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Full length Research Paper

Design and synthesis of some new theophylline linked amides and schiff's bases as bronchodilatros and anti-inflammatory agents

***[1]Alaa M. Hayallah, [2]Suzan A. Isper and [2]Amira S. Al-Okosh**

[1]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[2]Department of Applied Pharmaceutical Sciences, Faculty of Pharmacy, Al-Israa University, Amman, Jordan

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Chronic airway inflammation plays a key role in asthma. This fundamental feature has been included in the most recent definitions of the disease. 8-substituted ronchodi especially theophylline derivatives are well known as potent bronchodilators for the relief of acute asthma and play pronounced role in inflammation process. The present work involves the design and synthesis of new 8-substituted theophylline derivatives. The chemical structures of these compounds were elucidated by IR, ^{1}H NMR, elemental analyses, and FAB-MS spectral data. 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 aminophylline as a standard. Also, anti-inflammatory activity of the target compounds was investigated using indomethacin as a reference drug. Results showed that some of the tested compounds have good anti-inflammatory activity. A pharmacophore model was computed to get useful insight on the essential structural features of bronchodilator activity.

Keywords: Theophylline, bronchodilator, anti-inflammatory activity.

INTRODUCTION

Allergic asthma, a chronic airway disease involves bronchial epithelium, mucus-secreting glands, lung parenchyma, and infiltrating inflammatory leukocytes, has the characteristics of lung inflammation, airway hyper responsiveness and mucus overproduction (Hamid et al., 2009). It is now widely accepted that chronic airway inflammation plays a key role in asthma (Bousquet et al., 2000). This fundamental feature has been included in the most recent definitions of the disease: hence the Global Strategy for Asthma Management and Prevention reports that "asthma is a chronic inflammatory disease of the airways in which many cell types play a role, in particular mast cells, eosinophils and T-lymphocytes. In susceptible individuals the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or early morning. These symptoms are usually associated with widespread but variable airflow obstruction that is at least partly reversible either spontaneously or with treatment.The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli" (www.ginasthma.com). Based on this consensus all treatment guidelines focus on the importance of anti-inflammatory drugs (mainly inhaled corticosteroids) to control the disease process (Barnes, 1995). Searching for new bronchodilators with anti-inflammatory effect remains an important and challenging target.

It has previously been reported that several 8-aralkylthiotheophylline and triazole-based theophylline derivatives showed either more or equipotent activity to aminophylline reference drug (Elgaher et al., 2009; Hayallah et al., 2001; Hayallah et al., 2012). We reported previously that 1,8-disubstituted purine-2,6-diones were reported as potent analgesic and anti-inflammatory agents through adenosine receptor antagonism (Hayallah et al., 2002; Abo Salem et al., 2004; Bilkei-Gorzo et al., 2008) and 3,6-disubstituted thiazolo[2,3-*f*]purine-2,4-

*Corresponding Author E-mail: alaa_hayalah@yahoo.com

Diones as potent analgesic and also anti-inflammatory agents (Hayallah, 2007).

Based on these data, and in view of a pharmacophore model, some new theophylline linked amides and Schiff's bases as ronchodilators and anti-inflammatory Agents were designed and synthesized.

Experimental Section

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 (mp) were determined on an electrothermal Stuart Scientific SMP1 (UK) melting point apparatus and were uncorrected. Thin-layer chromatography (TLC, R_f values) was carried out using TLC aluminium sheets kieselgel 60 F_{254} (MERCK) and dichloromethane/methanol (9.5:0.5) (9:1) or 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. NMR spectra were recorded on Bruker DPX 300 MHz spectrometer at Jordan University, Amman, Jordan and on a Varian EM-360 60 MHz spectrometer at Faculty of Pharmacy, Assiut University, Egypt. DMSO-d_6 was used as a solvent, unless otherwise specified, 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. Protons of NH, and OH groups were confirmed by D_2O. The FAB-MS were determined using or JOEL JMS600 mass spectrometer at the Unit of Microanalysis, Assiut University, Egypt. The microanalyses for C, H, N and S were performed using Euro Elemental analyzer (Italy) at Faculty of Pharmacy, Jordan University, Amman, Jordan.

8-(4-Carboxymethyloxy)-1, 3-dimethyl-phenylxanthine 5

6-Amino-1, 3-dimethyl-5-(carboxymethyloxybenzylidene) aminouracil 4 (2.5 g, 7.53 mmol) was suspended at 0 °C in thionyl chloride (70 Ml). The reaction mixture was refluxed for 1.5 h and then the mixture was stirred at rt overnight. Thionyl chloride was distilled off, the residue was suspended in ice water, then filtered, and the washed with water to afford the expected product, which recrystallized from DMF/H_2O affording compound **5** as white crystals in 75 % yield.

^{1}H NMR (300 MHz, DMSO-d_6): 3.2 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.2 (s, 2H, O-CH_2), 7.1 (d, *J* = 8.5 Hz, 2H, Ar-CH), 8.0 (d, *J* = 8.5 Hz, 2H, Ar-CH), 13.6 (s, 1H, N7-H).

8-(4-Methoxycarbonylmethyloxy)-1,3-dimethyl-phenylxanthine 7

8-(4-Carboxymethyloxy)-1,3-dimethyl-phenylxanthine 5 was stirred at 70 °C in thionyl chloride (70 Ml) for 5 h. The excess thionyl chloride was distilled off affording the acyl chloride derivative **6**. The obtained acid chloride was directly refluxed in absolute methanol (30 Ml) for 2 h. The mixture was cooled and the formed precipitate was was collected by filtration. The product was recrystallized from DMF/H_2O to afford compound **7** as white crystals in 64 % yield.

^{1}H NMR (300 MHz, DMSO-d_6): 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 3.7 (s, 3H, O-CH_3), 4.2 (s, 2H, O-CH_2), 7.1 (d, *J* = 8.5 Hz, 2H, Ar-CH), 8.2 (d, *J* = 8.5 Hz, 2H, Ar-CH), 13.5 (s, 1H, N7-H).

General methods for synthesis of amide compounds 8-11

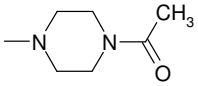
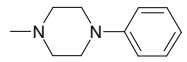
Method A (acyl chloride method).

A solution of compound 5 (0.7 g, 2.12 mmol) in 15 Ml of thionyl chloride was stirred at 70 C for 4 h, then the excess of thionyl chloride was removed by evaporation under reduced pressure. To the residue a solution of ethyl amine or acetyl piprazine (4.24 mmol) in 30 Ml of anhydrous pyridine: dichloromethane (1:1, v/v) was added. The reaction mixture was stirred at room temperature for 48 h and subsequently evaporated under *vaccu*. The product was recrystallized for methanol: dichloromethane (8:2) to afford compounds 8 and 10 respectively. Physical and microanalytical data are given in Table 1.

Method B (ester method)

8-(4-Methoxycarbonzlmethyloxy)-1,3-dimethyl-phenylxanthine 7 (0.5 mmol) was dissolved at 150 °C in DMF, then the solution was cooled to 60 °C and (0.1 mmol) of butylamine or phenylpiperazine was added. After stirring overnight at 60 °C and for 2 days at rt, the reaction mixture was concentrated. The formed precipitate was filtered off and washed with water and methanol affording the expected products. The products were cryatallized from dichloromethane/methanol to afford compound 9 and 11 respectively. Physical and microanalytical data are given in table 1.

Table 1. Physical and microanalytical data of compounds 8-11*.

No.	R	Yield %	mp (ºC) Crystal. solvent	Mol. formula (Mol. wt)		Microanalyses Calcd. %	Found %
8	$-NHC_2H_5$	40	285-287 DMF/H_2O	$C_{17}H_{19}N_5O_4.H_2O$ (375.39)	C	54.39	54.10
					H	5.65	5.70
					N	18.66	18.40
9	$-NH(CH_2)_3CH_3$	41	244-247 CH_2Cl_2	$C_{19}H_{23}N_5O_4$ (385.43)	C	59.21	58.75
					H	6.01	6.30
					N	18.17	18.05
10		70	308-309 DMF/H_2O	$C_{21}H_{24}N_6O_5.H_2O$ (458.48)	C	55.01	54.65
					H	5.73	5.90
					N	18.33	18.15
11		45	315-317 CH_2Cl_2	$C_{25}H_{26}N_6O_4$ (474.52)	C	63.28	62.85
					H	5.52	5.70
					N	17.71	17.30

*All new compounds were further confirmed by mass spectroscopy.

8-[(4-ÓÓÓEthylaminocarboxymethyloxy) phenyl)-1,3-dimethyl] xanthine 8

^{1}HNMR: (300 MHz, DMSO,d6): σ 0.90 (t, 3H, *j*=7.2, $CH_2\underline{CH_3}$), 3.1 (q, 2H, *j*=7.2, $\underline{CH_2}CH_3$), 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.5(s, 2H, O-CH_2), 7.01 (d, 2H, *J*=8.5 Hz, Ar-H), 8.01 (d, 2H, *J*=8.5 Hz, Ar- H), 8.15 (s, 1H, N-H amide), 13.5 (s, 1H, N-7H).
FAB-MS (*m/z*, % base): 358.83 (M^++1, 8.5), 287.95 (15.4), 249.12 (26.7), 228.04 (61.4), 213.05 (30.9), 166.01 (51.7), 118.99 (19.9), 95.02 (70.8), 69.04 (73.8), 51.02 (100).

8-[(4-Butylaminocarboxymethyloxyphenyl)-1,3-dimethyl] xanthine 9.

^{1}HNMR: (300 MHz, DMSO,d6): σ 0.85 (t, 3H, *J*=7.2 Hz, $CH_2CH_2CH_2\underline{CH_3}$), 1.00 (m, 2H, $CH_2CH_2\underline{CH_2}CH_3$), 1.45 (m, 2H, Hz $CH_2\underline{CH_2}CH_2CH_3$), 3.20 (t, 2H, *J*=7.2 Hz, $\underline{CH_2}CH_2CH_2CH_3$), 3.45 (s, 3H, N1-$CH_3$), 3.7(s, 3H, N3-$CH_3$), 4.81 (s, 2H, O-$CH_2$), 7.00 (d, 2H, *J*=8.5Hz, Ar-H), 8.00 (d, 2H, *J*=8.5 Hz, Ar- H), 8.80 (s, 1H, N-H amide).
FAB-MS (*m/z*, % base): 386.00 (M^++1, 2.2), 311.31 (19.1), 249.09 (9.6), 230.05 (65.2), 228.06 (78.5), 166.01 (51.1), 150.07 (22.4), 130.00 (41.2), 95.03 (66.1), 69.03 (71.3), 51.03 (100).

8-[4-[(4-Acetyl-piperazine-1-yl]-2-oxo ethoxy phenyl-1,3-dimethyl-]xanthine 10.

^{1}H-NMR: (300 MHz, DMSO, D6): σ 2.2 (s, 3H, O=C-$\underline{CH_3}$), 3.2 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 3.2-3.5 (m, 4H, pip.H), 4.7 (s, 4H, pip. H), 4.9 (s, 2H, O$\underline{CH_2}$), 7.1 (d, *j*=8.5 HZ, 2H, 2', 6', Ar-H), 8.0 (d, *j*=8.5 HZ, 2H, 3', 5', Ar-H).
FAB-MS (*m/z*, % base): 441.60 (M^++1, 3.2), 417.42 (12.7), 318.98 (18.1), 251.06 (21.9), 227.99 (69.6), 165.99 (54.2), 94.99 (100).

1, 3 Dimethyl-8[4-[(4-phenyl-piperazin-1-yl)-2-oxo-ethoxy phenyl]xanthine 11.

^{1}H-NMR: (300 MHz, DMSO, D6): σ 3.2 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 3.6-3.7 (m, 4H, pip.H), 3.9-4.0 (m, 4H, pip.H), 4.9 (s, 2H, O-$\underline{CH_2}$), 7.0 (d, *j*=8.5 HZ, 2H, 2', 6', Ar-H), 7.1-7.2 (m, 1H, Ar-H), 7.5-7.8 (m, 2H, Ar-H), 8.0 (d, *j*=8.5 HZ, 2H, 3', 5', Ar-H), 8.2-8.3 (m, 1H, Ar-H), 8.7 (s, 1H, Ar-H), 13.5 (s, H1, N-7H).
FAB-MS (*m/z*, % base): 475 (M^++1, 0.5), 249.07 (30.6), 230.04 (58.8), 211.03 (26.9), 165.99 (49.8), 150.04 (25.6), 129.98 (34.7), 113.04 (29.0), 95.01 (68.3), 69.02 (76.7), 51.01 (100).

General method for synthesis of Schiff's base compounds 15-24.

To a solution of 1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl thioaceto-hydrazide 14 (0.5 g, 1.76 mmol) in ethanol (20 ml), an equimolar of the appropriate aldehyde (1.76 mmol) was added followed by 3-4 drops of acetic acid. The reaction mixture was refluxed for 8-12 h with TLC monitoring until the start is vanished. The formed precipitate was filtered, and dried in oven. The products were crystallized from the suitable solvent to afford target compounds 15-24. Physical and microanalytical data are given in Table 2.

Table 2. Physical and microanalytical data of compounds 12-24*

No.	R	Yield %	mp (ºC) Crystal. solvent	Mol. formula (Mol. wt)		Microanalyses Calcd. %	Found %
15	H	80	265-266 Ethanol	$C_{16}H_{16}N_6O_3S$ (372.41)	C	51.47	51.05
					H	4.33	4.55
					N	22.57	22.10
					S	8.61	8.40
16	4-Br	85	291-292 Ethanol	$C_{16}H_{15}BrN_6O_3S$ (451.30)	C	42.58	42.32
					H	3.35	3.64
					N	18.62	18.43
					S	7.10	6.90
17	4-Cl	82	271-272 Ethanol	$C_{16}H_{15}ClN_6O_3S$ (406.85)	C	47.24	46.90
					H	3.72	3.95
					N	20.66	20.00
					S	7.88	---
18	2-F	85	267-268 Methanol	$C_{16}H_{15}FN_6O_3S$ (390.40)	C	49.23	49.45
					H	3.87	3.70
					N	21.53	21.15
					S	8.21	7.80
19	2-NO_2	88	285-286 Ethanol	$C_{16}H_{15}N_7O5S$ (417.41	C	46.04	45.85
					H	3.62	3.80
					N	23.49	23.05
					S	7.68	7.32
20	2-Br	87	285-286. Ethanol	$C_{16}H_{15}BrN_6O_3S$ (451.30)	C	42.58	42.20
					H	3.35	3.55
					N	18.62	18.15
					S	7.10	7.30
21	2-Cl	83	275-276 Ethanol	$C_{16}H_{15}ClN_6O_3S$ (406.85)	C	47.24	46.80
					H	3.72	3.90
					N	20.66	2035
					S	7.88	----
22	4-F	84	278-269 Ethanol	$C_{16}H_{15}FN_6O_3S$ (390.40)	C	49.23	48.75
					H	3.87	4.10
					N	21.53	21.10
					S	8.21	8.37
23	4-$N(NH_3)_2$	78	272-273 Ethanol	$C_{18}H_{21}N_7O_3S$ (415.48)	C	52.04	15.90
					H	5.09	5.00
					N	23.60	23.18
					S	7.72	7.60
24	4-O-CH_2-COOH	82	258-259 Ethanol	$C_{18}H_{18}N_6O_6S$ (446.44)	C	48.43	47.95
					H	4.06	4.35
					N	18.82	18.40
					S	7.18	6.80

*All new compounds were further confirmed by mass spectroscopy.

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purine-8-yl)thio]-N-(phenyl-imino)acetamide 15.

^{1}H-NMR: (DMSO, D6): σ 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.5 (s, 2H, S-$\underline{CH_2}$), 7.3-7.6 (m, 3H, Ar-H), 7.6-7.8 (m, 2H, Ar-H), 8.0 (s, 1H, N=$\underline{CH}$), 8.2 (s, 1H, Amide H), 11.5 (s, H1, N-7H).

FAB-MS (*m/z*, % base): 373.09 (M^++1, 1.5), 212.98

(22.0), 185.04 (48.2), 149.11 (19.8), 93.05 (100).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N-(4-bromophenylimino) cetamide 16.

^{1}H-NMR: (DMSO, D6): σ 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.5 (s, 2H, S-$\underline{CH_2}$), 7.5-7.6 (d, , *j*=8.5 HZ, 2H, 2', 6', Ar-H), 7.6-7.7 (d, , *j*=8.5 HZ, 2H, 3', 5', Ar-H),7.9 (s, 1H, N=$\underline{CH}$), 8.2 (s, 1H, Amide H), 11.7 (s, 1H, N7-H).
FAB-MS (*m/z*, % base): 453 (M^++2, 1.1), 452.31 (M^++1, 1.3), 249.12 (34.5), 230.10 (42.4), 166.02 (34.8), 131.06 (28.2), 101.06 (28.6), 95.04 (45.6), 69.03 (52.4), 57.09 (38.5), 51.03 (100).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purine-8-yl)thio]-N-(4-chlorophenylimino) acetamide 17.

^{1}H-NMR: (DMSO, D6): σ 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.5 (s, 2H, S-$\underline{CH_2}$), 7.3-8.0 (m, 4H, Ar-H), 8.2 (s, 1H, N=$\underline{CH}$), 8.7 (s, 1H, Amide H), 11.7 (s, 1H, N7-H).
FAB-MS (*m/z*, % base): 408.38 (M^++2, 0.7), 407.5 (M^++1, 2.2), 287.10 (12.5), 228.04 (63.7), 130.00 (42.5), 95.02 (64.2), 69.05 (72.1), 57.06 (40.8), 51.03 (100).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purine-8-yl)thio]-N-(2-fluoro-phenylimino) acetamide 18.

^{1}H-NMR: (DMSO, D6): σ 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.4 (s, 2H, S-$\underline{CH_2}$), 7.0-7.6 (m, 3H, Ar-H), 7.8-8.0 (m, 1H, Ar-H), 8.2 (s, 1H, N=$\underline{CH}$), 8.4 (s, 1H, Amide H), 11.9 (s, 1H, N7-H).
FAB-MS (*m/z*, % base): 391.99 (M^++1, 7.1), 311.28 (13.3), 253.05 (34.7), 228.02 (57.3), 166.00 (49.9), 131.03 (41.7), 95.02 (85.9), 69.04 (96.0), 57.05 (61.2), 51.02 (100).

2-[(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purine-8-yl)thio]-N-(2-nitro-phenylimino) acetamide 19.

^{1}H-NMR: (DMSO, D6): σ 3.1 (s, 3H, N1-CH_3), 3.3 (s, 3H, N3-CH_3), 4.3 (s, 2H,
S-$\underline{CH_2}$), 7.3-7.6 (m, 3H, Ar-H), 7.6-8.0 (m, 1H, Ar-H), 8.2 (s, 1H, N=$\underline{CH}$), 8.4 (s, 1H, Amide H), 11.8 (s, 1H, N7-H).
FAB-MS (*m/z*, % base): 417.2 (M^+, 3.8), 184.99 (62.7), 93.05 (100).

2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N-(2-bromo-phenylimino) acetamide 20.

^{1}HNMR: (DMSO,d6): σ 3.25 (s, 3H, N1-CH_3), 3.45 (s, 3H, N3-CH_3), 4.45 (s, 2H, S-CH_2), 7.20-7.50 (m, 2H, Ar-H), 7.60-7.90(m, 2H, Ar-H), 8.25 (s, 1H, N=CH), 8.50 (s, 1H, NH-amide), 11.7 (s, 1H, N7-H).
FAB-MS (*m/z*, % base): 453.2 (M^++2, 5.4), 452.5 (M^++1, 7.3), 353.75 (13.0), 287.02 (17.1), 230.06 (99.0), 166.00 (50.9), 129.99 (45.7), 95.04 (96.9), 51.03 (100).

2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N-(2-chloro-phenylimino) acetamide 21.

^{1}HNMR: (DMSO,d6): σ 3.2 (s, 3H, N1-CH_3), 3.5 (s, 3H, N3-CH_3), 4.35 (s, 2H, S-CH_2), 7.4-7.6 (m, 2H, Ar-H), 7.7-8.0 (m, 2H, Ar-H), 8.25 (s, 1H, N=CH), 8.55 (s, 1H, NH-amide), 11.85 (s, 1H, N-7H).
FAB-MS (*m/z*, % base): 408.38 (M^++2, 0.7), 407.5 (M^++1, 2.3), 287.10 (12.5), 228.04 (63.7), 130.00 (42.5), 95.02 (64.2), 69.05 (72.1), 57.06 (40.8), 51.03 (100).

2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N-(4-fluoro-phenylimino) acetamide 22

^{1}HNMR: (DMSO,d6): σ 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.4 (s, 2H, S-CH_2), 7.0 -7.4 (m, 2H, Ar-H), 7.5-7.9 (m, 2H, Ar-H), 8.0 (s, 1H, N=CH), 8.2 (s,1H, NH-amide), 11.5 (s, 1H, N-7H).
FAB-MS (*m/z*, % base): 391.99 (M^++1, 7.2), 311.28 (13.6), 253.05 (34.7), 228.02 (57.3), 166.00 (49.9), 131.03 (41.7), 95.02 (85.9), 69.04 (96.0), 57.05 (61.2), 51.02 (100).

2-[(1,3dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N-(4-N,N-dimethylaminophenylimino)acetamide 23

^{1}HNMR: (DMSO,d6): σ 3 (s, 6H, 4` N(CH_3)$_2$), 3.2 (s, 3H, N1-CH_3), 3.4 (s, 3H, N3-CH_3), 4.4 (s, 2H, S-CH_2), 6.4 -6.8 (d, 2H, *j*=8.5 Hz, Ar-H), 7.2 -7.5 (d, 2H, *j*=8.5
Hz, Ar-H), 7.8 (s, 1H, N=CH), 8.0 (s, 1H, NH-amide), 12.3 (s, 1H, N-7H).
FAB-MS (*m/z*, % base): 416.24 (M^++1, 11.5), 249.06 (38.1), 230.04 (75.1), 228.05 (58.3), 165.01 (28.5), 129.97 (32.0), 95.04 (88.3), 69.04 (45.5), 57.11 (30.4), 51.02 (100).

2-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl)thio]-N(*p*-carboxymethyloxyphenylimino)acetamide 24

^{1}HNMR: (DMSO,d6): σ 3.20 (s, 3H, N1-CH_3), 3.50 (s, 3H, N3-CH_3), 4.50 (s, 2H, O-CH_2), 4.80 (s, 2H, S-CH_2), 6.90 (d, 2H, *j*= 8.5Hz, Ar-H), 7.60 (d, 2H, *j*= 8,5 Hz, Ar-H), 8.10 (s, 1H, N=CH), 8.30 (s, 1H, NH-amide), 11.45 (s, 1H, N-7H).

Bronchodilator Activity

The method of (Kesler and Canning, 1999) was utilized with minor modifications (Grosa et al., 1989). 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) (Baziard-Mouysset et al., 1995) or aminophylline as a reference standard were administered (2.5-10 mg/kg *ip*), acetylcholine was administered again three to five minutes later. The effects of the test compounds were expressed as mean of percentage inhibition of five experiments ± SEM of the induced bronchospasm for three doses (2.5, 5, and 10 mg/kg body weight), 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. Results are shown in Table 3.

Acute Toxicity (LD_{50})

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. LD_{50} was calculated on the number of animals showing decreased muscle tone, and laboured respiration signs according to reported methods (Grosa et al., 1989; Litchfield et al., 1949; Akhila et al., 2007).

Anti-inflammatory Activity

The anti-inflammatory activity of the newly synthesized compounds was determined according to paw induced edema method[18] in comparison to indomethacin as a reference drug. The test is based on the pedal inflammation in rat paws induced by sub plantar injection of carrageenan suspension (0.2 mL of 1% solution in normal saline) in the right hind paw of the rats.

Male albino rats were divided into groups (5/group). The thickness of rat paw was measured by Varnier Caliper (SMIEC, China) before and after 1 h of carrageenan injection to determine the induced inflammation. The tested compounds of a dose (50 mg/kg) were injected i.p. to the animals. The control group received a vehicle (1% NaCMC) while the reference group received indomethacin (10 mg /kg).

Results of anti-inflammatory activity of the tested compounds and the reference drug were listed in (Table 4 and Figure 1, 2).

The percentage of edema and percentage of edema inhibition were calculated (Valencia et al., 1994) where:

$$\% \text{ Variation (edema)} = \frac{(V_R - V_L) \times 100}{V_R}$$

$$\% \text{ Edema inhibition} = \frac{(V_R - V_L) \text{ control} - (V_R - V_L) \text{ treated} \times 100}{(V_R - V_L) \text{ control}}$$

V_R: Average right paw thickness, V_L: Average left paw thickness.

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

Benzylidene derivative 4 was prepared from 5,6-diamino-1,3-dimethyluracil 3 by reaction with 4-carboxymetyloxy benzaldehyde in ethanol at reflux temperature(Mueller et al., 1993) as illustrated in Figure 3. Subsequent ring closure of compound 4 by stirring in thionyl chloride overnight, followed by refluxing 1 h afforded 8-(4-carboxymethyloxy)-phenyl-1,3-dimethylxanthine 5. The structure was proved using ^{1}H-NMR and it was in

Table 3. Inhibitory effect of compounds **8-11**, and **15-24** 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*
	2.5	21.1±1.2	
8	5	46.3±1.4	6.0
	10	76.8±1.7	
	2.5	26.1±1.3	
9	5	44.2±1.5	6.6
	10	68.5±0.95	
	2.5	22.1±1.2	
10	5	46..7±15	6.2
	10	75.6±1.7	
	2.5	26.1±1.4	
11	5	44.2±1.2	6.5
	10	75.6±1.6	
	2.5	12.5±1.0	
15	5	30.7±1.5	11.9
	10	41.3±1.8	
	2.5	28.3±2.1	
16	5	38.6±2.6	7.6
	10	62.1±1.9	
	2.5	20.5±1.2	
17	5	40.1±1.5	7.3
	10	63.9±1.8	
	2.5	15.8±0.55	
18	5	34.5±1.2	8.6
	10	56.2±1.4	
	2.5	16.9±1.2	
19	5	36.5±1.6	8.4
	10	56.4±1.8	
	2.5	21.1±1.4	
20	5	38.7±1.3	7.8
	10	59.8±1.4	
	2.5	21.2±1.5	
21	5	38.5±1.7	6.6
	10	61.8±1.6	
	2.5	14.8±1.3	
22	5	26.6±1.4	11.8
	10	42.8±1.2	
	2.5	21.5±0.5	
23	5	40.7±1.5	7.1
	10	65.4±1.4	
	2.5	19.7±1.3	
24	5	42.6±1.5	6.8
	10	68.5±1.6	
	2.5	22.6±1.3	
Aminophlline	5	48.4±1.3	5.8
	10	78.5±1.2	

accordance with reported data (Jacbobson et al., 1985). This compound was prepared by (Jacbobson et al., 1985) using anhydrous ferric chloride; cyclization using thionyl chloride was easier in purification and afforded higher

Table 4. % of edema inhibition of compounds **8-11** and **15-24** in comparison to indomethacin

Compd. No.	% of Edema Inhibition					
	0.5h	1h	2h	3h	4h	5h
8	15.44	38.32	63.85	70.45	72.35	71.8
9	15.54	36.52	60.55	68.55	70.45	71
10	14	39	59.5	62	64.5	70
11	17.44	40.32	65.8	74.25	75.25	75.7
15	8	42.5	56.25	57	60	58
16	10	40	58.5	60	63.5	64
17	6	18.75	48.75	56.25	56.25	61
18	7	22	30.5	40	48.5	50
19	5.5	23.7	31.65	39.5	51.3	51.3
20	9	21	29.5	43	46.5	52
21	16.5	20.5	34	60.5	64.5	65
22	10.5	10.5	24	73.5	73.5	64
23	18	39.5	48	55.5	56	58
24	14	42	57.5	60	61.5	65
Control	--	--	--	--	--	--
Indometh.	14.28	33.18	66.59	78.21	78.68	80.12

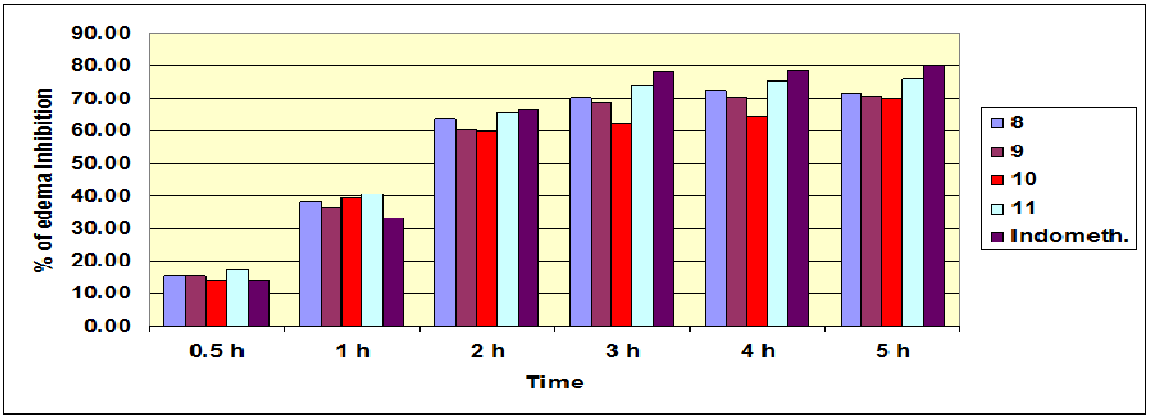


Figure 1. Inhibitory effect of compounds 8-11 and indomethacin on carrageenan induced paw edema in rats.

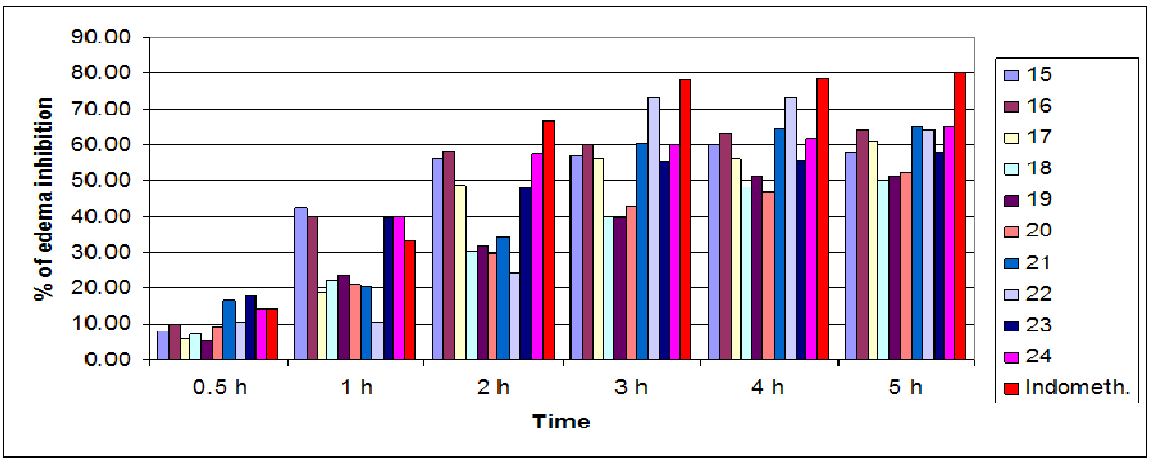


Figure 2. Inhibitory effect of compounds 15-24 and indomethacin on carrageenan induced paw edema in rats.

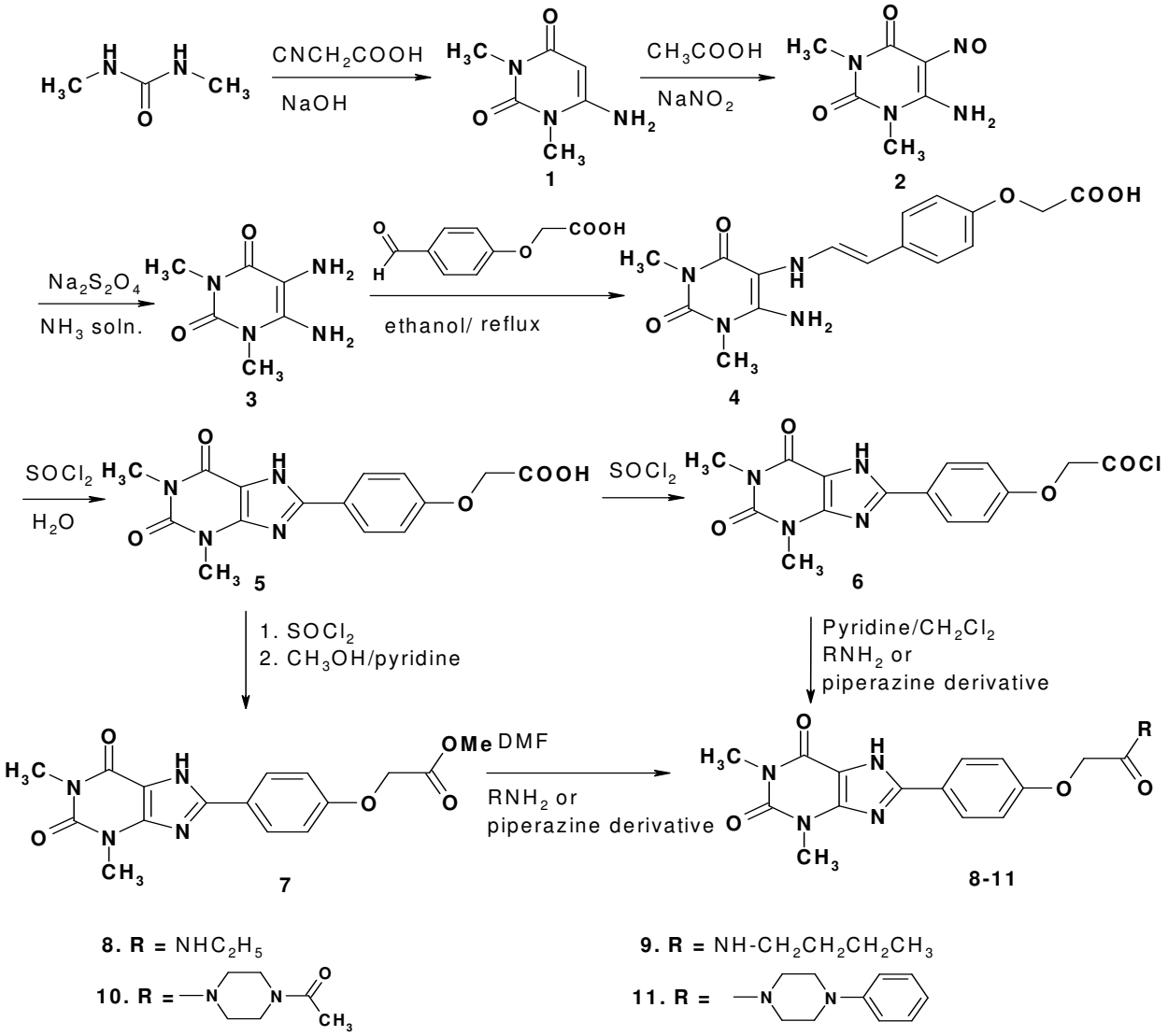


Figure 3. Synthetic pathway of compounds **8-11.**

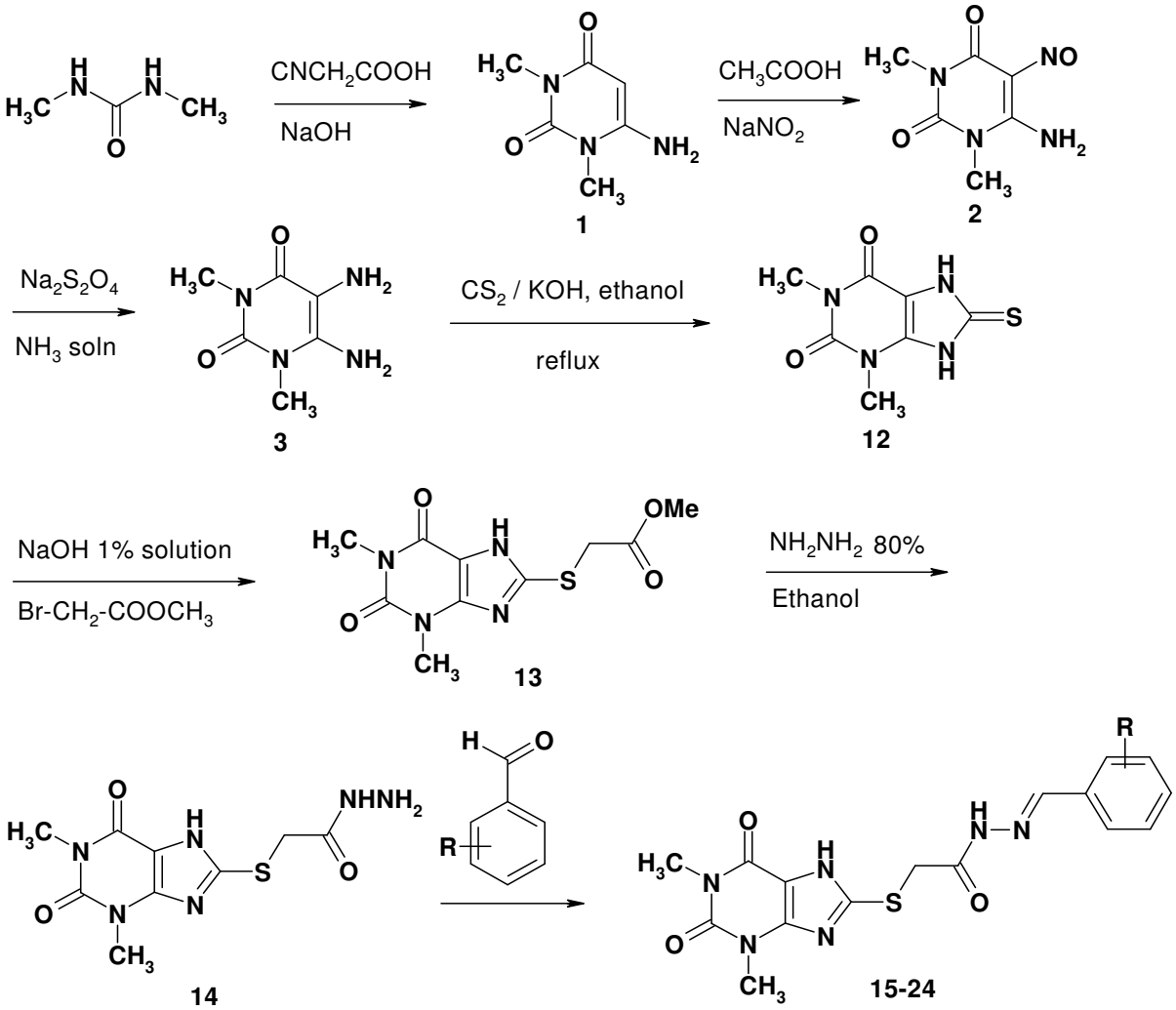
yields (Hayallah et al., 2002; Senga et al., 1977).

Xanthine amide derivatives 8-11 by two different methods Figure 3. Amide derivatives 8, 10 were prepared by conversion of compound 5 to its acid chloride 6 by refluxing in thionyl chloride for 4 h at 70 °C. After distillation of excess thionyl chloride, the rsidue was dissolved in a mixture of anhydrous pyridine/ dichloromethane (1:1), and then the appropriate amine was added in analogy with reported procedure (Hayallah et al., 2002; Kim et al., 2000).

Amide derivatives 9, 11 were obtained by direct reaction of xanthine methyl ester 7 with amine derivatives in hot dimethylformamide at 150 °C and then the reaction mixture was stirred overnight at 60 °C and for 2 days at room temperature. The structures of these derivatives were verified using ^{1}H-NMR, mass spectroscopy, and elemental analyses, as illustrated above and displayed in table 1.

The ^{1}H-NMR spectra of these derivatives 8, 9 were characterized by appearance of singlet around δ 8.15 and 8.80 ppm corresponding to amide proton and the new introduced moieties ethyl and butyl respectively. In case of compounds 10 and 11 two multiplets around δ 3.2-3.5 and 4-4.5 ppm each one equivalent to four protons and these corresponding to piperazine moiety. In addition, appearance of singlet at δ 2.2 ppm corresponding to acetyl CH_3 in compound 10 and multiplet signals in aromatic region corresponding to phenyl group in compound 11 are a signs of target amide compounds formation.

The intermediate 1,3-dimethyl-8-thioxo-3,7,8,9-tetrahydro-1H-purine-2,6- dione 12 was prepared from compound 3, as illustrated in Figure 4, by reaction with CS2 in anhydrous DMF, based on previous reported



15 R = H
16 R = 4-Br
17 R = 4-Cl
18 R = 2-F
19 R = 2-NO_2
20 R = 2-Br
21 R = 2-Cl
22 R = 4-F
23 R = 4-$N(CH_3)_2$
24 R = 4-O-CH_2-COOH

Figure 4. Synthetic pathway of compounds **15-24.**

methods.(Elgaher et al., 2009; Hayallah et al., 2011; Hayallah, 2007) The structure was confirmed using IR and 1H-NMR. Compound 13 was prepared by reaction the aqueous solution of compound 12 in sodium hydroxide 1% with an equimolar amount of methyl bromoacetate. Reaction of 13 with hydrazine hydrate 80% in ethanol under reflux for 2 h afforded compound 14 as reported before (Hayallah, 2007).

Schiff's base compounds 15-24 were prepared by reacting 1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-yl thioaceto-hydrazide 14 with an equimolar of the appropriate aldehyde in ethanol and few drops of acetic acid. At reflux temperature to afford target compounds 15-24. These new derivatives were confirmed by ^{1}H-NMR, mass spectroscopy, and elemental analyses, as illustrated above and displayed in table 2. ^{1}H-NMR of these new Schiff's base compounds were characterized by appearance of singlet around δ 7.9-8.2 ppm corresponding to CH=N, singlet around δ 8.2-8.7 ppm corresponding to amide NH and signals corresponding to new introduced aromatic moiety. In addition, disappearance of the broad singlet at δ 7.6 ppm

Table 5. Calculated RMSD values of compounds 8-11

Compound	RMSD
8	0.2425
9	0.2430
10	0.2453
11	0.2464

corresponding to $NHNH_2$, these signs were an indication for formation of target compounds.

Pharmacology

Bronchodilator activity

Following Kesler and Canning's method, all the new 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-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 3.

Six compounds (8-11, 21 and 24) exhibited an anti-bronchoconstrictive activity nearly similar to that of aminophylline. The rest compounds showed good to moderate to good activity in comparison with aminophylline.

Regarding the results of amide derivatives 8-11 showed very good activity (ID_{50} values: 6.0-6.8 mg/kg). The biological activities of these derivatives are also in agreement with their calculated RMSD values (0.2425, 0.2430, 0.2453 and 0.2464) respectively as displayed in Table 5. This mean the activity is better with small amide substituents than bulky one, to increase the water solubility of lipophilic insoluble 8-phenyltheophylline derivative piperazinylamides containtained a basic nitrogen atom, which can be protonated leading to increased water solubility were prepared. N-Acetylpiperazine amide derivative 10 was higher in activity than phenylpiperazine 11 and this also in agreement with their calculated RMSD values table 5.

Schiff's base compounds 15-24 showed moderate to good activity. Two of them 11 and 24 exhibited an anti-bronchoconstrictive activity nearly similar to that of aminophylline. Introduction of substituents on Schiff's bases phenyl moiety enhanced the activity except the para flouro-substituted 22 Table 3. On the other hand, introduction of flour substituent at ortho position compound 18 showed better activity if compared to its analogs 22. Accordingly, the position for substitution at the phenyl group may be playing a role in activities of these series.

It is clear that amide derivatives which carry phenyl moieties directly at 8-position of theophylline exhibited better activities in comparison with Schiff's bases in which aryl moieties are separated by thioalkyl chain. These results are in agreement with what is known that 8-phenyl substitution at xanthine increase their affinity for all adenosine receptor subtypes, (Hayallah et al., 2002; Mueller and Stein, 1996; Bruns, 1991), which may play a role in the activities of these derivatives as bronchodilators and anti-inflammatory agents.

Anti-inflammatory Screening

Anti-inflammatory activity

All new synthesized compounds 8-11 and 15-24 were tested *in vivo* for their anti-inflammatory effect. They were tested using carragennan induced paw edema in rats using indomethacin (10 mg/kg) as a refrence drug (valencia et al., 1994). The results of tested compounds and indomethacin were presented as the time course dependent size of edema and percentage of edema inhibition at a dose (40 mg/kg) and time intervals 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h (table 4 and figure 1,2). All the tested compounds showed moderate to potent anti-inflammatory effect. The 8-amide derivatives of theophylline 8-11 showed significant activity in comparison to that of indomethacin, they exhibit inhibition of induced edema ranged from 72-76%, table 4 and figure 1. Phenylpiperazinyl amide derivative 11 was the most potent one within this series.

Schiff's base compounds 15-24 displayed moderate to potent anti-inflammatory activities, compounds 16, 21, 22 and 24 showed very good activities if compared to that of indomethacin. Anti-inflammatory activities of rest compounds were moderate to good and revealed edema inhibition ranged from 50-61% as illustrated in figure 2.

Acute toxicity (LD_{50})

Acute toxicity (LD_{50}) study was performed in mice via

Table 6. Acute toxicity in mice following intraperitoneal injection of compounds 8, 10, 21, and 24.

Compound	LD_{50}[a] (mg/kg)
8	>300
10	300
21	300
24	>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[25]

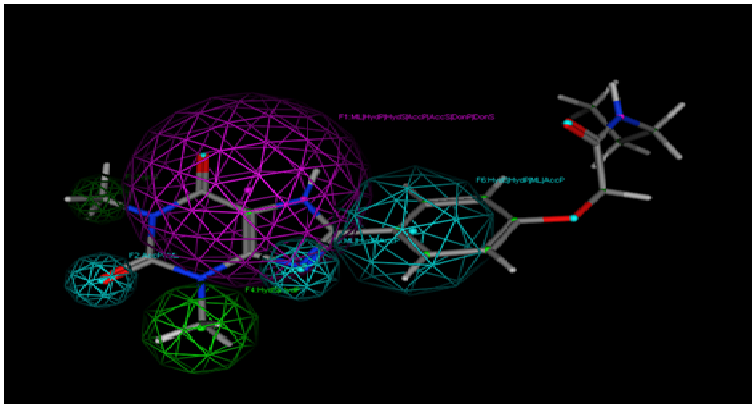


Figure 5. Mapping of compound **8** onto the hypothetical model.

intraperitoneal (*ip*) injection for the most active derivatives (compounds 8, 10, 21, and 24) and compared to aminophylline 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 (Peikov et al., 1994), Table 6. 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 well known that the actual molecular mechanism of action of xanthine derivatives as bronchodilators is still controversial (Howell, 1990; Branes at al., 1994), inhibition of phosphodiesterase III and IV isoenzymes relaxes smooth muscles in pulmonary arteries and air ways (Hall 1993), whereas antagonists of adenosine A_{2B} receptor proposed to have potential use as antiasthmatic agents (Feoktistov et al., 1998). 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. Therteen reported active ligands were selected as the training set. Two of them, theophylline and bamifylline are in therapeutic use. The receptor model and the pharmacophore query were built as we 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.

Mapping of compound **8** onto the pharmacophore model is shown in Figure 5. By inspection of Figure 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; oxygen atom, amide carbonyl, and 8-phenyl moiety fitted the region of HydS/HydP/ML/AccP, F6.

CONCLUSION

A number of new theophylline linked amides and Schiff's bases as bronchodilatros and anti-inflammatory agents. The chemical structures of these compounds were elucidated by IR, ^{1}H NMR, elemental analyses, and FAB-MS spectral data. 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 aminophylline as a standard. Some of them were potent candidates such as compounds **8-11**, **21** and **24**. Also, anti-inflammatory activity of the target compounds was investigated using indomethacin as a reference drug. Results showed that some of the tested compounds have good anti-inflammatory activity such as **8-11**, **16**, **21**, **22** and **24**. These results may be considered a corner stone and open the door towards design and synthesis of new derivatives as bronchodilators and anti-inflammatory drugs.

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