_3), 5.22 (s, 2H, SCH_2), 8.14 (d, J = 8.8 Hz, 2H, 3\`,5\`Ar-H), 8.42 (d, J = 8.8 Hz, 2H, 2\`,6\`Ar-H), 14.70 (br s, 1H, N7-H). High resolution EI-MS (*m/z*, % base): 407.9900 (M^+, 48) (calc. 407.9891), 410 (M^++2, 50), 390 (6), 225 (100), 211 (12), 182.9 (78), 154.9 (11), 99 (25).

1,3-Dimethyl-8-{[2-(4-nitrophenyl)-2-oxoethyl]thio}-3,7-dihydro-1H-purine-2,6-dione (20)

IR cm^{-1}: 3440 (N-H); 3050 (Ar-H); 2950 (C-H aliphatic); 1686, 1635 (C=O); 1534 (N-H); 1510, 1336 (NO_2); 843 (Ar-H). ^1H NMR (60 MHz): 3.46 (s, 3H, N1-CH_3), 3.54 (s, 3H, N3-CH_3), 5.38 (s, 2H, SCH_2), 8.75-9.30 (m, 4H, Ar-H), 14.76 (br s, 1H, N7-H).

General method for synthesis of N-(substituted)aryl/aralkyl/cycloalkyl/heteroaryl-2-chloroacetamides (21-53), N-methyl-N-phenyl-2-chloro-acetamide (54), and 2-chloro-1-(4-phenylpiperazin-1-yl)ethanone (55)

These compounds were prepared using reported methods[36-51]. Only compounds 44 and 52 were not reported, while compound 46 was prepared but its melting point was not reported, and characterized only by ^1H NMR[52].

N-(2-Acetyl-4-bromophenyl)-2-chloroacetamide (44)

Pale brown needles, yield 81%, m.p. 121-123°C (n-hexane), Rf 0.84, IR cm^{-1}: 3290 (N-H amide); 3115 (Ar-H); 2930 (C-H aliphatic); 1662, 1591 (C=O); 1567 (N-H); 828 (Ar-H). ^1H NMR (60 MHz, $CDCl_3$): 2.82 (s, 3H, 2-CH_3CO), 4.50 (s, 2H, $COCH_2Cl$), 8.22 (dd, *J* = 8.9, 2 Hz, 1H, 5Ar-H), 8.57 (d, *J* = 2 Hz, 1H, 3Ar-H), 9.29 (d, *J* = 8.9 Hz, 1H, 6Ar-H), 13.30 (s, 1H, amide-H).

(±)-2-Chloro-N-(1-phenylethyl)-acetamide (46)

White needles, yield 82%, m.p. 70-72°C (n-hexane), Rf 0.73, IR cm^{-1}: 3240 (N-H amide); 3035 (Ar-H); 2860 (C-H aliphatic); 1641 (C=O); 1536 (N-H); 741, 687 (Ar-H). ^1H NMR (60 MHz, $CDCl_3$): 1.64 (d, *J* = 7 Hz, 3H, $CHC\underline{H}_3$), 4.28 (s, 2H, $COCH_2Cl$), 5.58 (m, 1H, $HNC\underline{H}CH_3$), 7.45 (br s, 1H, amide-H), 7.85 (s, 5H, Ar-H).

2-Chloro-N-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)acetamide (52)

White solid, yield 85%, m.p. 178-180°C (ethanol), Rf 0.38, IR cm^{-1}: 3315 (N-H amide); 3130 (Ar-H); 2940 (C-H aliphatic); 1709, 1644 (C=O); 1597 (C=C); 1517 (N-H). ^1H NMR (60 MHz): 3.36 (s, 3H, N3-CH_3), 3.52 (s, 3H, N1-CH_3), 5.24 (s, 2H, $COCH_2Cl$), 9.14 (s, 1H, pyrimidinyl-CH), 11.66 (s, 1H, amide-H).

General method for synthesis of compounds 56-90

To a stirred solution of compound **6** (1 g, 4.7 mmol) in aqueous NaOH 1% (20 mL), the appropriate N-(substituted)aryl/aralkyl/cycloalkyl/heteroaryl-2-chloroacetamide or *N*-methyl-*N*-phenyl-2-chloroacetamide (**54**) or 2-chloro-1-(4-phenylpiperazin-1-yl)ethanone (**55**) (4.7 mmol) dissolved in the least amount of ethanol was added. The reaction mixture was stirred at the ambient temperature for 12 hrs, and then cooled in a refrigerator for 3 hrs. The product was filtered, washed with water, diethyl ether, dried, and crystallized from the appropriate solvent. Physical and microanalytical data are given in Table 1.

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-phenylacetamide (56)

IR cm^{-1}: 3468 (N-H); 3260 (N-H amide); 3045 (Ar-H); 2870 (C-H aliphatic); 1691, 1643 (C=O); 1529 (N-H); 743, 707 (Ar-H). ^1H NMR (60 MHz): 3.62 (s, 3H, N1-CH_3), 3.80 (s, 3H, N3-CH_3), 4.54 (s, 2H, SCH_2), 7.42-8.45 (m, 5H, Ar-H), 11.26 (s, 1H, amide-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-methylphenyl)acetamide (57)

IR cm^{-1}: 3450 (N-H); 3245 (N-H amide); 3045 (Ar-H); 2870 (C-H aliphatic); 1692, 1645 (C=O); 1530 (N-H); 805 (Ar-H). ^1H NMR (60 MHz): 2.40 (s, 3H, 4`-CH_3), 3.44 (s, 3H, N1-CH_3), 3.62 (s, 3H, N3-CH_3), 4.40 (s, 2H, SCH_2), 7.54 (d, *J* = 8.7

Hz, 2H, 3`,5`Ar-H), 7.95 (d, *J* = 8.7 Hz, 2H, 2`,6`Ar-H), 10.91 (s, 1H, amide-H), 14.50 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-methoxyphenyl)acetamide (58)

IR cm^{-1}: 3465 (N-H); 3270 (N-H amide); 3040 (Ar-H); 2930 (C-H aliphatic); 1691, 1641 (C=O); 1533 (N-H); 1248, 1046 (C-O); 815 (Ar-H). ^{1}H NMR (60 MHz): 3.42 (s, 3H, N1-CH_3), 3.65 (s, 3H, N3-CH_3), 3.95 (s, 3H, 4`-$OCH_3$), 4.42 (s, 2H, $SCH_2$), 7.31 (d, *J* = 8.7 Hz, 2H, 3`,5`Ar-H), 7.96 (d, *J* = 8.7 Hz, 2H, 2`,6`Ar-H), 10.66 (s, 1H, amide-H), 14.43 (br s, 1H, N7-H).

N-(4-Chlorophenyl)-2-[(1,3-di-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (59)

IR cm^{-1}: 3450 (N-H); 3320 (N-H amide); 3110 (Ar-H); 2970 (C-H aliphatic); 1712, 1655 (C=O); 1528 (N-H); 833 (Ar-H). ^{1}H NMR (60 MHz): 3.53 (s, 3H, N1-CH_3), 3.75 (s, 3H, N3-CH_3), 4.57 (s, 2H, SCH_2), 8.00 (d, *J* = 8.9 Hz, 2H, 3`,5`Ar-H), 8.34 (d, *J* = 8.9 Hz, 2H, 2`,6`Ar-H), 11.27 (s, 1H, amide-H), 14.64 (br s, 1H, N7-H).

N-(4-Bromophenyl)-2-[(1,3-di-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (60)

IR cm^{-1}: 3445 (N-H); 3320 (N-H amide); 3115 (Ar-H); 2947 (C-H aliphatic); 1711, 1656 (C=O); 1527 (N-H); 830 (Ar-H). ^{1}H NMR (60 MHz): 3.46 (s, 3H, N1-CH_3), 3.69 (s, 3H, N3-CH_3), 4.52 (s, 2H, SCH_2), 7.78-8.30 (m, 4H, Ar-H), 11.26 (s, 1H, amide-H), 14.66 (br s, 1H, N7-H).

N-(4-Iodophenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (61)

IR cm^{-1}: 3385 (N-H); 3288 (N-H amide); 3120 (Ar-H); 2970 (C-H aliphatic); 1710, 1657 (C=O); 1522 (N-H); 826 (Ar-H). ^{1}H NMR (60 MHz): 3.57 (s, 3H, N1-CH_3), 3.77 (s, 3H, N3-CH_3), 4.57 (s, 2H, SCH_2), 7.98 (d, *J* = 8.8 Hz, 2H, 2`,6`Ar-H), 8.28 (d, *J* = 8.8 Hz, 2H, 3`,5`Ar-H), 11.16 (s, 1H, amide-H), 14.46 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-nitrophenyl)acetamide (62)

IR cm^{-1}: 3395 (N-H); 3280 (N-H amide); 3115 (Ar-H); 2875 (C-H aliphatic); 1709, 1688 (C=O); 1538 (N-H); 1488, 1326 (NO_2); 854 (Ar-H). ^{1}H NMR (60 MHz): 3.40 (s, 3H, N1-CH_3), 3.59 (s, 3H, N3-CH_3), 4.52 (s, 2H, SCH_2), 8.34 (d, *J* = 8.7 Hz, 2H, 2`,6`Ar-H), 8.76 (d, *J* = 8.7 Hz, 2H, 3`,5`Ar-H), 11.70 (s, 1H, amide-H), 14.58 (br s, 1H, N7-H).

N-(4-Hydroxyphenyl)-2-[(1,3-di-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (63)

IR cm^{-1}: 3436 (N-H); 3348 (broad O-H); 3255 (N-H amide); 3080 (Ar-H); 2955 (C-H aliphatic); 1673, 1626 (C=O); 1502 (N-H); 1256 (C-O); 813 (Ar-H). ^{1}H NMR (60 MHz): 3.52 (s, 3H, N1-CH_3), 3.70 (s, 3H, N3-CH_3), 4.39 (s, 2H, SCH_2), 7.24 (d, *J* = 8.9 Hz, 2H, 3`,5`Ar-H), 7.94 (d, *J* = 8.9 Hz, 2H, 2`,6`Ar-H), 9.66 (br s, 1H, 4`-OH), 10.64 (s, 1H, amide-H), 14.48 (br s, 1H, N7-H).

4-({[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-acetyl}amino)benzoic acid (64)

IR cm^{-1}: 3395 (N-H); 3336-2525 (broad O-H); 3250 (N-H amide); 3040 (Ar-H); 2870 (C-H aliphatic); 1702, 1664, 1633 (C=O); 1519 (N-H); 849 (Ar-H). ^{1}H NMR (60 MHz): 3.43 (s, 3H, N1-CH_3), 3.62 (s, 3H, N3-CH_3), 4.48 (s, 2H, SCH_2), 8.16 (d, J = 8.7 Hz, 2H, 3`,5`Ar-H), 8.43 (d, J = 8.7 Hz, 2H, 2`,6`Ar-H), 10.10 (br s, 1H, COOH), 11.28 (s, 1H, amide-H), 14.20 (br s, 1H, N7-H).

N-(4-Acetylphenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (65)

IR cm^{-1}: 3455 (N-H); 3240 (N-H amide); 3100 (Ar-H); 2975 (C-H aliphatic); 1711, 1682, 1664 (C=O); 1526 (N-H); 825 (Ar-H). ^{1}H NMR (60 MHz): 2.73 (s, 3H, 4`-$CH_3$CO), 3.47 (s, 3H, N1-$CH_3$), 3.64 (s, 3H, N3-$CH_3$), 4.54 (s, 2H, $SCH_2$), 8.18 (d, $J$ = 8.7 Hz, 2H, 2`,6`Ar-H), 8.48 (d, $J$ = 8.7 Hz, 2H, 3`,5`Ar-H), 11.26 (s, 1H, amide-H), 14.42 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(2-methylphenyl)acetamide (66)

IR cm^{-1}: 3465 (N-H); 3235 (N-H amide); 3105 (Ar-H); 2975 (C-H aliphatic); 1712, 1641, 1619 (C=O); 1529 (N-H); 744 (Ar-H). ^{1}H NMR (60 MHz): 2.31 (s, 3H, 2`-CH_3), 3.43 (s, 3H, N1-CH_3), 3.67 (s, 3H, N3-CH_3), 4.46 (s, 2H, SCH_2), 7.42-8.11 (m, 4H, Ar-H), 10.39 (s, 1H, amide-H), 14.59 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(2-methoxyphenyl)acetamide (67)

IR cm^{-1}: 3485 (N-H); 3260 (N-H amide); 3045 (Ar-H); 2875 (C-H aliphatic); 1690, 1673, 1639 (C=O); 1524 (N-H); 1250, 1043 (C-O); 740 (Ar-H). ^{1}H NMR (60 MHz): 3.50 (s, 3H, N1-CH_3), 3.66 (s, 3H, N3-CH_3), 4.07 (s, 3H, 2`-O$CH_3$), 4.54 (s, 2H, $SCH_2$), 7.50 (m, 3H, 3`,4`,5`Ar-H), 8.63 (m, 1H, 6`Ar-H), 10.16 (s, 1H, amide-H), 14.65 (br s, 1H, N7-H).

N-(2-Ethoxyphenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (68)

IR cm^{-1}: 3435 (N-H); 3260 (N-H amide); 3045 (Ar-H); 2880 (C-H aliphatic); 1694, 1649, 1638 (C=O); 1525 (N-H); 1228, 1038 (C-O); 738 (Ar-H). ^{1}H NMR (60 MHz): 1.35 (t, J = 7.5 Hz, 3H, $CH_2C\underline{H_3}$), 3.44 (s, 3H, N1-CH_3), 3.65 (s, 3H, N3-CH_3), 4.36 (q, J = 7.5 Hz, 2H, 2`-$OC\underline{H_2}CH_3$), 4.51 (s, 2H, $SCH_2$), 7.19-7.80 (m, 3H, 3`,4`,5`Ar-H), 8.57 (m, 1H, 6`Ar-H), 10.01 (s, 1H, amide-H), 14.58 (br s, 1H, N7-H).

N-(2-Chlorophenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (69)

IR cm^{-1}: 3455 (N-H); 3205 (N-H amide); 3035 (Ar-H); 2865 (C-H aliphatic); 1701, 1655, 1633 (C=O); 1525 (N-H); 748 (Ar-H). ^{1}H NMR (60 MHz): 3.44 (s, 3H, N1-CH_3), 3.65 (s, 3H, N3-CH_3), 4.54 (s, 2H, SCH_2), 7.43-8.43 (m, 4H, Ar-H), 10.54 (s, 1H, amide-H), 14.64 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(2-nitrophenyl)acetamide (70)

IR cm^{-1}: 3440 (N-H); 3325 (N-H amide); 3050 (Ar-H); 2945 (C-H aliphatic); 1690, 1640 (C=O); 1534 (N-H); 1488, 1328 (NO_2); 739 (Ar-H). ^1H NMR (60 MHz): 3.41 (s, 3H, N1-CH_3), 3.64 (s, 3H, N3-CH_3), 4.49 (s, 2H, SCH_2), 7.64-8.64 (m, 4H, Ar-H), 11.32 (s, 1H, amide-H), 14.54 (br s, 1H, N7-H).

N-(2-Hydroxyphenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (71)

IR cm^{-1}: 3400 (N-H); 3340 (broad O-H); 3270 (N-H amide); 3045 (Ar-H); 2860 (C-H aliphatic); 1693, 1637 (C=O); 1534 (N-H); 1249 (C-O); 739 (Ar-H). ^1H NMR (60 MHz): 3.52 (s, 3H, N1-CH_3), 3.77 (s, 3H, N3-CH_3), 4.52 (s, 2H, SCH_2), 7.38 (m, 3H, 3`,4`,5`Ar-H), 8.51 (m, 1H, 6`Ar-H), 10.14 (s, 1H, amide-H), 10.49 (br s, 1H, 2`-OH), 14.25 (br s, 1H, N7-H).

2-({[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-acetyl}amino)benzoic acid (72)

IR cm^{-1}: 3450 (N-H); 3320-2500 (broad O-H); 3228 (N-H amide); 3115 (Ar-H); 2904 (C-H aliphatic); 1696, 1666, 1628 (C=O); 1531 (N-H); 750 (Ar-H). ^1H NMR (60 MHz): 3.42 (s, 3H, N1-CH_3), 3.60 (s, 3H, N3-CH_3), 4.52 (s, 2H, SCH_2), 7.68 (m, 1H, 5`Ar-H), 8.14 (m, 1H, 4`Ar-H), 8.59 (d, J = 8.7 Hz, 1H, 3`Ar-H), 9.16 (d, $J$ = 8.7 Hz, 1H, 6`Ar-H), 10.46 (br s, 1H, COOH), 12.55 (s, 1H, amide-H), 14.20 (br s, 1H, N7-H).

N-(2-Acetylphenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (73)

IR cm^{-1}: 3430 (N-H); 3280 (N-H amide); 3145 (Ar-H); 2970 (C-H aliphatic); 1691, 1646, 1634 (C=O); 1515 (N-H); 750 (Ar-H). ^1H NMR (60 MHz): 2.82 (s, 3H, 2`-$CH_3CO$), 3.48 (s, 3H, N1-$CH_3$), 3.63 (s, 3H, N3-$CH_3$), 4.52 (s, 2H, $SCH_2$), 7.68 (m, 1H, 5`Ar-H), 8.10 (m, 1H, 4`Ar-H), 8.54 (d, $J$ = 8.7 Hz, 1H, 3`Ar-H), 9.01 (d, J = 8.7 Hz, 1H, 6`Ar-H), 12.46 (s, 1H, amide-H), 14.45 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(3-methylphenyl)acetamide (74)

IR cm^{-1}: 3465 (N-H); 3265 (N-H amide); 3045 (Ar-H); 2865 (C-H aliphatic); 1692, 1645 (C=O); 1528 (N-H); 782, 742 (Ar-H). ^1H NMR (60 MHz): 2.42 (s, 3H, 3`-CH_3), 3.46 (s, 3H, N1-CH_3), 3.64 (s, 3H, N3-CH_3), 4.45 (s, 2H, SCH_2), 7.22-8.07 (m, 4H, Ar-H), 10.86 (s, 1H, amide-H), 14.62 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(3-methoxyphenyl)acetamide (75)

IR cm^{-1}: 3440 (N-H); 3235 (N-H amide); 3065 (Ar-H); 2925 (C-H aliphatic); 1687, 1651 (C=O); 1547 (N-H); 1251, 1043 (C-O); 772, 735 (Ar-H). ^1H NMR (60 MHz): 3.53 (s, 3H, N1-CH_3), 3.71 (s, 3H, N3-CH_3), 4.07 (s, 3H, 3`-OCH_3), 4.26 (s, 2H,

SCH_2), 6.95-8.10 (m, 4H, Ar-H), 12.36 (s, 1H, amide-H), 14.18 (br s, 1H, N7-H).

N-(3-Chlorophenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (76)

IR cm^{-1}: 3440 (N-H); 3265 (N-H amide); 3040 (Ar-H); 2865 (C-H aliphatic); 1691, 1643 (C=O); 1521 (N-H); 782, 741 (Ar-H). ^1H NMR (60 MHz): 3.44 (s, 3H, N1-CH_3), 3.63 (s, 3H, N3-CH_3), 4.44 (s, 2H, SCH_2), 7.39-8.40 (m, 4H, Ar-H), 11.09 (s, 1H, amide-H), 14.39 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(3-nitrophenyl)acetamide (77)

IR cm^{-1}: 3455 (N-H); 3245 (N-H amide); 3045 (Ar-H); 2870 (C-H aliphatic); 1694, 1648 (C=O); 1521 (N-H); 1483, 1343 (NO_2); 800, 736 (Ar-H). ^1H NMR (60 MHz): 3.48 (s, 3H, N1-CH_3), 3.67 (s, 3H, N3-CH_3), 4.55 (s, 2H, SCH_2), 7.87-9.34 (m, 4H, Ar-H), 11.45 (s, 1H, amide-H), 14.50 (br s, 1H, N7-H).

N-(3-Hydroxyphenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (78)

IR cm^{-1}: 3420 (N-H); 3350 (broad O-H); 3190 (N-H amide); 3045 (Ar-H); 2860 (C-H aliphatic); 1707, 1639 (C=O); 1536 (N-H); 1220 (C-O); 779, 739 (Ar-H). ^1H NMR (60 MHz): 3.46 (s, 3H, N1-CH_3), 3.62 (s, 3H, N3-CH_3), 4.44 (s, 2H, SCH_2), 6.72-7.92 (m, 4H, Ar-H), 9.96 (s, 1H, amide-H), 10.78 (s, 1H, 3`-OH), 14.46 (br s, 1H, N7-H).

N-(2-Acetyl-4-bromophenyl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (79)

IR cm^{-1}: 3450 (N-H); 3164 (N-H amide); 3060 (Ar-H); 2880 (C-H aliphatic); 1691, 1643 (C=O); 1539 (N-H); 837 (Ar-H). ^1H NMR (60 MHz): 2.82 (s, 3H, 2`-$CH_3CO$), 3.48 (s, 3H, N1-$CH_3$), 3.64 (s, 3H, N3-$CH_3$), 4.54 (s, 2H, $SCH_2$), 8.31 (dd, *J* = 10, 3 Hz, 1H, 5`Ar-H), 8.66 (d, *J* = 2 Hz, 1H, 3`Ar-H), 8.94 (d, *J* = 8.9 Hz, 1H, 6`Ar-H), 12.50 (s, 1H, amide-H), 14.68 (br s, 1H, N7-H).

N-Benzyl-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (80)

IR cm^{-1}: 3475 (N-H); 3260 (N-H amide); 3045 (Ar-H); 2860 (C-H aliphatic); 1704, 1638 (C=O); 1532 (N-H); 738, 691 (Ar-H). ^1H NMR (60 MHz): 3.50 (s, 3H, N1-CH_3), 3.63 (s, 3H, N3-CH_3), 4.31 (s, 2H, SCH_2), 4.63 (d, *J* = 7 Hz, 2H, $HNC\underline{H_2}$), 7.77 (s, 5H, Ar-H), 9.29 (t, *J* = 6 Hz, 1H, amide-H), 14.42 (br s, 1H, N7-H).

(±)-2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(1-phenylethyl)acetamide (81)

IR cm^{-1}: 3465 (N-H); 3260 (N-H amide); 3050 (Ar-H); 2870 (C-H aliphatic); 1701, 1633 (C=O); 1536 (N-H); 742, 690 (Ar-H). ^1H NMR (60 MHz): 1.49 (d, *J* = 7 Hz, 3H, $CHC\underline{H_3}$), 3.50 (s, 3H, N1-CH_3), 3.64 (s, 3H, N3-CH_3), 4.26 (s, 2H, SCH_2), 5.30 (m, 1H, $HNC\underline{H}CH_3$), 7.77 (s, 5H, Ar-H), 9.26 (d, *J* = 7 Hz, 1H, amide-H), 14.43 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(2-phenylethyl)acetamide (82)

IR cm^{-1}: 3475 (N-H); 3265 (N-H amide); 3050 (Ar-H); 2945 (C-H aliphatic); 1693, 1646 (C=O); 1537 (N-H); 739, 692 (Ar-H). ^{1}H NMR (60 MHz): 2.91 (t, *J* = 7 Hz, 2H, $CH_2C\underline{H}_2Ph$), 3.48 (s, 3H, N1-CH_3), 3.65 (s, 3H, N3-CH_3), 3.68 (m, 2H, $HNC\underline{H}_2$ CH_2), 4.18 (s, 2H, SCH_2), 7.74 (s, 5H, Ar-H), 8.82 (t, *J* = 6 Hz, 1H, amide-H), 14.44 (br s, 1H, N7-H).

N-Cyclohexyl-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (83)

IR cm^{-1}: 3435 (N-H); 3270 (N-H amide); 2900 (C-H aliphatic); 1706, 1633 (C=O); 1535 (N-H). ^{1}H NMR (60 MHz): 0.74-2.17 (m, 10H, cyclohexyl-$(CH_2)_5$), 3.49 (s, 3H, N1-CH_3), 3.74 (s, 4H, N3-CH_3& $HNC\underline{H}$), 4.23 (s, 2H, SCH_2), 8.64 (d, *J* = 7 Hz, 1H, amide-H), 14.44 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-1-naphthylacetamide (84)

IR cm^{-1}: 3460 (N-H); 3210 (N-H amide); 3040 (Ar-H); 2875 (C-H aliphatic); 1696, 1641 (C=O); 1532 (N-H); 782 (Ar-H). ^{1}H NMR (60 MHz): 3.48 (s, 3H, N1-CH_3), 3.66 (s, 3H, N3-CH_3), 4.64 (s, 2H, SCH_2), 7.75-8.80 (m, 7H, Ar-H), 10.93 (s, 1H, amide-H), 14.49 (br s, 1H, N7-H).

N-(1H-Benzimidazol-2-yl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (85)

IR cm^{-1}: 3412 (N-H); 3170 (N-H amide); 3055 (Ar-H); 2940 (C-H aliphatic); 1694, 1644, 1628 (C=O); 1536 (N-H). ^{1}H NMR (60 MHz): 3.52 (s, 3H, N1-CH_3), 3.71 (s, 3H, N3-CH_3), 4.68 (s, 2H, SCH_2), 7.55-8.47 (m, 4H, Ar-H), 9.68 (br s, 1H, amide-H), 10.03 (br s, 1H, N1`-H), 14.76 (br s, 1H, N7-H).

N-(1,3-Benzothiazol-2-yl)-2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide (86)

IR cm^{-1}: 3390 (N-H); 3120 (N-H amide); 3040 (Ar-H); 2930 (C-H aliphatic); 1687, 1644 (C=O); 1539 (N-H). ^{1}H NMR (60 MHz): 3.44 (s, 3H, N1-CH_3), 3.62 (s, 3H, N3-CH_3), 4.61 (s, 2H, SCH_2), 7.54-8.61 (m, 4H, Ar-H), 13.55 (s, 1H, amide-H), 14.65 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)acetamide (87)

IR cm^{-1}: 3472 (N-H); 3315 (N-H amide); 3065 (Ar-H); 2960 (C-H aliphatic); 1696, 1643 (C=O); 1618 (C=C); 1517 (N-H). ^{1}H NMR (60 MHz): 3.26 (s, 3H, N3`-$CH_3$), 3.32 (s, 3H, N1`-CH_3), 3.44 (s, 3H, N1-CH_3), 3.50 (s, 3H, N3-CH_3), 4.91 (s, 2H, SCH_2), 8.35 (s, 1H, pyrimidinyl-CH), 9.91 (br s, 1H, amide-H), 14.62 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(pyridin-2-yl)acetamide (88)

IR cm^{-1}: 3405 (N-H); 3170 (N-H amide); 3080 (Ar-H); 2910 (C-H aliphatic); 1696, 1637 (C=O); 1555 (C=N); 1500 (N-H); 756 (Ar-H). ^1H NMR (60 MHz): 3.49 (s, 3H, N1-CH_3), 3.69 (s, 3H, N3-CH_3), 5.05 (s, 2H, SCH_2), 7.70-8.90 (m, 4H, pyridyl-H), 10.47 (br s, 1H, amide-H), 14.28 (br s, 1H, N7-H).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-methyl-N-phenylacetamide (89)

IR cm^{-1}: 3465 (N-H); 3060 (Ar-H); 2945 (C-H aliphatic); 1694, 1648 (C=O); 1541 (N-H); 732, 699 (Ar-H). ^1H NMR (60 MHz): 3.46 (s, 3H, N1-CH_3), 3.52 (s, 3H, $PhNCH_3$) 3.64 (s, 3H, N3-CH_3), 4.35 (s, 2H, SCH_2), 8.01 (s, 5H, Ar-H), 14.58 (br s, 1H, N7-H).

1,3-Dimethyl-8-{[2-oxo-2-(4-phenyl-piperazino)ethyl]thio}-2,3,6,7-tetra-hydro-1H-2,6-purinedione (90)

IR cm^{-1}: 3505 (N-H); 3035 (Ar-H); 2865 (C-H aliphatic); 1691, 1632 (C=O); 1530 (N-H); 739, 688 (Ar-H). ^1H NMR (60 MHz): 3.39 (m, 4H, $(CH_2C\underline{H_2})_2NPh$), 3.45 (s, 3H, N1-$CH_3$), 3.67 (s, 3H, N3-$CH_3$), 3.92 (m, 4H, $(CH_2C\underline{H_2})_2NCO$), 4.70 (s, 2H, SCH_2), 7.14-8.06 (m, 5H, Ar-H), 14.38 (br s, 1H, N7-H).

Pharmacology

In-vivo studies on guinea pig tracheal smooth muscles

The method of Kesler and Canning[53] was utilized with minor modifications[54]. Male Hartley guinea pigs (300-400 g, House of Laboratory Animals, Faculty of Medicine, Assiut University) were anaesthetized with urethane (1 g/kg *ip*) and positioned ventral side up on a wooden pad. The trachea was connected to a pump for artificial respiration, stainless steel hooks were passed between two cartilage rings on either side of the trachea, one hook was sutured to a fixed bar and the other hook was sutured to an isometric force transducer (Universal oscillograph, Harvard, Fircroft way. Edenbridge. Kent.).

When the animals were stabilized, a bronchospasm was stimulated with acetylcholine (0.2 mg/kg *ip*). After two similar responses to spasm inducing injections, target compounds (dissolved in distilled water with a minimal amount of 1 N NaOH) or aminophylline as a reference drug were administered (2.5-10 mg/kg *ip*), acetylcholine was administered again three to five minutes later. The effects of the test compounds were evaluated with reference to the percentage reduction of the induced broncho-constriction. At the end of each experiment, animals were killed by cervical dislocation.

Acute toxicity

Groups of male adult albino mice (18-22 g, House of Laboratory Animals, Faculty of Medicine, Assiut University), each of five animals, were injected *ip* with 4 graded doses of the test compounds suspended in 0.5% carboxymethylcellulose. The

LD_{50} values were calculated according to Litchfield and Wilcoxon's method[55].

Microbiology

The antibacterial activity of all the target compounds was investigated *in-vitro* against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae* (clinical isolates obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University) using agar cup diffusion method[56] for susceptibility screening, and twofold dilution method[57] for MIC determination. Ampicillin was used as a reference drug, and DMSO was used as a solvent control.

Agar cup diffusion method

38 Grams of Mueller-Hinton agar medium (MH) (Hi-Media, M 001) were added to 1 L of distilled water, heated to boiling to dissolve the ingredients completely, and sterilized by autoclaving at 121°C for 30 minutes. High density inocula were made by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard unit with average turbidity 10^8 CFU/mL[58]. The sterile Petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50°C) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using sterile cork borer. One hundred µL of the tested compounds or ampicillin sodium (20 mg/mL in DMSO), or the blank solvent, were added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 hrs then the average diameters of the inhibition zones were measured in millimeters.

Minimum inhibitory concentration

The MIC was determined using twofold dilution method[57] for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog[59].

Receptor building and pharmacophore identification

All the computational works were carried out at the Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Receptor building and pharmacophore identification were performed on Molecular Operating Environment (MOE) version 2007.09, Chemical Computing Group Inc., 1010 Sherbrooke St. West, Suite 910, Montreal, Quebec, H3A 2R7, Canada. The program operated under "Microsoft Windows XP" operating system installed on an Intel Pentium IV PC with a 2.8 GHz processor and 512 Mb of RAM.

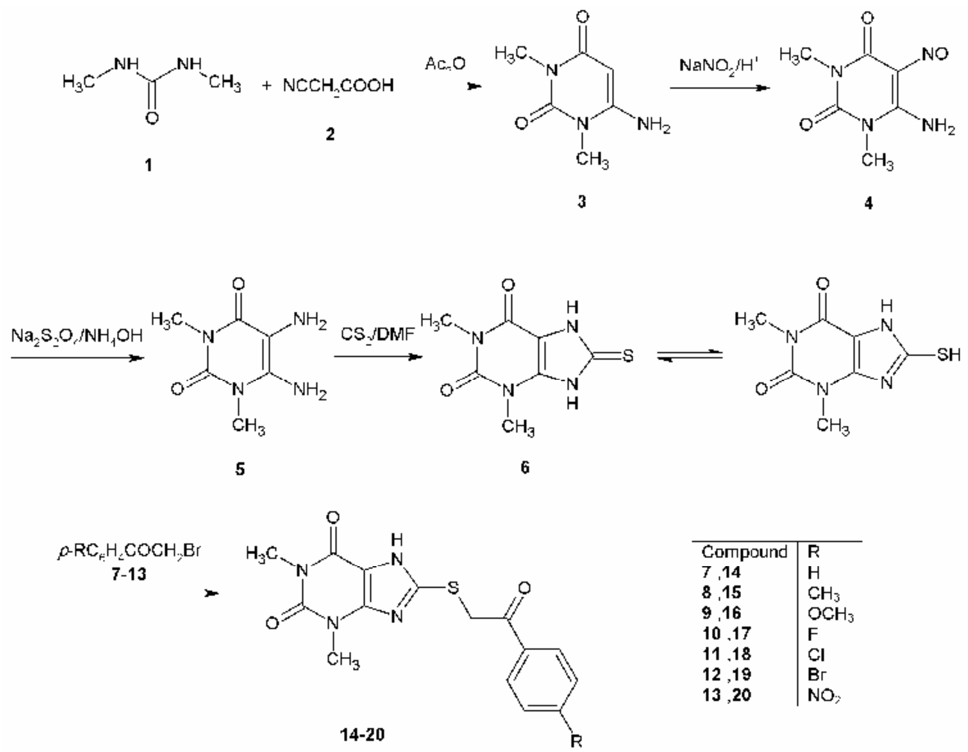
RESULTS AND DISCUSSION

Chemistry

The target compounds (**14-20**, **56-90**) were prepared by the reaction between 8-mercaptotheophylline (**6**)[29-31] with the appropriate synthetic reagents.

8-Mercaptotheophylline (**6**) has two tautomeric forms (thione-thiol tautomers) (Scheme 1), many reports about thione-thiol tautomerism in purine nucleus proved the existence of mercaptopurines and related compounds preferentially in the thione form, both in solution and in the solid state[60]. Here also, the thione form is the predominant tautomer, and that was confirmed by IR which showed two N-H stretching bands at 3455 and 3330 cm^{-1}, C=S stretching band at 1226 cm^{-1}, and the absence of an absorption at about 2600-2550 cm^{-1} region cited for SH group[61]. ^{1}H NMR showed two singlet signals at δ 3.16 and δ 3.35 ppm each equivalent to three protons characteristic to N1 and N3 methyl groups respectively, and two broad singlet signals at δ 12.97 and δ 13.39 ppm characteristic to N9-H and N7-H respectively. Also, ^{13}C NMR revealed the signal at δ 164.25 ppm corresponding to the thioketone group at C8 that is more deshielded than its usual value around δ 140 ppm, and it disappeared when applying DEPT technique. The EI-MS of compound **6** showed the molecular ion peak at *m/z* 212, and the base peak at *m/z* 99.

Compounds **14-20** were prepared by the interaction of equimolar amounts of compound **6** and the appropriate *p*-(un)substituted phenacylbromides (**7-13**) under the conditions of Schotten-Baumann reaction[62] (Scheme 1). Alkylation is thought to occur at the sulfur atom rather than N7 due to the greater nucleophilicity of sulfur atom, low temperature of the reaction, use of equimolar equivalent of alkylating agent, and aqueous medium[63&64]. Structures of compounds **14-20** were proved by IR, ^{1}H NMR, HRMS as well as by elemental analyses. EI-mass spectra of these compounds showed molecular ion peaks in agreement with their molecular formulae, and behaved in similar fragmentation patterns. It is noteworthy to mention that these compounds showed a fragment at $M^{+\cdot}$-18 corresponding to loss of a molecule of water with moderate intensity, the expulsion of a water molecule from the 8-aroylmethyl-thioxanthine series is not familiar, but can be explained by cyclodehydration mechanism through the removal of N7-hydrogen atom and one of the methylene group hydrogens together with the carbonyl group oxygen, and formation of thiazolo[2,3-*f*]xanthine derivatives (Fig. 1). This explanation is based on that these derivatives could be prepared actually by the same mechanism through using different dehydrating agents like phosphorus oxychloride ($POCl_3$)[29],



Scheme 1: Synthetic pathway for the preparation of compounds **14-20**.

ethanolic HCl[34], glacial acetic acid, and polyphosphoric acid (PPA)[65&66]. Proposed fragmentation pattern of compound **15** was chosen as a representative example and shown in Fig. 1.

Alkylation of **6** with *N*-(substituted)aryl/aralkyl/cycloalkyl/ heteroaryl-2-chloroacetamides (**21-53**)[36-52] in presence of aqueous ethanolic NaOH 1% furnished 2-[(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-acetamides (**56-88**) (Scheme 2). Also, 2-[(1,3-dimethyl-xanthin-8-yl)thio]-*N*-methyl-*N*-phenyl acetamide (**89**) and 1,3-dimethyl-8-{[2-oxo-2-(4-phenyl-piperazino)ethyl] thio}xanthine (**90**) were prepared by reaction of **6** with *N*-methyl-*N*-phenyl-2-chloroacetamide (**54**) or 2-chloro-1-(4-phenylpiperazin-1-yl)-ethanone (**55**) respectively (Scheme 2). Structures of **56-90** were proved by IR, ^{1}H NMR, and elemental analyses. IR spectra showed the appearance of a new N-H stretching band around 3260 cm^{-1} characteristic for monosubstituted amides, and absence of it in case of disubstituted amides (compounds **89** and **90**), in addition to the original N7-H stretching band of xanthine around 3455 cm^{-1}, presence of strong absorption bands at 1712-1619 cm^{-1}

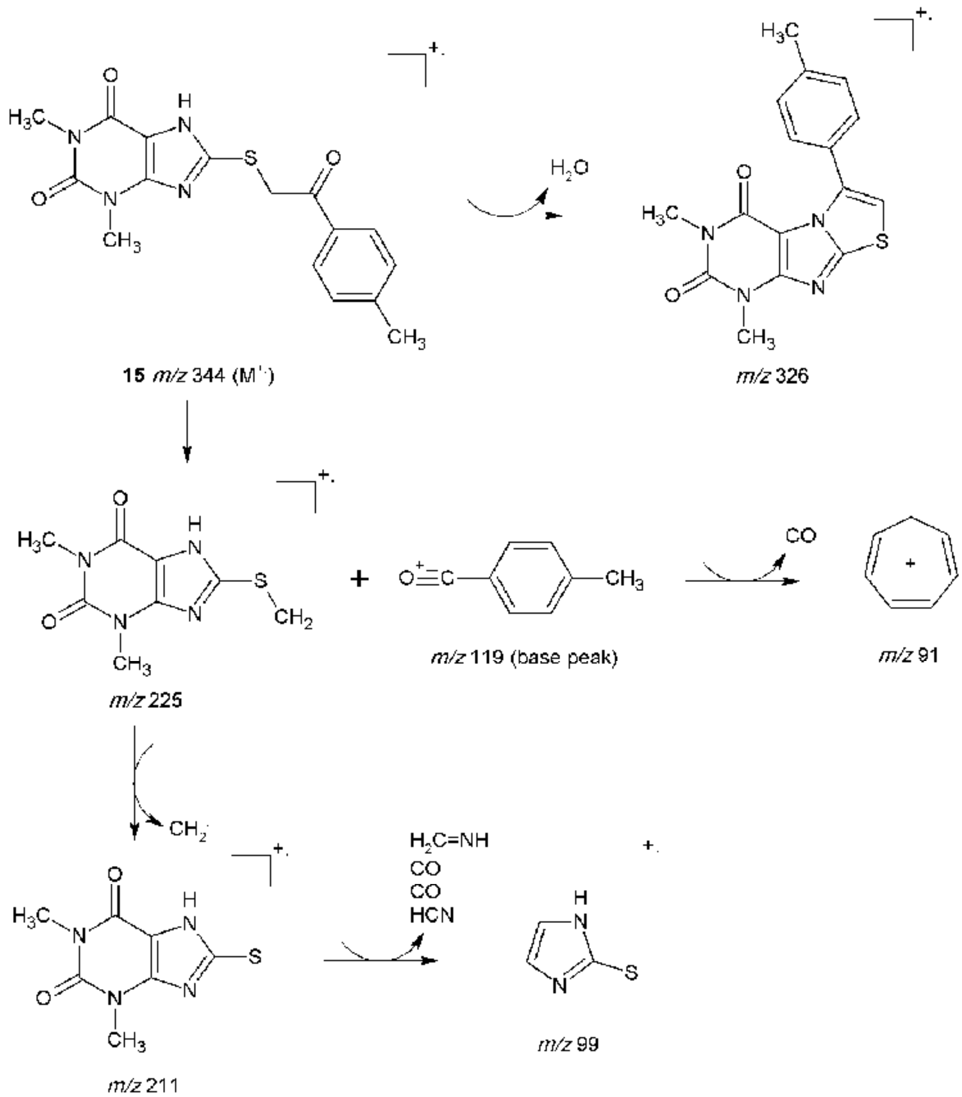
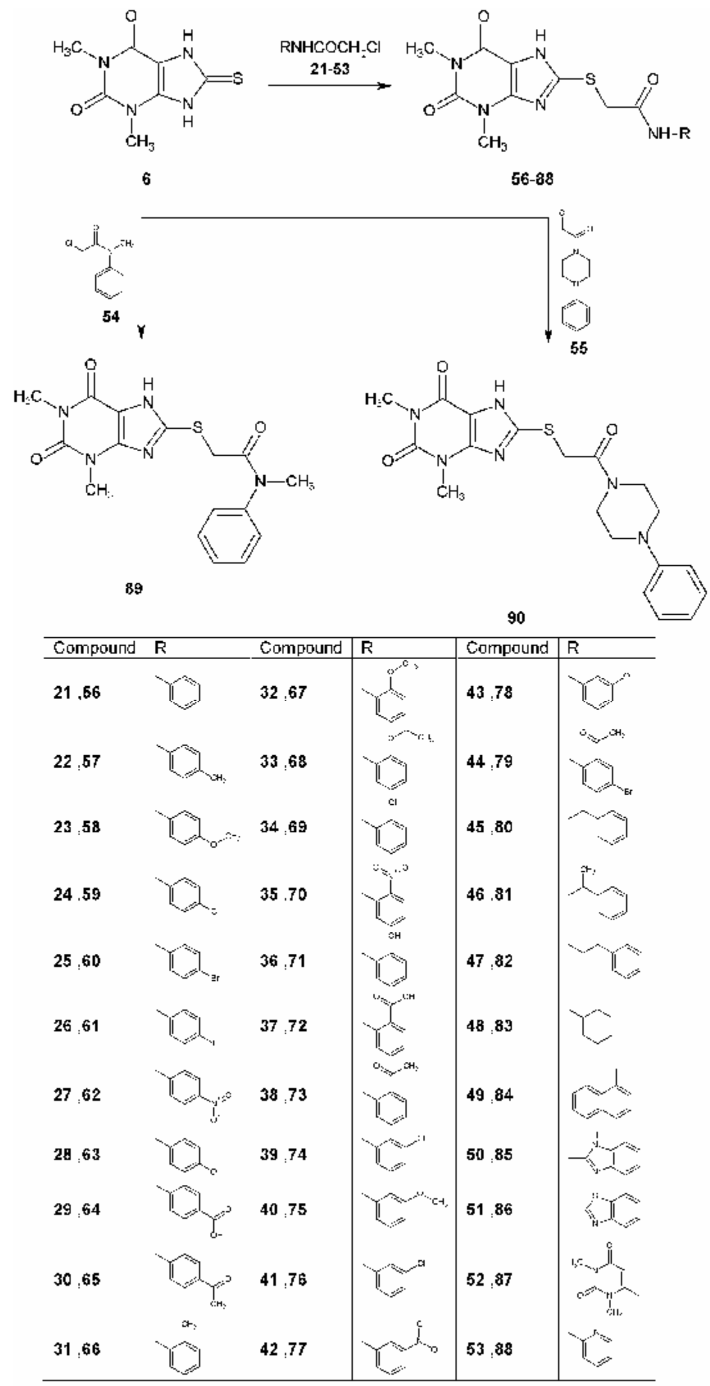


Fig. 1: Proposed mass fragmentation pattern of compound **15**.



Scheme 2: Synthetic pathway for the preparation of compounds **56-90**.

corresponding to C=O stretching bands, and also absence of C=S stretching band. ^1H NMR spectra showed the appearance of a singlet around δ 4.48 ppm corresponding to -S-CH_2- protons, also, singlet around δ 10.66 ppm equivalent to one proton corresponding to the monosubstituted amide group, and appearance of only one broad singlet around δ 14.49 ppm corresponding to N7-H. A multiplet at δ 6.92-8.94 ppm corresponding to the aromatic protons of most of the derivatives was also appeared.

Pharmacology

Thirty of the synthesized compounds were investigated for *in-vivo* anti-bronchospatic activity on acetylcholine induced bronchospasm in anaesthetized guinea-pigs according to Kesler and Canning method[53] in comparison to aminophylline as a reference drug. The anti-bronchoconsrictive effect was expressed as percentage inhibition (mean ± SEM) of bronchospasm for three doses (2.5, 5, and 10 mg/kg body weight), ID_{50} value (the dose of the drug causing 50% inhibition of bronchospasm) in each case was calculated by linear regression. Results are shown in Table 2.

Table 2: Inhibitory effect of compounds **6**, **14-18, 20, 56-60, 62, 64, 67, 68, 70, 72, 75, 77, 79-86, 89** & **90** and aminophyllin on acetylcholine induced bronchospasm in anaesthetized guinea-pigs.

Compound	Dose (mg/kg) *ip*	% Decrease of acetylcholine induced bronchospasm in guinea pigs	ID_{50} (mg/kg) *ip*
6	2.5 5 10	26.1±1.4 44.2±1.2 68.5±0.9	6.5
14	2.5 5 10	18.4±1.2 38.6±2.6 62.1±1.9	7.6
15	2.5 5 10	28.3±2.1 53.3±1.8 88.2±2	5
16	2.5 5 10	22.1±1.2 47.4±1.9 76±2	6
17	2.5 5 10	6.2±0.6 13±1.2 19.7±1.5	>20
18	2.5 5 10	14.7±1.2 26.7±1.9 42.9±1.1	11.8

Table 2: Continued

Compound	Dose (mg/kg) *ip*	% Decrease of acetylcholine induced bronchospasm in guinea pigs	ID_{50} (mg/kg) *ip*
20	2.5 5 10	8.1±0.7 14.6±1.4 25±1.4	>20
56	2.5 5 10	2±0.2 8±0.7 12.5±0.6	>20
57	2.5 5 10	- 3.3±0.1 5±0.3	>20
58	2.5 5 10	10.9±1 28±1.5 51±1	9.6
59	2.5 5 10	25.8±1.4 46.2±1.5 76±1.2	5.9
60	2.5 5 10	19.7±1.3 42.7±1.6 68.6±1.6	6.8
62	2.5 5 10	19±0.5 35±1.1 55.1±1.8	8.7
64	2.5 5 10	20.8±1.2 38.5±1.2 60.1±1.4	7.8
67	2.5 5 10	21.6±1.2 40.6±1.6 65.3±1.5	7.1
68	2.5 5 10	20.5±1.2 44.5±1.3 69.2±1.8	6.6
70	2.5 5 10	20.3±1.1 39.9±1.3 61.5±1.7	7.6
72	2.5 5 10	26.8±1.3 47.9±1.6 77±1.4	5.7
75	2.5 5 10	13.8±0.8 30.4±1.6 48.9±1.5	10

Table 2: Continued

Compound	Dose (mg/kg) *ip*	% Decrease of acetylcholine induced bronchospasm in guinea pigs	ID_{50} (mg/kg) *ip*
77	2.5 5 10	20.1±1.5 36.5±1.6 56.6±2	8.4
79	2.5 5 10	2.5±0.2 9.9±0.9 17.7±1.3	>20
80	2.5 5 10	18.7±1.2 41.7±1.7 65.4±1.5	7.1
81	2.5 5 10	20.5±1.2 40.1±1.5 63.9±1.8	7.3
82	2.5 5 10	19.7±1.3 36.4±1.4 52.3±1.5	9.1
83	2.5 5 10	20.9±1.2 45.8±1.7 74.9±1.8	6.2
84	2.5 5 10	- 9±0.9 13±1.2	>20
85	2.5 5 10	21.2±1.6 38.5±1.7 61.8±1.9	7.6
86	2.5 5 10	15.8±1 34.9±1.4 55.1±1.3	8.7
89	2.5 5 10	20.4±1.3 38.5±1.6 61.8±1.6	7.6
90	2.5 5 10	11.7±1.1 18.6±1.1 25.6±1.2	>20
Aminophylline	2.5 5 10	22.5±1.1 48.6±1.4 78.8±1.1	5.8

The data were presented as mean ± SEM (*n*= 5).

Fifteen compounds (**6**, **14**, **15**, **16**, **59**, **60**, **67**, **68**, **70**, **72**, **80**, **81**, **83**, **85** and **89**) exhibited an anti-bronchoconstrictive activity nearly similar to that of aminophylline. Compounds **15**, **16**, **59**, and **72** showed either more significant or equivalent effect to aminophylline.

In view of these results, presence of free mercapto group at 8-position in compound **6** (ID_{50} 6.5 mg/kg) does not inhibit significantly the broncho-dilator activity of the parent drug (theophylline) (ID_{50} 5.8 mg/kg).

In the 8-aroylmethylthioxanthine series (compounds **14**-**20**), introd-uction of *p*-methyl function (compound **15**) or *p*-methoxy group (compound **16**) presents the most active compounds (ID_{50} values: 5 mg/kg**,** and 6 mg/kg respectively), while presence of the strong electron withdrawing groups (compounds **17** and **20**) highly decrease the bronchodilator activity (ID_{50} values: >20 mg/kg).

Regarding the results of *N*-(substituted)phenyl-2-(theophyllin-8-ylthio)acetamide series (**56-79**), it can be concluded that among the various para-substituted phenyl groups, only the *p*-chloro (**59**) and *p*-bromo (**60**) derivatives have significant activity (ID_{50} values: 5.9 and 6.8 mg/kg respectively). All the ortho-substituted derivatives (**67**, **68**, **70**, and **72**) are also of significant activity (ID_{50} values: 7.1, 6.6, 7.6, and 5.7 mg/kg respectively), while the meta-substituted derivatives (**75** and **77**) showed moderate activity (ID_{50} values: 10 and 8.4 mg/kg respectively). The 2,4-disubstituted derivative (**79**) has a very weak activity (ID_{50} >20 mg/kg). Accordingly, the best position for substitution at the phenyl group is the ortho position that leads to active derivatives. Activity may be due to non planar orientation of the phenyl group with the 2-(theophyllin-8-ylthio)acetamide moiety due to presence of a bulky group at the ortho position. This assumption is in agreement with that of Baziard-Mouysset *et al.*[20] for the bronchodilator activity of various 8-substituted theophylline derivatives.

Results of *N*-aralkyl/cyclohexyl/naphthyl/heteroaryl (**80-88**) and *N*,*N*-disubstituted derivatives (**89** and **90**) indicate that the *N*-benzyl (**80**), *N*-1-phenylethyl (**81**), *N*-cyclohexyl (**83**), *N*-1*H*-Benzimidazol-2-yl (**85**), and *N*-methyl-*N*-phenyl (**89**) derivatives showed significant activity (ID_{50} values: 7.1, 7.3, 6.2, 7.6, and 7.6 mg/kg respectively), and all of them retain non planar structures. The rest of compounds, especially *N*-1-naphthyl (**84**) and 8-(4-phenylpipera-zinocarbonylmethylthio)theophylline (**90**), are of weak activity (ID_{50} values: >20 mg/kg) which may be attributed to their bulky substituents and steric interaction with the receptor binding site.

Acute toxicity (LD_{50}) study was performed in mice via intraperitoneal (*ip*) injection for the most active derivatives (compounds **15**, **16**, **59**, and **72**) and compared to amino-phylline as a reference drug. The obtained experimental data showed that all the test compounds didn't record significant toxicity with LD_{50} = 300 mg/kg in comparison with the standard drug aminophylline LD_{50} =

180 mg/kg[67] (Table 3). The maximal toxicity was observed after 12 hrs, when the animals showed decreased muscle tone and laboured respiration signs.

Table 3: Acute toxicity in mice following intraperitoneal injection of compounds **15**, **16**, **59**, and **72**.

Compound	$LD_{50}{}^{a}$ (mg/kg)
15	300
16	300
59	300
72	300
Aminophylline	180^{b}

(*a*): LD_{50} was calculated on the number of animals showing decreased muscle tone, and laboured respiration signs.
(*b*): as reported[67]

Microbiology

Antibacterial activity of all the synthesized target compounds was investigated *in-vitro* against the Gram-positive bacteria methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus cereus*, and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* using the agar diffusion assay[56]. MIC of the active compounds also calculated in comparison to ampicillin as a reference drug (Table 4).

Analysis of the results showed that compound **6** (MIC 77 μg/mL) has an equipotent activity with respect to ampicillin (MIC 69 μg/mL) against MRSA, and a moderate activity against the other bacterial strains (MIC 153-168 μg/mL).

Table 4: Antibacterial activity of the test compounds.

Compound	*In-vitro* activity-inhibition zone in mm (MIC in μg/mL)			
	Methicillin resistant *Staphylococcus aureus* (MRSA)	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*
6	20 (77)	13 (168)	14 (160)	16 (153)
14	21 (73)	-	-	-
15	20 (76)	-	-	-
16	22 (70)	25 (50)	23 (61)	26 (40)
17	26 (39.1)	27 (31.6)	28 (25)	23 (60)
18	20 (75)	-	-	-
19	26 (40)	28 (31.6)	23 (63.1)	21 (70)
20	21 (73)	15 (158)	12 (173)	11 (178)
56	-	-	-	-
57	20 (76)	13 (169)	11 (177)	7 (195)
58	-	-	-	-

Table 4: Continued

Compound	*In-vitro* activity-inhibition zone in mm (MIC in µg/mL)			
	Methicillin resistant *Staphylococcus aureus* (MRSA)	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*
59	-	-	-	-
60	15 (158)	-	-	-
61	-	-	-	-
62	-	-	-	-
63	17 (126)	15 (158)	16 (154)	15 (158)
64	-	-	-	-
65	-	-	-	-
66	-	-	-	-
67	-	-	-	-
68	16 (153)	17 (126)	20 (76)	21 (73)
69	15 (158)	13 (166)	11 (177)	15 (158)
70	-	-	-	-
71	-	-	-	-
72	20 (75)	22 (63.1)	18 (79)	17 (125)
73	14 (160)	13 (168)	12 (170)	15 (157)
74	16 (155)	17 (125)	16 (155)	12 (170)
75	-	-	-	-
76	15 (158)	-	-	-
77	14 (161)	13 (166)	-	-
78	20 (77)	19 (86)	15 (157)	12 (171)
79	-	-	-	-
80	14 (161)	-	-	-
81	-	-	-	-
82	-	-	-	-
83	-	-	-	-
84	16 (154)	23 (61)	-	-
85	-	-	-	-
86	17 (125)	22 (70)	15 (158)	-
87	-	-	-	-
88	-	-	-	-
89	19 (85)	20 (75)	21 (73)	20 (76)
90	-	-	-	-
Ampicillin	20 (69)	22 (60)	23 (50)	20 (70)
DMSO	-	-	-	-

(-): means no antibacterial activity at the studied concentration.

Regarding the 8-aroylmethylthio-xanthine series (compounds **14-20**), all the compounds exhibited comparable or better activities (MIC 39.1-76 µg/mL) against MRSA than that of ampicillin. Compounds **16**, **17**, and **19** were the most active compounds (MIC 25-70 µg/mL) against all the test bacterial strains, and even more potent than ampicillin (MIC 50-70 µg/mL). Compounds **14**, **15**, **18**, and **20** have weak activity (MIC 158-178 µg/mL) or inactive against *B. cereus* and all the Gram-negative bacteria strains.

Results of compounds **56-90** revealed that compounds **72** and **89** showed a significant activity (MIC 63.1-125 µg/mL) against all the tested bacterial strains. Compounds **57**, **63**, **68**, **69**, **73**, **74**, **78**, and **86** showed a moderate activity (MIC 70-195 µg/mL) against both Gram-positive and Gram-negative bacterial strains, while compounds **60**, **76**, **77**, and **84** showed a moderate activity (MIC 154-166 µg/mL) against only Gram-positive bacteria (MRSA and *B. cereus*). It is noteworthy to mention that compound **84** was equipotent to ampicillin against *B. cereus* (MIC 61 µg/mL).

Receptor building and pharmacophore identification

Since the actual molecular mechanism of action of xanthine derivatives as bronchodilators is still controversial[68&69], inhibition of phosphodiesterase III and IV isoenzymes relaxes smooth muscles in pulmonary arteries and air ways[70], whereas antagonists of adenosine A_{2B} receptor proposed to have potential use as antiasthmatic agents[71]. However, it is necessary to guess the important attributes of the active site to design better drugs. One way to suggest the properties of the active sites is to assume that they are complementary to active lead molecules. Before the receptor model can be built, the lead molecules must be aligned so that the active functional groups of the molecules are overlapping in space. All the computational works were performed on Molecular Operating Environment (MOE) version 2007.09, Chemical Computing Group Inc., software. Thirteen reported active ligands (compounds **a-m**)[19-21&72] (Fig. 2), were selected as the training set. Two of them, theophylline (**a**) and bamifylline (**b**), are in therapeutic use. They were sketched using molecular builder of MOE, and each structure was subjected to energy minimization up to gradient of 0.01 Kcal/mol Å using the MMFF94 force field. The training set molecules were aligned using MOE's Flexible Alignment. Alignment had the lowest strain energy, U, and the highest S value was selected to build the receptor model and the pharmacophore query.

Partial charges were computed using Gasteiger (PEOE) charges method. Molecular surface was computed and was shown in Figure 3. The receptor would have complementary regions to the color shown (rose, H-bonding; green, hydrophobic; blue, mild polar). The

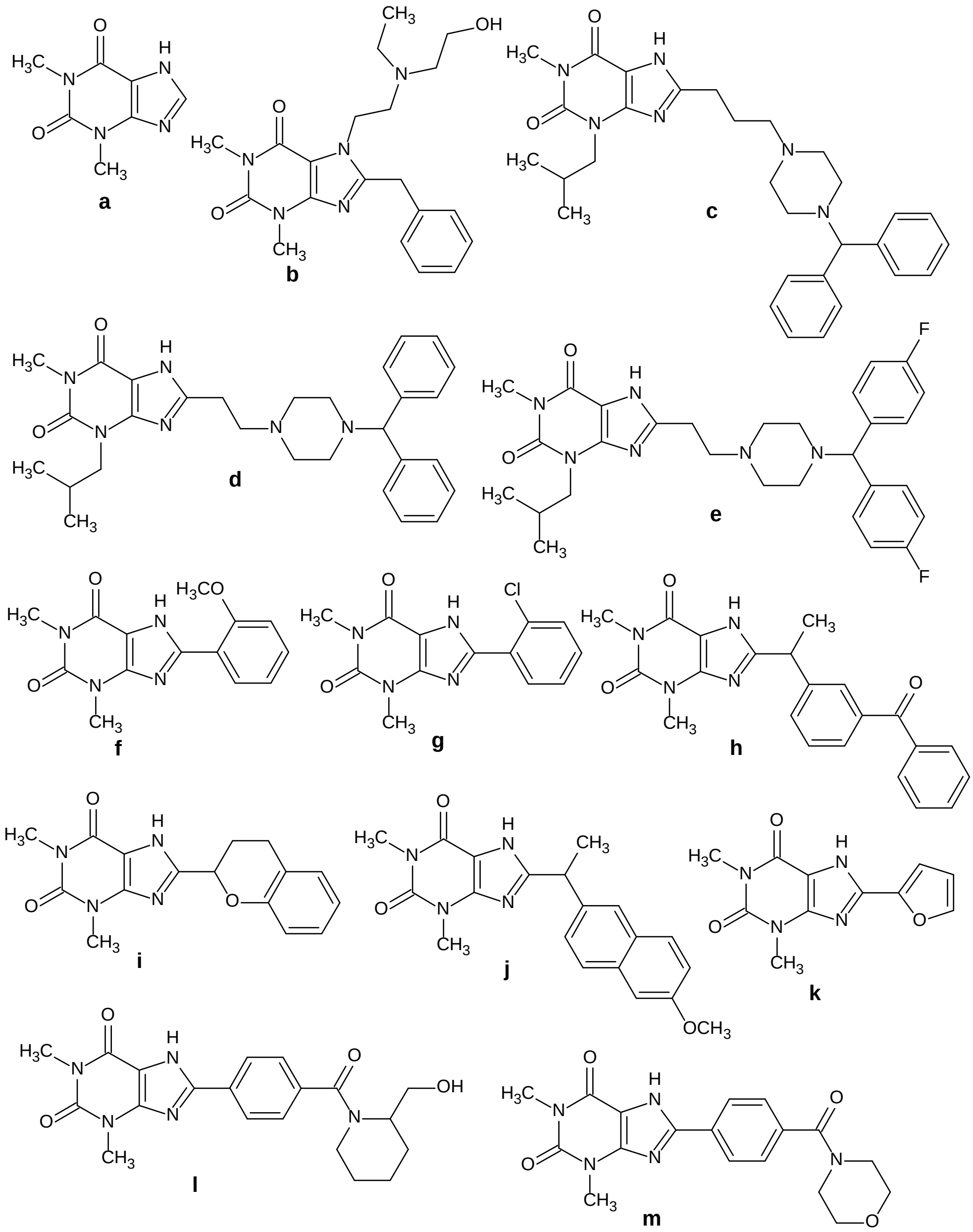


Fig. 2: Structures of the selected compounds (**a-m**) as the training set molecules.

hydrophobic (green) region of ligand surface should be corresponding to hydrophobic area at the receptor pocket. Regions colored rose shows the location of the lone pairs on the carbonyls or hydroxyl group on the ligands. The receptor model would have hydrogen bond donors placed to interact with the lone pairs on the ligand.

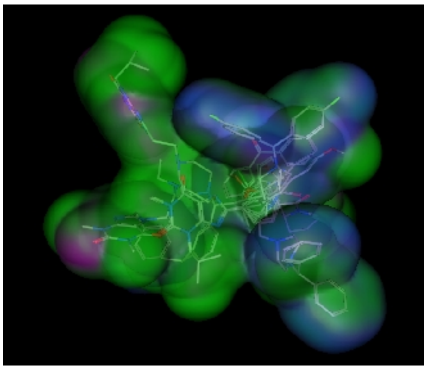


Fig. 3: Molecular surface of the training set.

Electrostatic map (Fig. 4) was also computed, it is calculated using Poisson Boltzmann equation[73]. The resulting contours are prediction for the type of interactions that might stabilize ligand binding: white dots, hydrophobic interaction; red lines, hydrogen bond acceptors or regions of positive electrostatic potential on the underlying molecule; blue lines, hydrogen bond donors or negative electrostatic potential on the underlying molecule.

Pharmacophore model was constructed based on those reported potent bronchodilators. The aim of this approach is to gain useful insights into ligand-receptor interactions, and to identify pharmacophoric structural features of the active ligands, and also to use this model for searching molecular data bases in order to find new structural categories, a process known as virtual (in silico) screening[74].

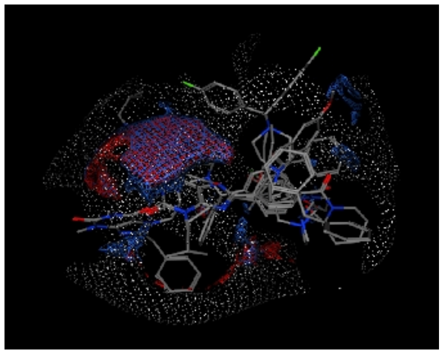


Fig. 4: Electrostatic map of the training set.

A pharmacophore query was created using the Pharmacophore Query Editor of MOE. The scheme used was PPCH-All (Planar-Polarity-Charge-Hydrophobicity). Under this scheme, the pharmacophore query was composed of six features (F1-F6). The pharmacophoric features and distances between them in Å are shown in Figure 5. Under this scheme, ML denotes metal ligator, HydS denotes non-planar hydrophobic region (sp^3), HydP denotes planar hydrophobic region (sp^2), AccP denotes planar H-bond acceptor (sp^2), DonP denotes planar H-bond donor (sp^2).

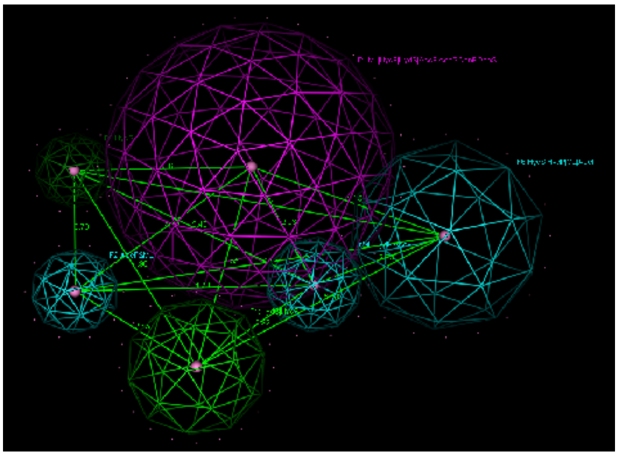


Fig. 5: Pharmacophore features and distances.

A pharmacophore search was done for our target compounds, the output of the pharmacophore search contains RMSD, i.e., the root mean square distance between the query features and their corresponding ligand target points. The smaller the RMSD, the better fitting the query compound has. Results are shown in Table 5, it was exciting to find that the first ten hits having the least RMSD values were for those with the most potent bronchodilator activity. Mapping of compound **15** onto the pharmacophore model is shown in Figure 6. By inspection of Figure 6, it can be seen that the chemical functionalities of the hypothesis are all matched by the chemical groups of the molecule: N1 atom, imidazole ring, and C6 carbonyl group fitted the region of ML/HydP/HydS/AccP/AccS/DonP/DonS, F1; C2 carbonyl group fitted the region of AccP/ML, F2; N9 atom fitted the region of ML/HydP/AccP, F3; N3-methyl group fitted the region of HydS/HydP, F4; N1-methyl group fitted the region of HydS, F5; sulfur atom and methylene group fitted the region of HydS/HydP/ML/AccP, F6.

Table 5: RMSD values of the hit set.

Compound	RMSD
83	0.2118
59	0.2118
67	0.2120
72	0.2121
60	0.2122
68	0.2125
80	0.2126
16	0.2132
15	0.2137
6	0.2143
14	0.2893
85	0.3466
81	0.3779
70	0.3907
82	0.3974
89	0.4182
79	0.4256
17	0.4495
84	0.4755
90	0.5296

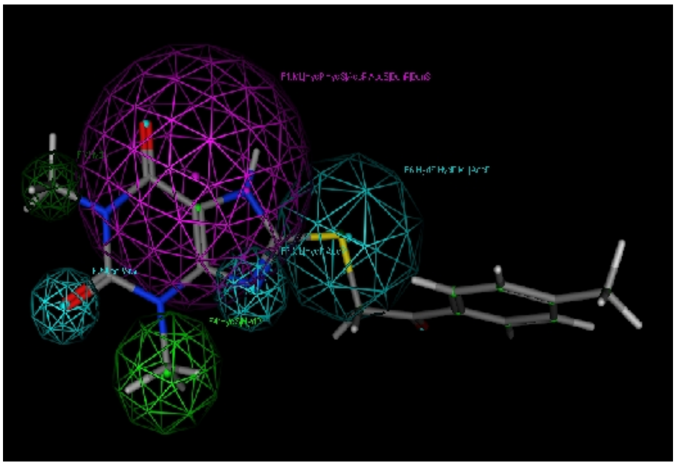


Fig. 6: Mapping of compound **15** onto the hypothetical model.

Conclusion

In this work, we report the synthesis of 8-mercaptotheophylline (**6**) by a simple procedure with an excellent yield. Forty-two final target compounds were synthesized including 8-aroylmethylthiotheophylline derivatives, 2-[(theophyllin-8-yl)thio]-*N*-substituted acetamide derivatives, and 2-[(theophyllin-8-yl)thio]-*N*,*N*-disubstituted acetamide derivatives. The anti-bronchoconstrictive activity study revealed that fifteen compounds exhibited significant anti-bronchoconstrictive activity. Compounds **15**, **16**, **59**, and **72** were either more effective than or equal to aminophylline. Moreover, none of these derivatives showed significant toxicity up to 300 mg/kg. The antibacterial activity studies revealed that compounds **16**, **17**, and **19** showed more potent antibacterial activity than ampicillin against both Gram-positive and Gram-negative bacteria. Most of the test compounds showed superior activity against Gram-positive bacteria to the Gram-negative ones. It is noteworthy to mention that compounds **16** and **72** exhibited both promising antibronchoconstrictive and antibacterial activities. A pharmacophore model was constructed to identify essential structural features responsible for bronchodilator activity.

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