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Design and Synthesis of New 8-Anilide Theophylline Derivatives as Bronchodilators and Antibacterial Agents

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Theophylline derivatives have long been recognized as potent bronchodilators for the relief of acute asthma. Recently, it was found that bacterial infection has a role in asthma pathogenesis. The present work involves the design and synthesis of 8-substituted theophylline derivatives as bronchodilators and antibacterial agents. The chemical structures of these compounds were elucidated by IR, ^{1}H-NMR, mass spectrometry, and elemental analyses. The bronchodilator activity was evaluated using acetylcholine-induced bronchospasm in guinea pigs, and most of the compounds showed significant anti-bronchoconstrictive activity in comparison with standard aminophylline. In addition, 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 possessed significant antibacterial activity. A pharmacophore model was computed to obtain useful insight into the essential structural features of bronchodilator activity. A structure activity relationship was also discussed.

Key words: Theophylline, 1,3,8-Trisubstituted-purine-2,6-diones, Antibacterial activity, Bronchodilators

INTRODUCTION

Asthma is a heterogeneous syndrome of intermittent wheezing and airway inflammation that affects 300 million individuals worldwide. Although its causes are unknown, many studies suggest a role of microbiota in its etiology (Cookson, 2004). Viral infections are important inducers of seasonal exacerbations of asthma (Johnston et al., 1995), but there is circumstantial evidence that bacterial infections may also play a role. Asymptomatic neonates whose throats are colonized with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* are at increased risk for recurrent wheezing and asthma early in life (Bisgaard et al., 2007). These same bacteria have consistently been associated with exacerbations of both asthma (Kraft, 2000) and chronic obstructive pulmonary disease (COPD) (Sethi et al., 2002). The response of asthmatics to antibiotics also suggests the importance of acute and chronic bacterial infections in the pathogenesis of disease (Blasi and Johnston, 2007). There is epidemiologic evidence that the prevalence of asthma and allergic diseases has significantly increased over the last three decades, particularly in children and young adults, and thus making allergic diseases a very common chronic health problem (Burr et al., 1989; Yunginger et al., 1992; Woolcock and Peat, 1997; von Mutius et al., 1999; Jedrychowski et al., 2004; Pawlinska-Chmara et al., 2008).

Searching for new bronchodilators with antimicrobial effect remains an important and challenging target due to the emergence of resistant strains of bacteria to the major classes of antibacterial agents.

It has previously been reported that several 8-aralkylthiotheophylline (Fig. 1 I) and triazole-based theophylline derivatives showed either more or equipotent activity to aminophylline and ampicillin as reference drugs (Elgaher et al., 2009; Hayallah et al., 2011).

Based on these data, and in view of a pharmacophore

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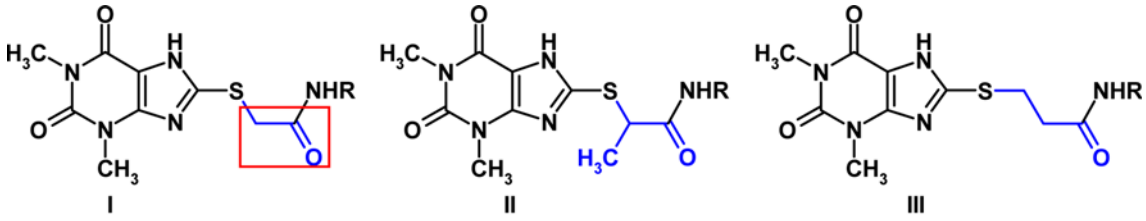


Fig. 1. Reported and targeted derivatives.

model, we designed and synthesized several 8-substituted theophylline derivatives (Fig. 1 II, and III) as novel bronchodilators with potent antibacterial activity.

MATERIALS AND METHODS

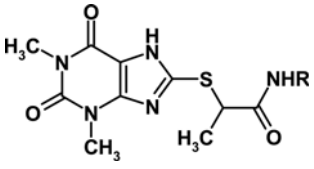
Chemistry

Reagents used for synthesis were purchased from Sigma-Aldrich and MERCK. All solvents were obtained from commercial suppliers and used without further purification. Melting points (mp) were determined on an electrothermal Stuart Scientific SMP1 melting point apparatus and were uncorrected. Thin-layer chromatography (TLC, R_f values) was carried out using TLC aluminum sheets kieselgel 60 F_{254} (MERCK) and dichloromethane-methanol (9.5:0.5) as a mobile phase. Visualization was effected with ultraviolet lamp Spectroline ENF-240C/F at short wavelength (λ = 254 nm). Not all chemical yields were optimized and they generally represented the findings of a single experiment. IR spectra were recorded on a Shimadzu spectrophotometer (IR-470) as potassium bromide discs at the Faculty of Pharmacy, Assiut University. NMR spectra were recorded on a Varian EM-360 60 MHz spectrometer at the Faculty of Pharmacy, Assiut University. DMSO-d_6 was used as a solvent, unless otherwise specified, the chemical shifts were given in δ (ppm), and coupling constants (J) were 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. Protons of NH and OH groups were confirmed by D_2O. The EI-MS were determined using or JOEL JMS600 mass spectrometer at the Unit of Microanalysis, Assiut University. The microanalyses for C, H, N, and S were performed on Perkin-Elmer 240 elemental analyzer at the Unit of Microanalysis, Faculty of Science, Assiut University.

General method for synthesis of compounds 22-38

In a stirred solution of compound **4** (0.5 g, 2.35 mmol)

Table I. Physical and microanalytical data of compounds **22-38**



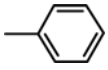
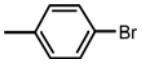
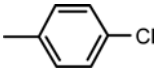
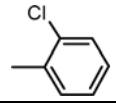
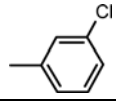
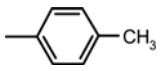
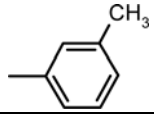
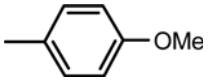
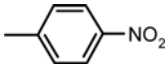
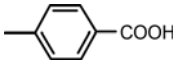
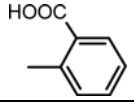
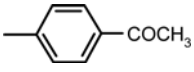
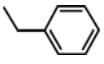
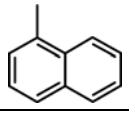
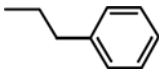
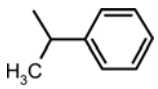
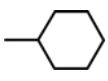
No.	R	Yield %	mp (°C) Crystal. solvent	Mol. formula (Mol. wt)		Microanalyses Calcd. %	Found %
22		82	238-240 Ethanol	$C_{16}H_{17}N_5O_3S$ (359.41)	C H N S	53.47 4.77 19.49 --	53.23 5.02 19.37 --
23*		83	265-267 Ethanol	$C_{16}H_{16}BrN_5O_3S$ (438.31)	C H N S	43.85 3.68 15.98 7.32	43.35 4.11 15.88 7.05
24*		85	277-279 aq. ethanol 30%	$C_{16}H_{16}ClN_5O_3S.H_2O$ (411.87)	C H N S	46.66 4.41 17.00 7.78	46.43 4.92 16.78 7.93

Table I. Continued

No.	R	Yield %	mp (°C) Crystal. solvent	Mol. formula (Mol. wt)	Microanalyses		
						Calcd. %	Found %
25		84	266-268 aq. methanol 20%	$C_{16}H_{16}ClN_5O_3S$ (393.85)	C H N S	48.79 4.09 17.78 8.14	48.54 4.47 17.68 7.95
26		80	258-260 aq. methanol 20%	$C_{16}H_{16}ClN_5O_3S$ (393.85)	C H N S	48.79 4.09 17.78 8.14	48.91 4.24 17.55 8.22
27		87	246-248 dec. aq. methanol 20%	$C_{17}H_{19}N_5O_3S$ (373.44)	C H N S	54.68 5.13 18.75 8.59	54.24 5.35 18.54 8.40
28*		78	234-236 dec. aq. methanol 20%	$C_{17}H_{19}N_5O_3S$ (373.44)	C H N S	54.68 5.13 18.75 8.59	54.30 5.45 18.45 8.10
29		79	258-260 dec. aq. ethanol 30%	$C_{17}H_{19}N_5O_4S$ (389.44)	C H N S	52.43 4.92 17.98 8.23	52.65 5.25 17.80 7.95
30*		84	263-265 aq. ethanol 20%	$C_{16}H_{16}N_6O_5S.H_2O$ (422.43)	C H N S	45.49 4.30 19.89 7.59	45.20 4.43 19.95 7.20
31*		77	275-277 dec. aq. ethanol 20%	$C_{17}H_{17}N_5O_5S$ (403.42)	C H N S	50.61 4.25 17.36 7.95	50.15 4.68 17.40 8.14
32		73	255-257 aq. ethanol 20%	$C_{17}H_{17}N_5O_5S$ (403.42)	C H N S	50.61 4.25 17.36 7.95	50.30 4.70 17.50 7.65
33*		79	270-272 ethanol	$C_{18}H_{19}N_5O_4S.1/2\ H_2O$ (410.55)	C H N S	52.66 4.92 17.06 7.79	52.20 4.95 16.80 7.65
34		80	222-224 dec. ethanol	$C_{17}H_{19}N_5O_3S$ (373.44)	C H N S	54.68 5.13 18.75 8.59	55.12 4.88 18.53 8.34
35		90	249-251 ethanol	$C_{20}H_{19}N_5O_3S$ (409.47)	C H N S	58.67 4.68 17.10 7.83	58.32 4.79 17.30 8.35
36		82	230-232 ethanol	$C_{18}H_{21}N_5O_3S$ (387.46)	C H N S	55.80 5.46 18.07 8.28	55.42 5.73 18.15 7.90
37		80	237-239 dec. ethanol	$C_{18}H_{21}N_5O_3S$ (387.46)	C H N S	55.80 5.46 18.07 8.28	55.37 5.85 17.86 8.40
38		87	257-269 dec. ethanol	$C_{16}H_{23}N_5O_3S.H_2O$ (383.48)	C H N S	50.12 6.57 18.26 8.36	49.80 6.90 18.10 8.55

*****These compounds were further confirmed by mass spectrometry.

in aqueous NaOH 1% (20 mL), an appropriate amount of N-(substituted)aryl/aralkyl or cycloalkyl-2-bromopropio-namides **5**-**21** (2.35 mmol) dissolved in the least amount of ethanol was added. The reaction mixture was stirred at the ambient temperature of 12 h, and then cooled in a refrigerator for 3 h. The product was filtered, washed with water, diethyl ether, dried, and crystallized from the appropriate solvent to afford the desired products **22**-**38**. Physical and micro-analytical data are given in Table I.

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-phenyl propionamide 22
IR cm^{-1}: 3470 (N-H); 3265 (N-H amide); 3045 (Ar-H); 2870 (C-H aliphatic); 1693, 1643 (C=O); 1529 (N-H); 745, 710 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.5 (d, J = 7 Hz, 3H, CH_3), 3.3 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.5 (q, J = 6.9 Hz, 1H, CH), 7.9-7.7 (m, 5H, phenyl C-H), 10.6 (s, 1H, amide N-H).

N-(4-Bromophenyl)-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)propionamide 23
IR cm^{-1}: 3450 (N-H); 3230 (N-H amide); 3080 (Ar-H); 2940 (C-H aliphatic); 1711, 1643 (C=O); 1529 (N-H); 835 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 7 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.6 (q, J = 6.9 Hz, 1H, CH), 7.2 (d, J = 8.8 Hz, 2H, phenyl C-H), 7.7 (d, J = 8.8 Hz, 2H, phenyl C-H), 10.8 (s, 1H, amide N-H). EI-MS (m/z, % base): 438.49 (M^+, 1.2), 440.56 (M^++2, 0.9), 265.61 (100), 237.76 (24.7), 199.91 (50.8), 197.81 (53.2).

N-(4-Chlorophenyl)-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)propionamide 24
IR cm^{-1}: 3450 (N-H); 3280 (N-H amide); 3110 (Ar-H); 2965 (C-H aliphatic); 1715, 1650 (C=O); 1530 (N-H); 833 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.8 (d, J = 6.9 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.6 (q, J = 6.9 Hz, 1H, CH), 7.7 (d, J = 8.7 Hz, 2H, phenyl C-H), 8.3 (d, J = 8.7 Hz, 2H, phenyl C-H), 11 (s, 1H, amide N-H). EI-MS (m/z, % base): 393.59 (M^+, 1.7), 395.06 (M^++2, 0.6), 265.75 (100), 237.81 (24.9), 126.85 (56.2).

N-(2-Chlorophenyl)-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)propionamide 25
IR cm^{-1}: 3455 (N-H); 3205 (N-H amide); 3030 (Ar-H); 2860 (C-H aliphatic); 1705, 1655, 1640 (C=O); 1530 (N-H); 748 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.7 (d, J = 6.9 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.6 (q, J = 6.9 Hz, 1H, CH), 7.6-8.4 (m, 4H, phenyl C-H), 10.6 (s, 1H, amide N-H), 14.7 (br s, 1H, N7-H).

N-(3-Chlorophenyl)-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)propionamide 26
IR cm^{-1}: 3445 (N-H); 3220 (N-H amide); 3040 (Ar-H); 2870 (C-H aliphatic); 1695, 1645 (C=O); 1525 (N-H); 782, 741 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.8 (d, J = 7 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.7 (q, J = 7 Hz, 1H, CH), 7.7-8.3 (m, 4H, phenyl C-H), 10.9 (s, 1H, amide N-H), 14.6 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-*p*-tolyl propionamide 27
IR cm^{-1}: 3450 (N-H); 3245 (N-H amide); 3040 (Ar-H); 2870 (C-H aliphatic); 1695, 1650 (C=O); 1530 (N-H); 805 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.8 (d, J = 6.8 Hz, 3H, CH_3), 2.5 (s, 3H, 4'-CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.9 (q, J = 6.8 Hz, 1H, CH), 7.6 (d, J = 8.5 Hz, 2H, phenyl C-H), 8.1 (d, J = 8.5 Hz, 2H, phenyl C-H), 10.9 (s, 1H, amide N-H), 14.7 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-*m*-tolyl propionamide 28
IR cm^{-1}: 3460 (N-H); 3255 (N-H amide); 3045 (Ar-H); 2890 (C-H aliphatic); 1695, 1655 (C=O); 1530 (N-H); 782, 742 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 6.8 Hz, 3H, CH_3), 2.25 (s, 3H, 3'-CH_3), 3.3 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.45 (q, J = 6.8 Hz, 1H, CH), 6.8-7.4 (m, 4H, Ar-H), 10.7 (s, 1H, amide-H), 14.7 (br s, 1H, N7-H). EI-MS (m/z, % base): 373.25 (M^+, 1.5), 239.02 (42.5), 150.04 (68.9), 134.07(100).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-(4-methoxyphenyl)propionamide 29
IR cm^{-1}: 3465 (N-H); 3265 (N-H amide); 3035 (Ar-H); 2930 (C-H aliphatic); 1693, 1640 (C=O); 1535 (N-H); 1248, 1040 (C-O); 815 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.7 (d, J = 6.9 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 3.8 (s, 3H, 4'-OCH_3), 4.9 (q, J = 6.9 Hz, 1H, CH), 6.9 (d, J = 8.5 Hz, 2H, phenyl C-H), 7.4 (d, J = 8.5 Hz, 2H, phenyl C-H), 10.2 (s, 1H, amide N-H), 14.6 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-(4-nitrophenyl)propionamide 30
IR cm^{-1}: 3395 (N-H); 3275 (N-H amide); 3110 (Ar-H); 2875 (C-H aliphatic); 1710, 1688 (C=O); 1538 (N-H); 1488, 1326 (NO_2); 854 (Ar). ^{1}H-NMR (60 MHz, DMSO-

d_6): 1.9 (d, J = 7 Hz, 3H, CH_3), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 4.8 (q, J = 7 Hz, 1H, CH), 7.9 (d, J = 8.6 Hz, 2H, phenyl C-H), 8.3 (d, J = 8.6 Hz, 2H, phenyl C-H), 10.9 (s, 1H, amide N-H), 14.7 (br s, 1H, N7-H). EI-MS (m/z, % base): 404.31 (M^+, 6.0), 265.90 (100), 236.95 (17.1), 137.96 (38.6).

4-[2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-propionylamino]-benzoic acid 31

IR cm^{-1}: 3395 (N-H); 3330-2530 (broad O-H); 1710, 1664, 1640 (C=O); 1520 (N-H); 849 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 7 Hz, 3H, CH_3), 3.3 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.6 (q, J = 7 Hz, 1H, CH), 7.5 (d, J = 8.7 Hz, 2H, phenyl C-H), 7.9 (d, J = 8.7 Hz, 2H, phenyl C-H), 10.9 (s, 1H, amide N-H), 12.7 (br s, 1H, COOH), 14.7 (br s, 1H, N7-H). EI-MS (m/z, % base): 402.95 (M^+, 5.3), 356.04 (13.8), 223.84 (28.6), 177.92 (100), 126.90 (5.5).

2-[2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-propionylamino]-benzoic acid 32

IR cm^{-1}: 3450 (N-H); 3325-2520 (broad O-H); 1698, 1666, 1640 (C=O); 1531 (N-H); 750 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 6.9 Hz, 3H, CH_3), 3.3 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.5 (q, J = 6.9 Hz, 1H, CH), 7.2-7.5 (m, 2H, phenyl C-H), 7.9 (d, J = 8.6 Hz, 1H, phenyl C-H), 8.4 (d, J = 8.6 Hz, 1H, phenyl C-H), 10.4 (br s, 1H, COOH), 10.7 (s, 1H, amide N-H), 14.3 (br s, 1H, N7-H).

N-(4-Acetylphenyl)-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-ylsulfanyl)propionamide (33)

IR cm^{-1}: 3455 (N-H); 3245 (N-H amide); 3100 (Ar-H); 2970 (C-H aliphatic); 1715, 1680, 1665 (C=O); 1525 (N-H); 825 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 7 Hz, 3H, CH_3), 2.5 (s, 3H, 4'-CH_3CO), 3.3 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.5 (q, J = 7 Hz, 1H, CH), 7.6 (d, J = 8.5 Hz, 2H, phenyl C-H), 7.8 (d, J = 8.5 Hz, 2H, phenyl C-H), 10.7 (s, 1H, amide N-H), 14.6 (br s, 1H, N7-H). EI-MS (m/z, % base): 401.24 (M^+, 4.5), 355.82 (11.1), 223.84 (28.6), 265.63 (100), 237.92 (36.6), 134.93 (43.0).

N-Benzyl-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl) propionamide 34

IR cm^{-1}: 3478 (N-H); 3265 (N-H amide); 3040 (Ar-H); 2865 (C-H aliphatic); 1704, 1638 (C=O); 1535 (N-H); 738, 691 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.6 (d, J = 7 Hz, 3H, CH_3), 3.3 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.4-4.6 (m, 4H, CH & NH-$\underline{CH_2}$), 7.2 (br s, 5H, phenyl C-H), 9.1 (t, J = 6 Hz, 1H, amide N-H), 14.4 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-naphthalen-1-yl propionamide 35

IR cm^{-1}: 3465 (N-H); 3210 (N-H amide); 3045 (Ar-H); 2875 (C-H aliphatic); 1698, 1640 (C=O); 1532 (N-H); 782 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.7 (d, J = 6.9 Hz, 3H, CH_3), 3.4 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.9 (q, J = 6.9 Hz, 1H, CH), 7.3-7.9 (m, 7H, naphthyl C-H), 10.3 (s, 1H, amide N-H), 14.5 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-phenethyl prpoionamide 36

IR cm^{-1}: 3470 (N-H); 3260 (N-H amide); 3050 (Ar-H); 2945 (C-H aliphatic); 1695, 1645 (C=O); 1535 (N-H); 739, 692 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.2-1.7 (m, 5H, CH_3 & $CH_2C\underline{H_2}Ph$), 3.4 (s, 3H, N1-CH_3), 3.7 (m, 5H, N3-CH_3 & $HNC\underline{H_2}$ CH_2), 4.8 (q, J = 6.9 Hz, 1H, CH), 7.5 (br. s, 5H, phenyl C-H), 8.6 (t, J = 6 Hz, 1H, amide-H), 14.7 (br s, 1H, N7-H).

2-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylsulfanyl)-N-(1-phenyl ethyl) prpoionamide 37

IR cm^{-1}: 3460 (N-H); 3265 (N-H amide); 3055 (Ar-H); 2870 (C-H aliphatic); 1701, 1633 (C=O); 1535 (N-H); 742, 690 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 1.5 (d, J = 7 Hz, 3H), 2.2 (d, J = 7 Hz, 3H), 3.4 (s, 3H, N1-CH_3), 3.6 (s, 3H, N3-CH_3), 4.6-5.0 (m, 2H, CH & CH), 7.3 (br s, 5H, phenyl C-H), 8.5 (d, J = 7 Hz, 1H, amide-H), 14.4 (br s, 1H, N7-H).

N-Cyclohexyl-2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl-sulfanyl) prpoionamide 38

IR cm^{-1}: 3440 (N-H); 3275 (N-H amide); 2900 (C-H aliphatic); 1710, 1640 (C=O); 1530 (N-H). ^{1}H-NMR (60 MHz): 0.74-2.17 (m, 13H, CH_3 and cyclohexyl-$(CH_2)_5$), 3.49 (s, 3H, N1-CH_3), 3.74 (s, 4H, N3-CH_3 & $HNC\underline{H}$), 8.64 (s, 1H, amide N-H), 14.44 (br s, 1H, N7-H).

General method for synthesis of compounds 43-46

In a stirred solution of compound **4** (0.5 g, 2.35 mmol) in aqueous NaOH 1% (20-25 mL), an appropriate amount of N-(substituted)aryl-3-chloropropionamides **39-42** (2.35 mmol) dissolved in the least amount of ethanol was added. The reaction mixture was stirred at an ambient temperature for 36-48 h and the reaction was monitored using TLC chromatography. The

Table II. Physical and microanalytical data of compounds **43-46**

No.	R	Yield %	mp (°C) Crystal. solvent	Mol. formula (Mol. wt)	Microanalyses		
						Calcd. %	Found %
43	4-Br	78	238-240 Ethanol	$C_{16}H_{17}N_5O_3S$ (359.41)	C	53.47	53.23
					H	4.77	5.02
					N	19.49	19.37
					S	–	–
44	4-Cl	77	265-267 Ethanol	$C_{16}H_{16}BrN_5O_3S$ (438.31)	C	43.85	43.35
					H	3.68	4.11
					N	15.98	15.88
					S	7.32	7.05
45	4-COOH	80	277-279 aq. ethanol 30%	$C_{16}H_{16}ClN_5O_3S.H_2O$ (411.87)	C	46.66	46.43
					H	4.41	4.92
					N	17.00	16.78
					S	7.78	7.93
46	2-COOH	81	266-268 aq. methanol 20%	$C_{16}H_{16}ClN_5O_3S$ (393.85)	C	48.79	48.54
					H	4.09	4.47
					N	17.78	17.68
					S	8.14	7.95

reaction mixture was cooled in a refrigerator for 6-8 h. The product was filtered, washed with water, diethyl ether, dried, and crystallized from the appropriate solvent to afford the desired products **43-46**. Physical and micro-analytical data are given in Table II.

3-(2,3,6,7-Tetrahydro-1,3-dimethyl-2,6-dioxo-1*H*-purin-8-ylthio)-N-(4-bromo-phenyl)propionamide 43

IR cm^{-1}: 3455 (N-H); 3285 (N-H amide); 3110 (Ar-H); 2970 (C-H aliphatic); 1715, 1650 (C=O); 1535 (N-H); 840 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 2.8 (t, 2H, J = 7.2 Hz, CH_2), 3.5 (s, 3H, N1-CH_3), 3.7 (s, 3H, N3-CH_3), 3.8 (t, 2H, J = 7.2 Hz, CH_2), 7.4 (d, J = 8.6 Hz, 2H, phenyl C-H), 8.1 (d, J = 8.6 Hz, 2H, phenyl C-H), 11 (s, 1H, amide N-H).

3-(2,3,6,7-Tetrahydro-1,3-dimethyl-2,6-dioxo-1*H*-purin-8-ylthio)-N-(4-chloro-phenyl)propionamide 44

IR cm^{-1}: 3450 (N-H); 3265 (N-H amide); 3070 (Ar-H); 2890 (C-H aliphatic); 1710, 1655, 1645 (C=O); 1530 (N-H); 810 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 2.7 (t, 2H, J = 7.2 Hz, CH_2), 3.5 (s, 3H, N1-CH_3), 3.6 (s, 3H, N3-CH_3), 3.7 (t, 2H, J = 7.2 Hz, CH_2), 7.1 (d, J = 8.5 Hz, 2H, phenyl C-H), 7.9 (d, J = 8.5 Hz, 2H, phenyl C-H), 10.8 (s, 1H, amide N-H).

4-[3-(2,3,6,7-Tetrahydro-1,3-dimethyl-2,6-dioxo-1*H*-purin-8-ylthio)propion-amido]benzoic acid 45

IR cm^{-1}: 3395 (N-H); 3330-2580 (broad O-H); 1710, 1664, 1640 (C=O); 1520 (N-H); 849 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 2.7 (t, 2H, J = 7.1 Hz, CH_2), 3.3 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 3.8 (t, 2H, J = 7.1 Hz, CH_2), 7.6 (d, J = 8.5 Hz, 2H, phenyl C-H), 7.9 (d, J = 8.5 Hz, 2H, phenyl C-H), 10.8 (s, 1H, amide N-H), 12.6 (br s, 1H, COOH), 14.5 (br s, 1H, N7-H).

2-[3-(2,3,6,7-Tetrahydro-1,3-dimethyl-2,6-dioxo-1*H*-purin-8-ylthio)propion-amido]benzoic acid 46

IR cm^{-1}: 3450 (N-H); 3325-2620 (broad O-H); 1698, 1666, 1640 (C=O); 1531 (N-H); 750 (Ar). ^{1}H-NMR (60 MHz, DMSO-d_6): 2.8 (t, 2H, J = 7.3 Hz, CH_2), 3.3 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 3.7 (t, 2H, J = 7.3 Hz, CH_2), 7.2-7.4 (m, 2H, phenyl C-H), 7.9 (d, J = 8.6 Hz, 1H, phenyl C-H), 8.5 (d, J = 8.6 Hz, 1H, phenyl C-H), 10.5 (br s, 1H, COOH), 10.7 (s, 1H, amide N-H), 14.4 (br s, 1H, N7-H).

Bronchodilator activity

The bronchodilator activity was carried out following Kesler and Canning's method (Kesler and Canning, 1999) with minor modifications (Grosa et al., 1989). Male Hartley guinea pigs, 300-400 g (House of Laboratory Animals, Faculty of Medicine, Assiut University) were anesthetized with urethane (1 g/kg *i.p.*) 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).

When the animals were stabilized, a bronchospasm was stimulated with acetylcholine (0.2 mg/kg *i.p.*). After two similar responses to spasm inducing injections, target compounds (dissolved in distilled water

with a minimal amount of 1 N NaOH) (Baziard-Mouysset et al., 1995) or aminophylline as a reference standard were administered (2.5-10 mg/kg *i.p.*), and acetylcholine was administered again three to five minutes later. The effects of the test compounds were expressed as means of percentage inhibition of five experiments ± S.E.M. of the induced bronchospasm for three doses (2.5, 5, and 10 mg/kg body weight). The ID_{50} value in each case was calculated by linear regression (Raeburn et al., 1994). At the end of each experiment, animals were killed by cervical dislocation.

Antibacterial activity

Organisms and culture conditions

Four bacterial species representing both Gram positive and Gram negative strains were used to test the antibacterial activity of the target compounds: methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae* (clinical isolates were obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University).

Agar cup diffusion method

The sterile Petri dishes were seeded with 100 µL of the microorganism: a specified amount of the molten Mueller-Hinton (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 a sterile cork borer. One hundred µL of the test compounds or ampicillin sodium (20 mg/mL in DMSO), or the solvent, were added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 h, the average diameters of the inhibition zones were then measured in millimeters.

Minimum inhibitory concentration

The MIC was determined using the two-fold dilution method (Scott, 1989) for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the test compounds. Extrapolation of the resulting straight line intersecting with the log concentration scale in the curve corresponds to log MIC, and MIC was obtained as antilog (Hewitt, 1977).

Acute toxicity (LD_{50})

Groups of five male adult albino mice, 18-22 g (House of Laboratory Animals, Faculty of Medicine, Assiut University), were injected *i.p.* with 4 graded doses of the test compounds suspended in 0.5% carboxymethylcellulose. LD_{50} was calculated based on the number of animals showing decreased muscle tone, and labored respiration signs using the Litchfield and Wilcoxon's method (Litchfield and Wilcoxon, 1949; Grosa et al., 1989; Akhila et al., 2007).

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 the 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 the "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 key intermediate 1,3-dimethyl-8-thioxo-3,7,8,9-tetrahydro-1H-purine-2,6-dione (**4**) was prepared from

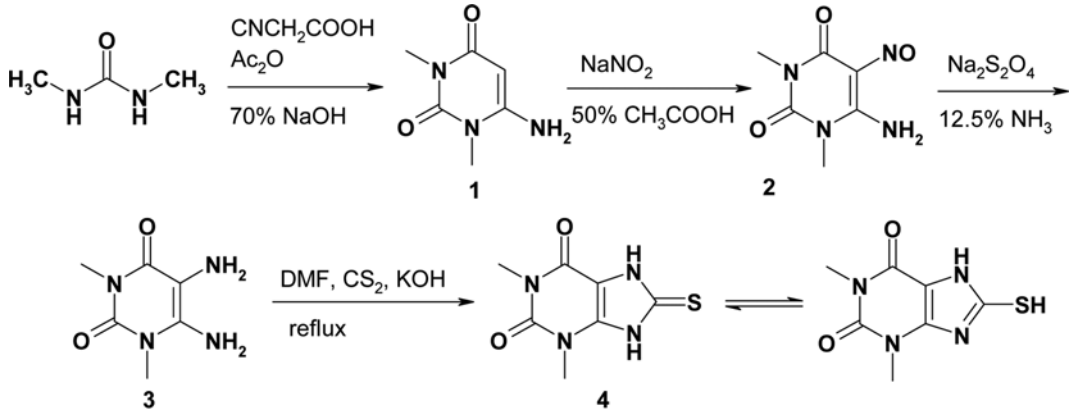


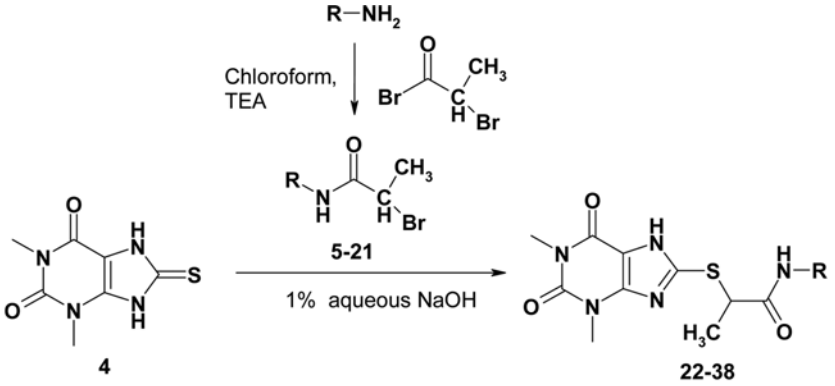
Fig. 2. Synthetic pathway for compound **4**.

compound **3**, as illustrated in Fig. 2, in reaction with CS2 in anhydrous DMF, based on previous reported methods (Hayallah, 2007, 2011; Elgaher et al., 2009). The structure was proven using IR and ^{1}H-NMR.

The intermediates (un)substituted 2-bromo-propionamides **5-21** and *N*-substituted-3-chloropropionamides **39-42** were prepared according to reported methods (Malatesta et al., 1962; Sadanandam et al., 1972; Snatzke and El-Abadelah, 1973; Snatzke et al., 1973; Fontanella et al., 1981; El-Abadelah, 1982; Malatesta et al., 1982; El-said et al., 1993), and their structures were verified using ^{1}H-NMR.

Alkylation of **4** with *N*-(substituted)aryl/aralkyl/cyclo-alkyl 2-bromo-propionamides **5-21** in the presence of aqueous ethanolic NaOH 1% furnished 2-[(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-2-propionamides **22-38** (Fig. 3). Structures of the new compounds **22-38** were verified by IR, ^{1}H-NMR, mass spectroscopy, and elemental analyses, as illustrated above and displayed in Table I. The IR spectra of the newly synthesized compounds showed the appearance of a new N-H stretching band around 3260 cm^{-1} characteristic of monosubstituted amides, 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} corresponding to C=O stretching bands, and also the absence of C=S stretching band. ^{1}H-NMR spectra of these compounds showed the appearance of a doublet around δ 1.5-1.9 ppm corresponding to -S-CH-$\underline{CH_3}$ protons; the quartet around δ 4.5-4.9 was equivalent to one proton of -S-$\underline{CH}$-CH_3 proton. In addition, the appearance of singlet at δ 10.66 ppm was equivalent to one proton corresponding to the monosubstituted amide group; broad singlet around δ 14.49 ppm corresponding to N7-H, and a multiplet at δ 6.92-8.94 ppm corresponding to the aromatic protons of most of the derivatives that also appeared. The aforementioned ^{1}H-NMR data are proof in the formation of the new derivatives.

[(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-3-propionamides **43-46**, (Fig. 4) were prepared by alkylation of theophylline-8-thione **4** with *N*-(substituted) aryl-3-bromo-propionamides **39-42** in the presence of aqueous ethanolic NaOH 1% (Dietz and Burgison, 1966) with slight modifications. Their structures were verified by IR, ^{1}H-NMR, and elemental analysis as illustrated above. The ^{1}H-NMR spectra of compounds **43-46** are characterized by the appearance of a pair of



No.	R	No.	R	No.	R
5, 22		11, 28		16, 33	
6, 23		12,29		17, 34	
7, 24		13,30		18, 35	
8, 25		14,31		19, 36	
9, 26		15,32		20, 37	
10, 27				21, 38	

Fig. 3. Synthetic pathway for the preparation of compounds **22-38**.

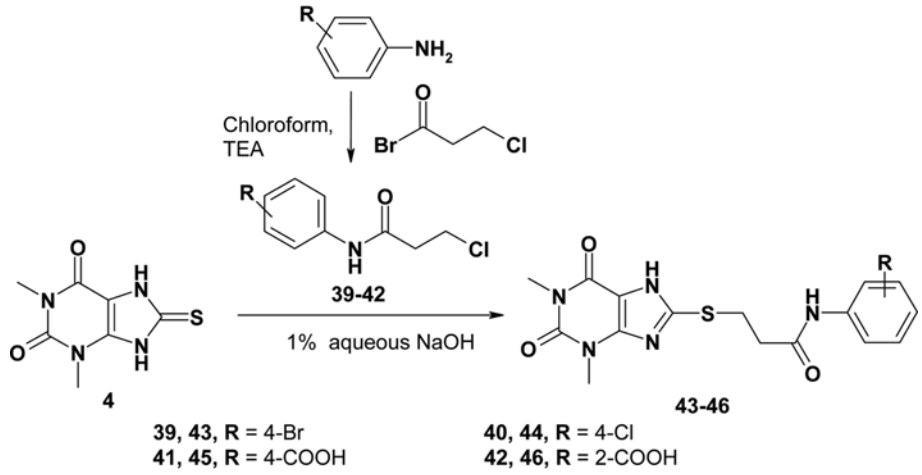


Fig. 4. Synthetic pathway for the preparation of compounds **43**-**46**.

triplet around δ 2.7-2.8 and δ 3.7-3.8 corresponding to OC-CH_2-CH_2-S respectively. In addition, the introduced aromatic moiety and the presence of N7-H signal is a strong support for an S-alkylation reaction rather than N-one. It is known that mercaptopurines undergo S-alkylation at a lower temperature, while N7-H is also attacked when the temperature is elevated (Lister, 1971). The preparation of new derivatives **43**-**46** takes a longer reaction time when compared to derivatives **22**-**38** due to the lower reactivity of *N*-substituted-3-chloropropionamides **39**-**42** in comparison to the isomers **5**-**21**.

Pharmacology

Bronchodilator activity

Following Kesler and Canning's method, all the synthesized compounds were investigated for *in vivo* anti-bronchospatic activity on acetylcholine-induced bronchospasm in anaesthetized guinea pigs in comparison to aminophylline as a reference drug. The anti-bronchoconstrictive effect was expressed as a percentage inhibition (mean ± S.E.M.) of bronchospasm for three doses (2.5, 5, and 10 mg/kg body weight). The 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 III.

Eight compounds (**23**, **24**, **31**, **32**, **37**, **38**, **45**, and **46**) exhibited an anti-bronchoconstrictive activity nearly similar to aminophylline. Compounds **24**, **32**, **37**, and **38** showed either a more significant or equivalent effect to aminophylline.

Regarding the results of *N*-(substituted)phenyl-2-(theophyllin-8-ylthio)-2-propionamides series **22**-**38**, the meta-substituted derivatives **26** and **28,** and the para-acetyl **33** revealed a very weak activity (ID_{50} >20 mg/kg). These results are similar to 2-[(1,3-Dimethyl-

Table III. Inhibitory effects of the test compounds on acetylcholine induced bronchospasm in anaesthetized guinea pigs

Compound	Dose mg/kg *i.p.*	% Decrease of acetylcholine induced bronchospasm in guinea pigs	ID_{50} mg/kg *i.p.*
Aminophylline	2.5	22.5 ± 1.1	5.8
	5	48.6 ± 1.4	
	10	78.8 ± 1.1	
22	2.5	5.8 ± 0.5	>20
	5	15.7 ± 1.19	
	10	18.0 ± 1.37	
23	2.5	18.5 ± 0.7	6.7
	5	40.7 ± 1.6	
	10	67.7 ± 1.2	
24	2.5	22.2 ± 1.3	6
	5	47.0 ± 1.7	
	10	76.0 ± 1.9	
25	2.5	20.5 ± 1.27	7.7
	5	38.6 ± 1.49	
	10	61.3 ± 1.67	
26	2.5	–	>20
	5	7.8 ± 0.56	
	10	11.9 ± 0.94	
27	2.5	19.7 ± 1.3	8.5
	5	35.7 ± 1.57	
	10	56.0 ± 1.85	
28	2.5	–	>20
	5	7.9 ± 0.51	
	10	12.3 ± 1.07	
29	2.5	20.6 ± 1.2	7.3
	5	39.2 ± 1.5	
	10	64.7 ± 1.9	

Table III. Continued

Compound	Dose mg/kg *i.p.*	% Decrease of acetylcholine induced bronchospasm in guinea pigs	ID_{50} mg/kg *i.p.*
30	2.5	11.5 ± 1.1	11.9
	5	31.7 ± 1.6	
	10	41.5 ± 1.8	
31	2.5	21.8 ± 1.1	6.6
	5	44.2 ± 1.51	
	10	69.8 ± 1.46	
32	2.5	26.1 ± 1.19	5.9
	5	47.1 ± 1.54	
	10	75.5 ± 1.83	
33	2.5	7.4 ± 0.65	>20
	5	15.8 ± 1.11	
	10	24.5 ± 1.38	
34	2.5	7.8 ± 0.47	17.1
	5	15.7 ± 1.27	
	10	29.4 ± 1.4	
35	2.5	19.0 ± 1.4	8.7
	5	35.7 ± 1.6	
	10	54.8 ± 1.47	
36	2.5	12.8 ± 1.04	13.7
	5	23.5 ± 1.24	
	10	37.5 ± 1.46	
37	2.5	26.7 ± 1.48	5.8
	5	47.4 ± 1.54	
	10	76.2 ± 1.8	
38	2.5	22.3 ± 1.33	6.1
	5	45.7 ± 1.50	
	10	75.5 ± 1.91	

Table IV. 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*
DMSO	–[a]	–	–	–
Ampicillin	22 (70)	22 (60)	27 (50)	20 (20)
Nalidixic acid	27 (30)	20 (67)	27 (25)	30 (20)
22	17 (–)	–	17	22
23	32 (22)	21 (73)	17 (120)	18 (125)
24	10	20 (75)	17 (110)	21 (63)
25	28 (31)	29 (33)	18 (126)	27 (45)
26	–	20	17	21
27	2	11	14	–
28	35 (20)	23 (66)	16 (140)	18 (130)
29	–	11	–	–
30	–	–	–	–
31	17 (125)	16 (153)	15 (158)	15 (158)
32	–	22 (72)	17 (168)	19 (75)
33	32 (27)	32 (23)	31 (25)	32 (25)
34	28 (30)	29 (43)	–	–
35	18 (115)	17 (143)	–	–
36	–	–	–	–
37	–	–	–	–
38	–	–	–	–
43	31 (22)	21 (75)	18 (110)	19 (115)
44	12	22 (75)	16 (90)	23 (63)
45	19 (120)	17 (133)	14 (148)	16 (159)
46	16 (122)	20 (78)	19 (162)	18 (78)

[a](–) means no antibacterial activity at the studied concentration.

xanthin-8-yl)thio]-*N*-substituted-acetamide derivatives (Elgaher et al., 2009), which ensures that the meta-substituted derivatives are the weakest derivatives. The biological activities of these derivatives are also in agreement with their calculated RMSD values (0.2950, 0.2815, and 0.2553, respectively) (Table V). Among the various para-substituted phenyl groups, the *p*-bromo **23**, *p*-chloro **24**, and p-COOH **31**, derivatives have significant activities (ID_{50} values: 6.7, 6.0, and 6.6 mg/kg, respectively). Several derivatives of p-substituted derivatives of *N*-(substituted)phenyl-2-(theophyllin-8-ylthio)-2-propionamides series, such as p-CH3 **27**, p-OCH3 **29**, and p-COOH **31,** showed enhanced ID_{50} values (8.5, 7.3, and 6.6 mg/kg, respectively) when compared to 2-[(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-acetamide derivatives (ID_{50} values: >20, 9.6 and 7.8 mg/kg respectively) (Elgaher et al., 2009). For naphthyl derivative **35** and 1-phenethyl derivative **37**, the ID_{50} values were markedly enhanced from >20 to 8.7 and from 7.3 to 5.8 mg/kg, respectively, in comparison with acetamide derivatives (Elgaher et al., 2009). On the other hand, 2-phenethyl derivative **36** demonstrated a lower activity (ID_{50} value: 13.7 mg/kg) when compared to the same analogue of acetamide derivative (ID_{50} value: 9.1 mg/kg, Elgaher et al., 2009). The selected ortho-substituted derivatives (**25** and **32**) showed significant activity (ID_{50} values: 7.7 and 5.9 mg/kg, respectively). Accordingly, the best position for substitution at the phenyl group is the para and ortho, which leads to active derivatives. Activity may be due to the non-planar orientation of the phenyl group with the 2-(theophyllin-8-ylthio)acetamide or propionamide moiety due to the presence of a bulky group at the ortho position. This assumption is in agreement with

Table V. RMSD values of some representative compounds

Compound	RMSD
22	0.2328
23	0.2138
24	0.2073
26	0.2950
28	0.2815
31	0.2053
32	0.2096
33	0.2553
43	0.3320
44	0.2464
45	0.4147
46	0.2207

Baziard-Mouysset et al. (1995) for the bronchodilator activity of various 8-substituted theophylline derivatives.

[(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-3-propionamides **43**-**46** were synthesized according to the calculated RMSD values (Table V) for these compounds and their isomers [(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-2-propionamide series, which showed significant activity. These derivatives **43**-**46** showed a decrease of the bronchodilator activity of three derivatives **43**, **44**, and **45** (ID_{50} values: 8.8, 6.7, and 8.7 mg/kg, respectively), in comparison with **23**, **24**, and **31** of the [(1,3-Dimethylxanthin-8-yl)thio]-*N*-substituted-2-propionamide series which showed ID_{50} values 6.7, 6.0, and 6.6 mg/kg, respectively (Table III). The ortho-substituted carboxy derivative **46** is the only one that retained its high bronchodilator activity (ID_{50} values: 6.2 mg/kg) if compared to its isomer **32** (ID_{50} values: 6.0 mg/kg). This may be because one of the best positions for substitution at the phenyl group is the ortho position, which leads to active derivatives (Elgaher et al., 2009). The activity may be due to a non-planar orientation of the phenyl group with the 2-(theophyllin-8-ylthio)acetamide and 2-(theophyllin-8-ylthio)-propionamide moiety due to the presence of a bulky group at the ortho position. This assumption is in agreement with that of (Baziard-Mouysset et al., 1995), for the bronchodilator activity of various 8-substituted theophylline derivatives. In addition, the hydrogen bond of carboxy group at this position may play a vital role for the biological activity of these derivatives as new potential bronchodilator agents. Finally, an increase in the carbon chain between the 8-sulfur and the amide function group may lead to more potent bronchodilator agents of this series. The pharmacophore study, calculating both RMSD and biological results, also indicated that the increase of carbon chain length from acetamide to iso-propionamide derivatives is preferable to the straight chain one.

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 (Clark et al., 1981). MIC of the active compounds was also calculated in comparison to nalidixic acid and ampicillin as a reference drug (Table IV).

Analysis of the antibacterial results showed that compounds **23**, **25**, **28**, **33**, **34**, and **43** exhibited comparable or better activities (MIC 22-30 μg/mL) against MRSA than ampicillin and nalidixic acid. They were also active against *B. cereus* (MIC 23-75 μg/mL), several of which, including **25**, **33**, and 34, were even more potent than ampicillin and nalidixic acid (MIC 23-43 μg/mL). Compound **33** was the most active compound against all bacterial strains (MIC 23-27 μg/mL).

These compounds showed a significant activity (MIC 45-158 μg/mL) against Gram-negative bacteria strains *E. coli* and *K. pneumoniae* except for compound **34**, which did not show any activity against Gram-negative bacteria. These results indicate that these derivatives showed better activity against Gram-positive bacteria when compared to the Gram-negative one.

Compounds **44**-**46** showed a significant to moderate activity (MIC 63-159 μg/mL) against both Gram-positive and Gram-negative bacterial strains, while compound **35** showed a moderate activity (MIC 115-143 μg/mL) against only Gram-positive bacteria (MRSA and *B. cereus*) and compound **24** was active against all bacteria strains except *B. cereus*.

In a trial to find a possible mechanism of action for the title compounds, the following reported studies on the interaction of DNA or RNA with xanthine derivatives were considered. It was found that caffeine inhibits the incorporation of adenine and thymidine in the synthesis of DNA (Labbe and Nolan, 1987); it also inhibits the synthesis of DNA (Sandlie et al., 1980) and RNA (Pérez et al., 1994). Among the three xanthine derivatives, theophylline showed a greater binding efficacy with RNA than theobromine and caffeine (Johnson et al., 2003). G-C and A-U bases of RNA were the targets for binding. At the same time, the complexation occurred with the G-C and A-T bases and PO_2 group of DNA through hydrogen bonding (Nafisi et al., 2008). Considering these data, we suggest that the mechanism of action of the target xanthine

derivatives as antibacterial agents is through the inhibition of nucleic acid synthesis.

Acute toxicity (LD_{50})

Acute toxicity (LD_{50}) study was performed in mice via intraperitoneal (*i.p.*) injection for the most active derivatives (compounds **24**, **32**, **37**, and **38**) and compared to aminophylline as a reference drug. The obtained experimental data showed that not all of the test compounds recorded significant toxicity with LD_{50} = 300 mg/kg in comparison with the standard drug aminophylline LD_{50} = 180 mg/kg (Peikov et al., 1994) (Table VI). The maximal toxicity was observed after 12 h, when the animals showed decreased muscle tone and strenuous respiration signs.

Receptor building and pharmacophore identification

It is known that the actual molecular mechanism of action of xanthine derivatives as bronchodilators is controversial (Howell, 1990; Barnes and Pauwels, 1994). The inhibition of phosphodiesterase III and IV isoenzymes relaxes smooth muscles in pulmonary arteries and air ways (Hall, 1993), while antagonists of adenosine A_{2B} receptors were proposed to have potential use as antiasthmatic agents (Feoktistov et al., 1998). However, it is necessary to determine 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 overlap in space. All the computational works were performed on the Molecular Operating Environment (MOE) version 2007.09, Chemical Computing Group Inc. Thirteen reported active ligands were selected as the training set. Two of them, theophylline and bamifylline, were in therapeutic use. The receptor model and the pharmacophore query were built as reported before (Elgaher et al., 2009).

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.

Table VI. Acute toxicity in mice following *ip* injection of compounds **24**, **32**, **37**, and **38**

Compound	LD_{50} mg/kg
24	300
32	300
37	>300
38	300
Aminophylline	180[a]

[a]LD_{50} value as reported (Peikov et al., 1994).

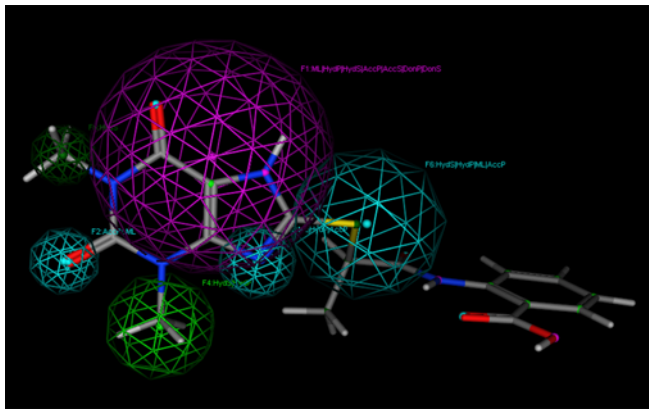


Fig. 5. Mapping of compound **32** onto hypothetical model.

The mapping of compound **32** onto the pharmacophore model is shown in Fig. 5. 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/HydS/HydP/AccP/AccS/ DonP/DonP, F1; C2 carbonyl group fitted the region of AccP/ML, F2; N9 fitted the region of ML/HydP/AccP, F3; N3 methyl group fitted the region of HydS/HydP, F4; N1-methyl group fitted the HydS, F5; sulfur atom and methylene group fitted the region of HydS/HydP/ML/AccP, F6.

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