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Der Pharma Chemica, 2014, 6(2):199-211
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Design and synthesis of some new purine-dione derivatives of potential anti-inflammatory activity

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ABSTRACT

*A new series of 2-[(1-substituted)-2,6-dioxo-2,3,6,7–tetrahydro-1H-purin-8-ylsulfanyl]-N-substituted acetamide **16-29**, and 1-substituted-8-[2-(4-substituted phenyl)-2-oxoethylsulfanyl]-3,7-dihydro-1H-purine-2,6-diones **37-50** were designed and synthesized. The target compounds **16-29** and **37-50** were prepared by reaction of 1-substituted-8-thioxo-3,7-dihydropurine-2,6-dione with equimolar amount of the appropriate substituted anilide or p-(un)substituted phenacyl bromide respectively. Structures of the new compounds were verified on the basis of their IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analyses. The newly synthesized compounds were tested for their anti-inflammatory effects and most of them showed good to excellent anti-inflammatory activity compared to indomethacin as a reference drug.*

Keywords: Purine; adenosine receptors; synthesis; anti-inflammatory

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INTRODUCTION

Rheumatic diseases are the most prevalent causes of disability, and non-steroidal anti-inflammatory drugs (NSAIDs) are still the most commonly used remedies. NSAIDs cause several serious adverse effects; the most important are gastric ulceration and renal injury. Thus, development of novel anti-inflammatory compounds with improved safety is still a necessity [1].

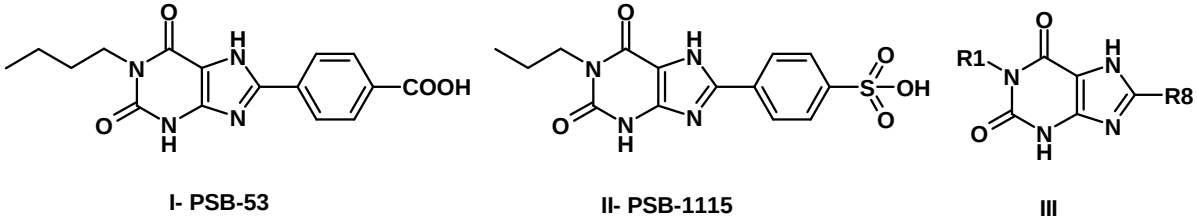
The pyrimidine and purine ring systems undoubtedly belong to the most ubiquitous heterocycles in nature, as they represent the main structure of many biologically significant compounds, including nucleosides and nucleotides. Several of the latter heterocycles possess a multitude of pronounced biological activities [2].

Moreover, the class of fused purines is considered to be attractive targets since their fundamental skeleton is analogous to naturally occurring purine alkaloids [3,4]. For this reason many analogues and derivatives of purine and pyrimidine have been synthesised and developed as pharmacologically active compounds [2]. Literature survey revealed that these classes of compounds have been reported as antitumor and anticancer amplifiers [5,6], phosphodiesterase inhibitors [7], antimicrobial agents [8-10], and anti-asthmatic agents [8-11]. Also, some 9-deazaxanthines represent promising highly adenosine antagonists potentially as anti-asthmatic agents [12,13]. This class of compounds has also potential antidiabetic through inhibition of phosphoenolpyruvate carboxykinase (PEPCK) [14].

On the other hand, some substituted pyrimido-purine diones were reported to act as atypical nonsteroidal anti-inflammatory agents [15,16]. Moreover, recently 1,8-disubstituted purine-2,6-diones [**PSB-53** (I)**, PSB-1115** (II) **and** (III), **chart 1**] were reported to possess potent analgesic and anti-inflammatory activity through adenosine receptor antagonism [4,13,17-19].

In addition to all the aforementioned findings, it was also reported that anilides are important binding blocks in both nature and chemical synthesis as they reported to be effective when used therapeutically for treatment of asthma [20], and as anti-HIV-1 agents [21]. Also some antiarthritic agents were derivatized as amide prodrug [22], and some used for treatment of atherosclerosis, septic shock and impotence through adenosine receptor agonistic action [23].

In view of the above facts and in continuation of our interest in the synthesis of new heterocyclic compounds comprising in their skeletons the 1,8-disubstituted purine-2,6-dione moieties and screening of their biological activities [4,10,13,17-19,24]. Herein we report the synthesis and anti-inflammatory activities of new 1,8-disubstituted purine-2,6-diones.



I- PSB-53 **II- PSB-1115** **III**

Chart 1: Structure of: I. PSB-53, II. PSB-1115 and compound III

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, SMP3, Staffordshire, UK) and were uncorrected. Pre-coated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Darmstadt, Germany) were used for TLC monitoring of reactions using CH_2Cl_2/CH_3OH 9:1 as mobile phase. Visualization of the spots was effected using an ultraviolet lamp (Spectroline, model CM-10, Seattle, USA) (λ= 254 nm) and/or iodine stain. IR spectra were carried out as KBr discs on a Shimadzu IR-470 Spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University. ^{1}H NMR spectra were performed on a JEOL JNMLA series FT NMR system (400 MHz, JEOL, Tokyo, Japan) at the Assiut University Unit of Trace Analyses or on a Varian EM-360L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in *δ*-values (ppm) relative to tetramethylsilane (TMS) as an internal standard using DMSO-d_6 as a solvent and deuterium oxide was used for the detection of exchangeable protons. ^{13}C NMR spectra were taken using JEOL JNMLA series FT NMR system (100 MHz, JEOL, Tokyo, Japan) at the Assiut University Unit of Trace Analyses, Assiut. Mass spectra were recorded with a Gas Chromatography Mass, Quadruple-2010 Plus (Shimadzu, Kyoto, Japan) at the unit of Microanalysis, Faculty of Science, Cairo University. ESI-HRMS were determined using Bruker Bio TOF III (ESI-TOF, Bruker, MA, USA) in the Genomic Research Centre, Academia Sinica, Taiwan. Elemental microanalyses were performed on a Vario elemental analyzer III (Vario, Hanau, Germany) at the unit of Microanalysis, Faculty of Science, Cairo University. Compounds 6-Amino-3-(2-fluorobenzyl)-1*H*-pyrimidine-2,4-dione **2**, 6-Amino-3-(2-substituted benzyl)-5-nitroso-1*H*-pyrimidine-2,4-dione **3-4**, 5,6-Diamino-3-(2-substituted benzyl)-1*H*-pyrimidine-2,4-dione **5-6** and 1-(2-Fluorobenzyl)-8-thioxo-3,7,8,9-tetrahydro-purine-2,6-dione **8** were prepared according to reported procedures[3,6,25]. The intermediates *N*-Substituted aryl-2-chloroacetamides **9-15** were prepared according to reported methods [23,26,27].The required phenacyl bromide derivatives **30-36** were prepared according to reported procedure [28,29].

2.1.1. *6-Amino-3-benzyl-1H-pyrimidine-2,4-dione **1***

A suspension of 6-aminouracil (5.0 g, 39.34 mmol) and of ammonium sulphate (0.20 g) were refluxed in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (40 ml) for 5 h, within this time the mixture became clear homogenous. Excess HMDS was distilled off at first under atmospheric pressure, then *in vacuo*. The product was allowed to cool to about 50°C, the required amount of benzyl chloride (4.1 ml, 35.41 mmol) was added. The reaction mixture was heated and cooled successively (to keep the temperature within 70-120ºC) for 5-6 h (increase of temperature lead to formation of disubstituted derivative), the reaction progress was monitored with TLC (eluent: CH_2Cl_2: MeOH, 9:1). The reaction was stopped when the disubstituted product started to appear on TLC. The mixture was allowed to cool to ambient temperature and a solution of sodium thiosulfate in water was added. The reaction mixture was cooled in an ice bath, and a saturated solution of sodium bicarbonate in water was added portionwise until effervescence ceased. The formed precipitate was filtered and washed with cold water then with diethyl ether. The product was purified using column chromatography (MeOH:CH_2Cl_2 = 1:99 to 2.5:97.5) as white crystals, m.p. 260-263ºC (reported m.p. is 252ºC, Müller, 1991); IR (KBr) ύ (cm^{-1}) 3400 (NH_2); 3200 (NH); 3065 (Ar-H); 2965 (C-H aliphatic); 1679, 1641 (C=O); 728, 691 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6) δ 4.60 (s, 1H, C5-H), 4.83 (s, 2H, N3-CH_2), 6.17 (s, 2H,

NH_2), 7.17 (s, 5H, Ar-H), 10.47 (br s, 1H, N1-H); Anal. Calc. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34; Found: C, 60.63; H, 5.40; N, 19.40.

2.1.2.*1-Substituted-8-thioxo-3,7.8,9-tetrahydropurine-2,6-dione* ***7-8***
Potassium hydroxide (0.3 g, 5.5 mmol) was dissolved in 40 ml absolute ethanol, then carbon disulfide (0.4 g, 5.5 mmol) was added followed by addition of 5,6-Diamino-3-(2-substituted benzyl)-1*H*-pyrimidine-2,4-dione (**5 or 6)** (5.5 mmol). The reaction mixture was refluxed for 5 h, then diluted with warm water (6 ml) with stirring, and then acetic acid 95% (0.5 ml) in water (1 ml) was added portionwise. The reaction mixture was allowed to cool in refrigerator for 3 h, the product was collected by filtration and then recrystallized from absolute ethanol to afford the compounds (**7-8)** as white crystals.

2.1.2.1. *1-Benzyl-8-thioxo-3,7,8,9-tetrahydro-purine-2,6-dione* ***7***
White crystals, m.p. 331-333ºC, yield 77%, IR (KBr) ύ (cm^{-1}) 3450 (NH); 3005 (Ar-H); 2855 (C-H aliphatic); 1712, 1633 (C=O); 736, 687 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6) δ 5.00 (s, 2H, N1-CH_2), 7.27 (s, 5H, Ar-H), 8.17 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43; S, 11.69; Found: C, 52.08; H, 4.10; N, 20.04; S, 11.43.

2.1.3.*2-[(1-substituted)-2,6-dioxo-2,3,6,7–tetrahydro-1H-purin-8-yl-sulfanyl]-N-substituted acetamide* ***16-29***
To a stirred solution of compounds **7** or **8** (5.5 mmol) in aqueous sodium hydroxide 1% w/v (25 ml), the appropriate *N*-substituted aryl-2-chloroacetamides **9-15** (5.5 mmol) dissolved in absolute ethanol (5 ml) was added portionwise. The reaction mixture was stirred at ambient temperature overnight, and then cooled in a refrigerator for 3 h. The product was filtered, washed with water then diethyl ether. The product was recrystallized from absolute ethanol to afford the target compounds **16-29**.

2.1.3.1. *2-(1-Benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl)-N-p-tolyl acetamide* ***16***
White crystals, m.p. 299-301ºC, yield 77%, IR (KBr) ύ (cm^{-1}) 3480 (N-H); 3275 (N-H amide); 3080 (Ar-H); 2955 (C-H aliphatic); 1709, 1646, 1616 (C=O); 807, 749, 702 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_3), 4.17 (s, 2H, SCH_2), 5.05 (s, 2H, N1-CH_2), 6.90-7.75 (m, 9H, Ar-H), 10.57 (s, 1H, amide-H), 12.50 (br s, 2H, N3-H and N7-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.33, 36.29, 42.81, 119.15, 126.79, 127.10, 128.16, 129.06, 132.45, 136.15, 137.76, 150.76, 154.05, 165.29; Anal. Calc. (%) for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; Found: C, 59.63; H, 4.58; N, 16.27.

2.1.3.2. N-(4-Acetylphenyl)-2-(1-benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl-sulfanyl) acetamide ***17***
White crystals, m.p. 302-304ºC, yield 85%, IR (KBr) ύ (cm^{-1}) 3285 (N-H); 3175 (N-H amide); 3050 (Ar-H); 2955 (C-H aliphatic); 1709, 1663, 1619 (C=O); 825, 748, 702 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.53 (s, 3H, $COCH_3$), 4.18 (s, 2H, SCH_2), 5.03 (s, 2H, N1-CH_2), 7.30 (s, 5H, Ar-H), 7.70 (d, *J* = 8.7 Hz, 2H, 2′,6′Ar-H), 8.00 (d, *J* = 8.7 Hz, 2H, 3′,5′Ar-H), 10.93 (s, 1H, amide-H), 12.33 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{22}H_{19}N_5O_4S$: C, 58.79; H, 4.26; N, 15.58; Found: C, 58.64; H, 4.70; N, 15.64.

2.1.3.3. *2-(1-Benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl)-N-(4-bromophenyl) acetamide* ***18***
White crystals, m.p. 300-302ºC, yield 45%, IR (KBr) ύ (cm^{-1}) 3470 (N-H); 3365 (N-H amide); 3075 (Ar-H); 2980 (C-H aliphatic); 1710, 1647, 1617 (C=O); 813, 748, 702 (Ar-H), 541 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.16 (s, 2H, SCH_2), 4.99 (s, 2H, N1-CH_2), 7.19-7.29 (m, 5H, Ar-H), 7.48 (d, *J* = 8.8 Hz, 2H, 2′,6′Ar-H), 7.54 (d, *J* = 8.8 Hz, 2H, 3′,5′Ar-H), 10.47 (s, 1H, amide-H), 11.99 (br s, 1H, N3-H), 13.51 (br s, 1H, N7-H); Anal. Calc. (%) for $C_{20}H_{16}BrN_5O_3S$: C, 49.39; H, 3.32; N, 14.40; S, 6.59; Found: C, 48.99; H, 3.74; N, 13.99; S, 6.20.

2.1.3.4. *2-(1-Benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl)-N-(4-chlorophenyl) acetamide* ***19***
White crystals, m.p. 314-316ºC, yield 65%, IR (KBr) ύ (cm^{-1}) 3425 (N-H); 3295 (N-H amide); 3065 (Ar-H); 2945 (C-H aliphatic); 1708, 1647, 1617 (C=O); 1090 (C-Cl); 818, 748, 718 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.10 (s, 2H, SCH_2), 5.10 (s, 2H, N1-CH_2), 7.37 (s, 5H, Ar-H), 7.47 (d, *J* = 8.4 Hz, 2H, 2′,6′ Ar-H), 7.73 (d, *J* = 8.4 Hz, 2H, 3′,5′ Ar-H), 10.90 (s, 1H, amide-H), 12.67 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{20}H_{16}ClN_5O_3S$: C, 54.36; H, 3.65; N, 15.85; S, 7.26; Found: C, 54.09; H, 3.82; N, 16.03; S, 6.96.

2.1.3.5. *2-(1-Benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl)-N-(4-methoxyphenyl) acetamide* ***20***
White crystals, m.p. 278-280ºC, yield 79%, IR (KBr) ύ (cm^{-1}) 3380 (N-H); 3235 (N-H amide); 3055 (Ar-H); 2915 (C-H aliphatic); 1700, 1630, 1620 (C=O); 1248 & 1023 (C-O); 820, 749, 718 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 4.20 (s, 2H, SCH_2), 5.13 (s, 2H, N1-CH_2), 7.00 (d, *J* = 8.2 Hz, 2H, 3′,5′Ar-H), 7.40 (s, 5H, Ar-H), 7.63 (d, *J* = 8.2 Hz, 2H, 2′,6′Ar-H), 10.63 (s, 1H, amide-H), 12.00 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{21}H_{19}N_5O_4S$: C, 57.66; H, 4.38; N, 16.01; S, 7.33; Found: C, 57.28; H, 4.31; N, 16.00; S, 7.00.

2.1.3.6. *N-Benzyl-2-(1-benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl) acetamide* ***21***
White crystals, m.p. 285-287ºC, yield 83%, IR (KBr) ύ (cm^{-1}) 3445 (N-H); 3260 (N-H amide); 3065 (Ar-H); 2955 (C-H aliphatic); 1704, 1643, 1632 (C=O); 750, 697 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.10 (s, 2H, SCH_2), 4.33 (d, 2H, NH-C$\underline{H_2}$-C_6H_5), 5.10 (s, 2H, N1-CH_2), 7.37 (s, 10H, Ar-H); 8.77 (t, 1H, amide-H); 12.67 (2 br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; S, 7.61; Found: C, 59.52; H, 4.59; N, 16.61; S, 7.36.

2.1.3.7. *2-(1-Benzyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl)-N-phenethyl-acetamide* ***22***
White crystals, m.p. 290-292ºC, yield 79%, IR (KBr) ύ (cm^{-1}) 3390 (N-H); 3260 (N-H amide); 3065 (Ar-H); 2945 (C-H aliphatic); 1700, 1638, 1621 (C=O); 748, 699 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.70 (t, 2H, NH-$CH_2C\underline{H_2}$-C_6H_5), 3.00-3.77 (m, 2H, NH-C$\underline{H_2}CH_2$-C_6H_5), 3.87 (s, 2H, SCH_2), 5.03 (s, 2H, N1-CH_2), 7.20 (s, 5H, Ar-H), 7.30 (s, 5H, Ar-H), 8.20 (t, 1H, amide-H), 12.50 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{22}H_{21}N_5O_3S$: C, 60.67; H, 4.86; N, 16.08; S, 7.36; Found: C, 61.01; H, 5.16; N, 15.95; S, 7.19.

2.1.3.8. *2-[1-(2-Fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl]-N-p-tolyl acetamide* ***23***
White crystals, m.p. 230-232ºC, yield 63%, IR (KBr) ύ (cm^{-1}) 3450 (N-H); 3370 (N-H amide); 3095 (Ar-H); 2945 (C-H aliphatic); 1709, 1680, 1620 (C=O); 1538 (N-H); 1224 (C-F); 808, 748 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.10 (s, 2H, SCH_2), 5.00 (s, 2H, N1-CH_2), 6.77-7.57 (m, 8H, Ar-H), 10.45 (s, 1H, amide-H), 12.50 (br s, 2H, N3-H and N7-H); ESI-HRMS (*m/z*): 440.1197 (M^++1) (calcd. 440.1187); Anal. Calc. (%) for $C_{21}H_{18}FN_5O_3S$: H, 4.13; N, 15.94; S, 7.30; Found: H, 4.02; N, 16.25; S, 7.23;

2.1.3.9. *N-(4-Acetylphenyl)-2-[1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl] acetamide* ***24***
White crystals, m.p. 279-281ºC, yield 69%, IR (KBr) ύ (cm^{-1}) 3465 (N-H); 3290 (N-H amide); 3095 (Ar-H); 2925 (C-H aliphatic); 1707, 1663, 1634 (C=O); 1592 (N-H); 1224 (C-F); 829, 747 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.57 (s, 3H, $COCH_3$), 4.23(s, 2H, SCH_2), 5.00 (s, 2H, N1-CH_2), 6.78-7.40 (m, 4H, Ar-H), 7.62 (d, *J* = 8.2 Hz, 2H, 2′,6′ Ar-H), 7.90 (d, *J* = 8.2 Hz, 2H, 3′,5′ Ar-H), 10.77 (s, 1H, amide-H), 12.00 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{22}H_{18}FN_5O_4S$: C, 56.52; H, 3.88; N, 14.98; Found: C, 56.62; H, 4.12; N, 15.12.

2.1.3.10. *N-(4-Bromophenyl)-2-[1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl] acetamide* ***25***
White crystals, m.p. 281-282ºC, yield 58%, IR (KBr) ύ (cm^{-1}) 3485 (N-H); 3260 (N-H amide); 3065 (Ar-H); 2950 (C-H aliphatic); 1711, 1647, 1617 (C=O); 1555 (N-H); 1225 (C-F); 812, 750 (Ar-H), 651 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.16 (s, 2H, SCH_2), 5.04 (s, 2H, N1-CH_2), 7.00-7.29 (m, 4H, Ar-H), 7.48 (d, *J* = 8.8 Hz, 2H, 2′,6′Ar-H), 7.54 (d, *J* = 8.8 Hz, 2H, 3′,5′Ar-H), 10.51 (s, 1H, amide-H), 12.03 (s, 1H, N3-H), 13.54 (s, 1H, N7-H); Anal. Calc. (%) for $C_{20}H_{15}BrFN_5O_3S$: C, 47.63; H, 3.00; N, 13.89; S, 6.36; Found: C, 47.55; H, 2.87; N, 13.52; S, 6.68.

2.1.3.11. *N-(4-Chlorophenyl)-2-[1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl] acetamide* ***26***
White crystals, m.p. 299-300ºC; yield 82%, IR (KBr) ύ (cm^{-1}) 3440 (N-H); 3290 (N-H amide); 3085 (Ar-H); 2965 (C-H aliphatic); 1711, 1647, 1618 (C=O); 1554 (N-H); 1225 (C-F); 1082 (C-Cl); 815, 750 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.17 (s, 2H, SCH_2), 5.04 (s, 2H, N1-CH_2), 7.00-7.29 (m, 4H, Ar-H), 7.36 (d, *J* = 8.8 Hz, 2H, 2′,6′Ar-H), 7.59 (d, *J* = 8.8 Hz, 2H, 3′,5′Ar-H), 10.51 (s, 1H, amide-H), 12.03 (s, 2H, N3-H), 13.53 (s, 1H, N7-H); EI-MS: *m/z*: 459.30 (M^+, 2.56%), 461.30(M^++2, 1.70%), 127 (100%). Anal. Calc. (%) for $C_{20}H_{15}ClFN_5O_3S$: C, 52.23; H, 3.29; N, 15.23; S, 6.97; Found: C, 52.36; H, 3.40; N, 15.05; S, 7.33.

2.1.3.12. *2-[1-(2-Fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl]-N-(4-methoxyphenyl) acetamide* ***27***
White crystals, m.p. 285-287ºC; yield 89%, IR (KBr) ύ (cm^{-1}) 3465 (N-H); 3275 (N-H amide); 3080 (Ar-H); 2955 (C-H aliphatic); 1710, 1637, 1616 (C=O); 1550 (N-H); 1225 (C-F); 1248 & 1080 (C-O); 819, 749 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 3H, OCH_3), 4.13 (s, 2H, SCH_2), 5.04 (s, 2H, N1-CH_2), 6.87 (d, J = 8.8 Hz, 2H, 3′,5′Ar-H), 6.99-7.29 (m, 4H, Ar-H), 7.47 (d, J = 8.7 Hz, 2H, 2′,6′Ar-H), 10.23 (s, 1H, amide-H), 12.03 (s, 1H, N3-H), 13.52 (s, 1H, N7-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.24, 37.06, 55.18, 113.94, 115.01, 115.22, 120.72, 124.44, 124.60, 127.78, 128.63, 131.91, 145.86, 147.77, 149.20, 150.78, 153.84, 155.44, 158.60, 161.10, 165.13, 167.17; EI-MS: *m/z*: 455.90 (M^+, 53.92%), 181 (100%). Anal. Calc. (%) for $C_{21}H_{18}FN_5O_4S$: C, 55.38; H, 3.98; N, 15.38; S, 7.04; Found: C, 55.09; H, 4.03; N, 15.13; S, 6.76.

2.1.3.13. *N-Benzyl-2-[1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl] acetamide* ***28***
White crystals, m.p. 283-284ºC; yield 68%, IR (KBr) ύ (cm^{-1}) 3410 (N-H); 3265 (N-H amide); 3065 (Ar-H); 2955 (C-H aliphatic); 1709, 1645, 1627 (C=O); 1543 (N-H); 1224 (C-F); 749, 691 (Ar-H); ^{1}H NMR (60 MHz, DMSO-

d_6): δ 4.08 (s, 2H, SCH_2), 4.60 (d, 2H, NH-C$\underline{H_2}$-C_6H_5), 5.23 (s, 2H, N1-CH_2), 7.00-7.67 (m, 9H, Ar-H), 8.67 (t, 1H, amide-H); 12.00 (s, 1H, N3-H), 13.57 (s, 1H, N7-H); Anal. Calc. (%) for $C_{21}H_{18}FN_5O_3S$: C, 57.39; H, 4.13; N, 15.94; Found: C, 57.08; H, 4.33; N, 15.91.

2.1.3.14. *2-[1-(2-Fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylsulfanyl]-N-phenethyl acetamide* ***29***
White crystals, m.p. 285-287ºC; yield 64%, IR (KBr) ύ (cm^{-1}) 3500 (N-H); 3395 (N-H amide); 3080 (Ar-H); 2960 (C-H aliphatic); 1710, 1650, 1626 (C=O); 1544 (N-H); 1226 (C-F); 748, 691 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.90 (t, 2H, NH-CH_2C$\underline{H_2}$-C_6H_5), 3.13-3.95 (m, 2H, NH-C$\underline{H_2}$$CH_2$-$C_6H_5$), 4.05 (s, 2H, SCH_2), 5.33 (s, 2H, N1-CH_2), 7.08-7.70 (m, 9H, Ar-H), 8.42 (t, 1H, amide-H), 12.50 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{22}H_{20}FN_5O_3S$: C, 58.27; H, 4.45; N, 15.44; Found: C, 58.36; H, 4.68; N, 15.69.

2.1.4.*1-substituted-8-[2-(4-substituted phenyl)-2-oxo ethylsulfanyl]-3,7-dihydro-1H-purine-2,6-diones* ***37-50***
To a stirred solution of compounds **7** or **8** (5.5 mmol) in aqueous sodium hydroxide 1% w/v (25 ml), the appropriate *p*-(un)substituted phenacyl bromide **30-36** (5.5 mmol) dissolved in absolute ethanol (5 ml) was added dropwise. The reaction mixture was stirred at the ambient temperature overnight, and then cooled in a refrigerator for 3 h. The product was filtered, washed with water then diethyl ether. The product was recrystallized from absolute ethanol to afford the target compounds **37-50**.

2.1.4.1. *1-Benzyl-8-(2-oxo-2-phenylethylsulfanyl)-3,7-dihydropurine-2,6-dione* ***37***
White crystals, m.p. 283-284ºC; yield 70%, IR (KBr) ύ (cm^{-1}) 3430 (N-H); 3075 (Ar-H); 2895 (C-H aliphatic); 1707, 1661, 1616 (C=O); 1542 (N-H); 745, 701 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.00 (br s, 4H, SCH_2 & N1-CH_2), 7.30 (s, 5H, Ar-H), 7.40-8.40 (m, 5H, Ar-H); 12.50 (s, 1H, N3- H), 13.50 (s, 1H, N7-H); Anal. Calc. (%) for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; S, 8.17; Found: C, 61.34; H, 4.08; N, 14.06; S, 7.91.

2.1.4.2. *1-Benzyl-8-[2-(4-bromophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***38***
White crystals, m.p. 305-306ºC; yield 62%, IR (KBr) ύ (cm^{-1}) 3445 (N-H); 3075 (Ar-H); 2895 (C-H aliphatic); 1708, 1663, 1616 (C=O); 1581 (N-H); 806, 748, 701 (Ar-H), 543 (C-Br); ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.87 (s, 2H, SCH_2), 5.00 (s, 2H, N1-CH_2), 7.15 (s, 5H, Ar-H), 7.58 (d, *J* = 8.8 Hz, 2H, 3′,5′Ar-H), 7.87 (d, *J* = 8.8 Hz, 2H, 2′,6′Ar-H), 12.00 (br s, 2H, N3-H and N7-H); EI-MS: *m/z*: 471.1 (M^+/81.48%), 452.1 (100%). Anal. Calc. (%) for $C_{20}H_{15}BrN_4O_3S$: C, 50.97; H, 3.21; N, 11.89; S, 6.80; Found: C, 50.60; H, 3.33; N, 11.64; S, 7.08.

2.1.4.3. *1-Benzyl-8-[2-(4-chlorophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***39***
White crystals, m.p. 291-293ºC; yield 69% , IR (KBr) ύ (cm^{-1}) 3415 (N-H); 3060 (Ar-H); 2875 (C-H aliphatic); 1708, 1663, 1616 (C=O); 1546 (N-H); 1088 (C-Cl); 809, 766, 702 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.10 (s, 2H, SCH_2), 5.20 (s, 2H, N1-CH_2), 7.50 (s, 5H, Ar-H), 7.83 (d, *J* = 8.2 Hz, 2H, 3′,5′Ar-H), 8.32 (d, *J* = 8.2 Hz, 2H, 2′,6′Ar-H), 12.50 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{20}H_{15}ClN_4O_3S$: C, 56.27; H, 3.54; N, 13.12; S, 7.51; Found: C, 56.00; H, 3.50; N, 13.58; S, 7.32.

2.1.4.4. *1-Benzyl-8-[2-(4-fluorophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***40***
White crystals, m.p. 300-302ºC; yield 68%, IR (KBr) ύ (cm^{-1}) 3400 (N-H); 3060 (Ar-H); 2875 (C-H aliphatic); 1707, 1664, 1616 (C=O); 1541 (N-H); 1230 (C-F); 823, 747, 702 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.93 (s, 2H, SCH_2), 4.97 (s, 2H, N1-CH_2), 7.17-7.60 and 7.73-8.33 (2m, 9H, Ar-H), 12.83 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{20}H_{15}FN_4O_3S$: C, 58.53; H, 3.68; N, 13.65; Found: C, 58.61; H, 3.70; N, 13.38.

2.1.4.5. *1-Benzyl-8-(2-oxo-2-p-tolyl-ethylsulfanyl)-3,7-dihydropurine-2,6-dione* ***41***
White crystals, m.p. 268-271ºC; yield 69%, IR (KBr) ύ (cm^{-1}) 3420 (N-H); 3100 (Ar-H); 2905 (C-H aliphatic); 1708, 1657, 1616 (C=O); 1546 (N-H); 801, 747, 702 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 4.95 (s, 2H, SCH_2), 4.99 (s, 2H, N1-CH_2), 7.23-7.29 (m, 5H, Ar-H), 7.36 (d, *J* = 7.8 Hz, 2H, 3′,5′Ar-H), 7.91 (d, *J* = 7.8 Hz, 2H, 2′,6′Ar-H), 11.89, (s, 1H, N3-H), 13.47 (s, 1H, N7-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.24, 40.13, 42.86, 107.80, 126.89, 127.17, 128.27, 128.52, 129.39, 132.78, 137.88, 144.35, 147.74, 149.07, 150.78, 153.86, 192.49; Anal. Calc. (%) for $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78; S, 7.89; Found: C, 61.95; H, 4.21; N, 13.97; S, 7.73.

2.1.4.6. *1-Benzyl-8-[2-(4-methoxyphenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***42***
White crystals, m.p. 292-293ºC; yield 71%, IR (KBr) ύ (cm^{-1}) 3495 (N-H); 3060 (Ar-H); 2875 (C-H aliphatic); 1707, 1641, 1620 (C=O); 1596 (N-H); 1265, 1067 (C-O); 791, 746, 701 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 4.93 (s, 2H, SCH_2), 4.98 (s, 2H, N1-CH_2), 7.19 -7.29 (m, 5H, Ar-H), 7.07 (d, *J* = 8.8 Hz, 2H, 3′,5′Ar-H), 7.99 (d, *J* = 8.8 Hz, 2H, 2′,6′Ar-H), 11.94 (s, 1H, N3-H), 13.47 (s, 1H, N7-H); EI-MS: *m/z*: 422 (M^+, 24.47%), 123 (100%). Anal. Calc. (%) for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26; Found: C, 59.57; H, 4.11; N, 13.33.

2.1.4.7. *1-Benzyl-8-[2-(4-nitrophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***43***
Yellowish-white crystals, m.p. 269-271ºC; yield 45%, IR (KBr) ύ (cm^{-1}) 3450 (N-H); 3025 (Ar-H); 2865 (C-H aliphatic); 1705, 1634, 1616 (C=O); 1556 (N-H); 1512, 1333 (NO_2); 845, 736, 691 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.10 (s, 4H, SCH_2 & N1-CH_2), 7.37 (s, 9H, Ar-H), 12.83 (br. s, 2H, N3-H and N7-H); ESI-HRMS (*m/z*): 438.0878 (M^++1) (calcd. 438.0867); Anal. Calc. (%) for $C_{20}H_{15}N_5O_5S.H_2O$: C, 52.74; H, 3.76; S, 7.04; Found: C, 52.86; H, 3.41; S, 7.28.

2.1.4.8. *1-(2-Fluorobenzyl)-8-(2-oxo-2-phenylethylsulfanyl)-3,7-dihydropurine-2,6-dione* ***44***
White crystals, m.p. 269-271ºC; yield 70%, IR (KBr) ύ (cm^{-1}) 3435 (N-H); 3065 (Ar-H); 2965 (C-H aliphatic); 1709, 1664, 1616 (C=O); 1543 (N-H); 1247 (C-F); 747, 688 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.97(s, 2H, SCH_2), 5.07 (s, 2H, N1-CH_2), 6.70-8.20 (m, 9H, Ar-H), 12.50 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{20}H_{15}FN_4O_3S$: C, 58.53; H, 3.68; N, 13.65; S, 7.81; Found: C, 58.47; H, 4.00; N, 13.27; S, 8.02.

2.1.4.9. *8-[2-(4-Bromophenyl)-2-oxoethylsulfanyl]-1-(2-fluorobenzyl)-3,7-dihydropurine-2,6-dione* ***45***
White crystals, m.p. 288-289ºC; yield 58%, IR (KBr) ύ (cm^{-1}) 3405 (N-H); 3000 (Ar-H); 2980 (C-H aliphatic); 1710, 1662, 1620 (C=O); 1557 (N-H); 1224 (C-F); 804, 751 (Ar-H); 550 (C-Br); ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.95 (s, 2H, SCH_2), 5.07 (s, 2H, N1-CH_2), 6.88 -7.50 (m, 4H, Ar-H), 7.58 (d, *J* = 8.2 Hz, 2H, 3′,5′Ar-H), 8.00 (d, *J* = 8.2 Hz, 2H, 2′,6′Ar-H), 12.30 (br s, 2H, N3-H and N7-H); EI-MS: *m/z*: 488.30 (M^+, 21.08%), 490.25(M^++2, 19.28%), 109.15 (100%). Anal. Calc. (%) for $C_{20}H_{14}BrFN_4O_3S$: C, 49.09; H, 2.88; N, 11.45; S, 6.55; Found: C, 48.90; H, 3.08; N, 11.27; S, 6.80.

2.1.4.10. *8-[2-(4-Chlorophenyl)-2-oxoethylsulfanyl]-1-(2-fluorobenzyl)-3,7-dihydropurine-2,6-dione* ***46***
White crystals, m.p. 277-279ºC; yield 62%, IR (KBr) ύ (cm^{-1}) 3455 (N-H); 3090 (Ar-H); 2980 (C-H aliphatic); 1710, 1667, 1616 (C=O); 1581 (N-H); 1229 (C-F); 1083 (C-Cl); 806, 749 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.00 (s, 2H, SCH_2), 5.10 (s, 2H, N1-CH_2), 6.90 -7.43 (m, 4H, Ar-H), 7.60 (d, *J* = 8.7 Hz, 2H, 3′,5′Ar-H), 8.07 (d, *J* = 8.7 Hz, 2H, 2′,6′Ar-H), 12.00 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{20}H_{14}ClFN_4O_3S$: C, 54.00; H, 3.17; N, 12.59; S, 7.21; Found: C, 54.11; H, 3.49; N, 12.43; S, 7.19.

2.1.4.11. *1-(2-Fluorobenzyl)-8-[2-(4-fluorophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***47***
White crystals, m.p. 292-294ºC; yield 63%, IR (KBr) ύ (cm^{-1}) 3350 (N-H); 3070 (Ar-H); 2880 (C-H aliphatic); 1710, 1666, 1618 (C=O); 1590 (N-H); 1230 (C-F); 825, 748 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.98 (s, 2H, SCH_2), 5.04 (s, 2H, N1-CH_2), 6.99 -7.43 (m, 4H, Ar-H), 8.10 (d, *J* = 8.2 Hz, 2H, 3′,5′Ar-H), 8.12 (d, *J* = 8.2 Hz, 2H, 2′,6′Ar-H), 11.95 (s, 1H, N3-H), 13.48 (s, 1H, N7-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.06, 40.13, 107.64, 115.01, 115.22, 115.83, 116.05, 124.42, 124.60, 127.73, 127.78, 128.65, 128.71, 131.45, 131.53, 132.07, 147.92, 149.13, 150.70, 153.71, 158.63, 161.06, 164.08, 166.59, 191.64; Anal. Calc. (%) for $C_{20}H_{14}F_2N_4O_3S$: C, 56.07; H, 3.29; N, 13.08; S, 7.48; Found: C, 55.84; H, 3.00; N, 12.90; S, 7.22.

2.1.4.12. *1-(2-Fluorobenzyl)-8-(2-oxo-2-p-tolyl-ethylsulfanyl)-3,7-dihydropurine-2,6-dione* ***48***
White crystals, m.p. 295-297ºC; yield 67%, IR (KBr) ύ (cm^{-1}) 3435 (N-H); 3100 (Ar-H); 2905 (C-H aliphatic); 1710, 1661, 1618 (C=O); 1549 (N-H); 1229 (C-F); 800, 750 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 4.87 (s, 2H, SCH_2), 5.00 (s, 2H, N1-CH_2), 6.62 -7.47 (m, 4H, Ar-H), 7.30 (d, *J* = 8.7 Hz, 2H, 3′,5′Ar-H), 7.87 (d, *J* = 8.7 Hz, 2H, 2′,6′Ar-H), 12.00 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{21}H_{17}FN_4O_3S$: C, 59.42; H, 4.04; N, 13.20; S, 7.55; Found: C, 59.06; H, 3.84; N, 12.94; S, 7.50.

2.1.4.13. *1-(2-Fluorobenzyl)-8-[2-(4-methoxyphenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***49***
White crystals, m.p. 273-275ºC; yield 47%, IR (KBr) ύ (cm^{-1}) 3450 (N-H); 3015 (Ar-H); 2960 (C-H aliphatic); 1710, 1649, 1618 (C=O); 1549 (N-H); 1250, 1165 (C-O); 1229 (C-F); 815, 749 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 4.93 (s, 2H, SCH_2), 5.33 (s, 2H, N1-CH_2), 6.97-7.57 (m, 6H, Ar-H), 8.00 (d, *J* = 8.6 Hz, 2H, 2′,6′Ar-H), 12.17 (br s, 2H, N3-H and N7-H); Anal. Calc. (%) for $C_{21}H_{17}FN_4O_4S$: C, 57.27; H, 3.89; N, 12.72; Found: C, 57.11; H, 3.54; N, 12.61.

2.1.4.14. *1-(2-Fluorobenzyl)-8-[2-(4-nitrophenyl)-2-oxoethylsulfanyl]-3,7-dihydropurine-2,6-dione* ***50***
Yellowish-white crystals, m.p. 270-273ºC; yield 51%, IR (KBr) ύ (cm^{-1}) 3390 (N-H); 3085 (Ar-H); 2955 (C-H aliphatic); 1703, 1675, 1645 (C=O); 1595 (N-H); 1514, 1335 (NO_2); 1221 (C-F); 841, 749 (Ar-H); ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.03 (s, 2H, SCH_2), 5.07 (s, 2H, N1-CH_2), 6.80 -7.73 (m, 4H, Ar-H), 8.23 (d, *J* = 8.5 Hz, 2H, 2′,6′Ar-H), 8.40 (d, *J* = 8.5 Hz, 2H, 3′,5′Ar-H), 12.00 (br s, 2H, N3-H and N7-H) ; Anal. Calc. (%) for $C_{20}H_{14}FN_5O_5S$: C, 52.75; H, 3.10; N, 15.38; S, 7.04; Found: C, 53.00; H, 3.07; N, 15.55; S, 7.44.

2.2. Anti-inflammatory activity

The rat paw thickness was measured with a Vernier calliper (SMIEC, Shangahai, China). Carrageenan (Sigma, USA), indomethacin (Liometacin® vial, Nile Company, Cairo, Egypt), sodium carboxymethylcellulose (NaCMC) (El Nasr Pharm. Company, Cairo, Egypt) and normal saline (Almottahedoon Pharma Company, Cairo, Egypt) were obtained from the local market. Male adult albino rats (120–150 g) were obtained from the animal house (Faculty of Medicine, Assiut University, Egypt). Animals were housed in separate cages 6 animals each, in temperature-controlled rooms at 25±2°C. Animals were allowed free access to rodent chow and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines [30] and Institutional Ethical Committee Approval was obtained.

The anti-inflammatory activity of the newly synthesized compounds **16-29** and **37-50** in addition to the intermediates **7** and **8** was evaluated according to the carrageenan induced paw edema method in comparison with indomethacin as a reference drug [31]. The test is based on pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 ml of 1 % solution in normal saline) into the right hind paw of the rats.

Male adult albino rats were divided into groups of four animals each. The rat paw thickness was measured before and 1 h after carrageenan injection to detect the carrageenan induced inflammation.

Test compounds **7, 8, 16-29** and **37-50** and indomethacin at a dose of 0.02 mmol/Kg were suspended in 1 % NaCMC in normal saline. Suspensions were injected *i.p.* (1 ml each) to rats 1 h after carrageenan injection. In addition, a control group received the vehicle 1 % NaCMC solution in normal saline (negative control).

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 h after injection of the test compounds, reference drug, and control and results were listed in **Table 1**.

The percentage of edema and percentages of edema inhibition were calculated according to the following [32]:

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

Where, VR: Average right paw thickness, VL: Average left paw thickness.

2.3. MOLECULAR DOCKING

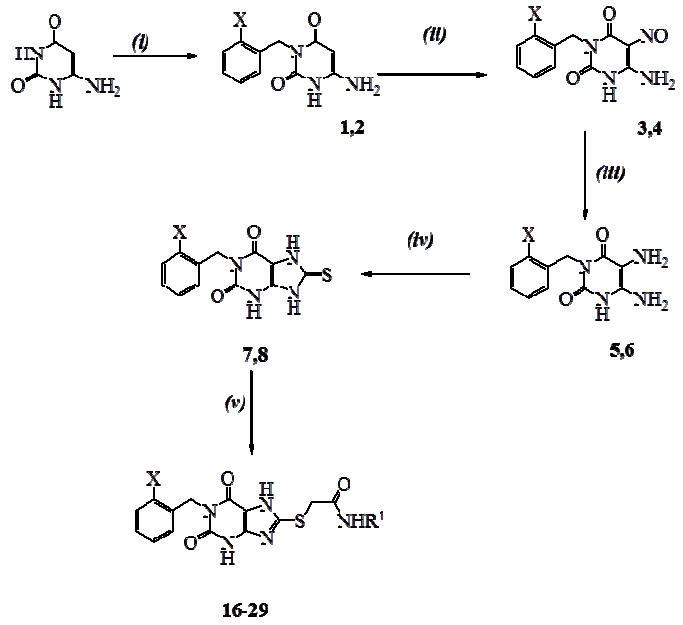
The docking studies were carried out at the Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, on a Dell Precision™ T3600 Workstation [Intel Xeon E5-1660 3.3GHz, 16GB 1600MHz DDR3, ECC RDIMM 1TB (7200RPM), 1GB NVIDIA Quadro 2000, Windows 7 Professional (64 Bit)] using Molecular Operating Environment (MOE) [33] as the computational software.

All minimizations were performed with MOE until RMSD gradient of 0.00001 Kcal mol^{-1} $Å^{-1}$ with MMFF94X force-field [34] and the partial charges were automatically calculated. The X-ray crystallographic structure of murine Cyclooxygenase-2 complexed with indomethacin (PDB code: 4COX) was obtained from protein data bank [35]. The enzyme was prepared for docking studies where: i) Protein structures were repaired using Ramchandran plot to ascertain the health of protein and then appropriately protonated in the presence of ligands using the Protonate 3D process [36] in MOE. ii) Ligand molecule was removed from the enzyme active site. iii) Hydrogen atoms were added to the structure with their standard geometry. iv) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. v) The obtained model was then used in predicting the ligand-enzyme interactions at the active site.

RESULTS AND DISCUSSION

3.1. Chemistry

The general route to obtain the target 1,8-disubstituted purine-2,6-diones (**16-29** and **37-50**) and their intermediates is presented in **Scheme 1** and **2**. The intermediate 6-amino-3-benzyl-1H-pyrimidine-2,4-dione (**1**) was prepared by modification of the reported procedure [37], by optimization of reaction temperature and reaction time to avoid the substitution at 1- and 5- positions and afforded selective alkylation at N-3 Position after purification using column chromatography. 6-Amino-3-(2-fluorobenzyl)-1H-pyrimidine-2,4-dione (**2**) was prepared as reported [3].



Comp. No.	X	R^1	Comp. No.	X	R^1
1, 3, 5, 7	H	-	2, 4, 6, 8	F	-
16	H	*p*-CH_3-C_6H_4	23	F	*p*-CH_3-C_6H_4
17	H	*p*-$COCH_3$-C_6H_4	24	F	*p*-$COCH_3$-C_6H_4
18	H	*p*-Br-C_6H_4	25	F	*p*-Br-C_6H_4
19	H	*p*-Cl-C_6H_4	26	F	*p*-Cl-C_6H_4
20	H	*p*-OCH_3-C_6H_4	27	F	*p*-OCH_3-C_6H_4
21	H	CH_2-C_6H_5	28	F	CH_2-C_6H_5
22	H	CH_2-CH_2-C_6H_5	29	F	CH_2-CH_2-C_6H_5

Reagents and conditions (i) o-X-$C_6H_4CH_2Br$/HMDS (ii) $NaNO_2$/50% CH_3COOH
(iii) $Na_2S_2O_4$/12.5% NH_3 (iv) CS_2/KOH/EtOH (v) $ClCH_2CONHR^1$/1% aqu. NaOH/EtOH

Scheme 1: Synthetic route of compounds 16-29

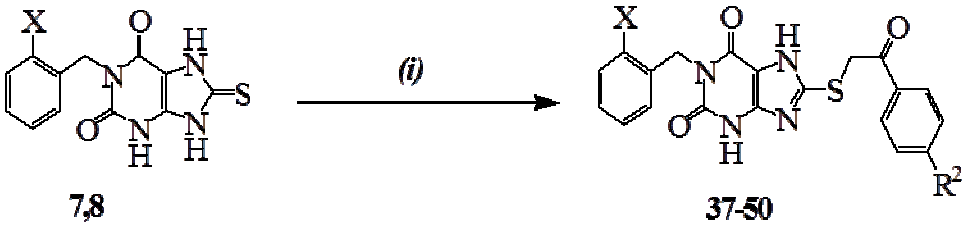
Nirosation of (**1** and **2**) afforded 6-amino-3-(2-(un)substituted benzyl)-5-nitroso-1H-pyrimidine-2,4-dione (**3** and **4**) respectively which subjected to reduction to yield 5,6-Diamino-3-(2-(un)substituted benzyl)-1*H*-pyrimidine-2,4-dione (**5-6**) respectively [3,25]. The produced 5,6-diaminouracil derivatives (**5-6**) were used, immediately, in the next step due to its sensitivity against light, oxygen, and moisture [25,38]. The key intermediates 1-benzyl-8-thioxo-3,7,8,9-tetrahydropurine-2,6-dione (**7**) and 1-(2-fluorobenzyl)-8-thioxo-3,7,8,9-tetrahydropurine-2,6-dione (**8**) were prepared from compounds (**5** and **6**) respectively as illustrated in **scheme 1**, by reaction of compounds **5** or **6** with carbon disulfide in ethanolic potassium hydroxide, based on previous reported methods [4,8,10].

The chemical structures of these compounds (**7** and **8**) were verified by IR and ^{1}H NMR. IR spectra of compound (**7**) is characterized by presence of characteristic bands of N-H at 3450 cm^{-1}, C=O groups at 1712 and 1633 cm^{-1} and a characteristic band at 3005 cm^{-1} (Ar-H stretching) in addition to its bending at 736, 687 cm^{-1}. Also, its ^{1}H NMR spectra showed the N1-CH_2 protons as singlet at δ 5.00 integrating for two protons, N3-H and N7-H at δ 8.17 which was exchangeable with D_2O, in addition to the aromatic protons at δ 7.27 ppm. Moreover the spectral data of compound (**8**) was in accordance with the reported data (Hayallah et al., 2008).
Alkylation of (**7** or **8**) with *N*-Substituted aryl-2-chloroacetamides (**9-15**) in the presence of aqueous ethanolic NaOH 1% afforded the target compounds 2-[(1-substituted)-2,6-dioxo-2,3,6,7–tetrahydro-1H-purin-8-yl-sulfanyl]-*N*-substituted acetamide (**16-29**).

Chemical structures of the derivatives (**16-29**) were elucidated depending on their IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental method of analyses. IR spectra of the new compounds (**16-29**) are characterized by presence of characteristic bands of N-H at 3285-3500 cm^{-1}, N-H amide at 3175-3395 cm^{-1} and C=O groups at 1616-1711 cm^{-1}. Moreover, compounds (**16-29**) showed the characteristic bands at 3050–3095 cm^{-1} (Ar-H stretching) in addition to its bending at 702–829 cm^{-1}. ^{1}H NMR spectra of compounds (**16-29**) showed the S-CH_2 protons resonated as a singlet at δ 3.87-4.23 ppm integrating for two protons, N1-CH_2 protons as a singlet at δ 5.00-5.33 integrating for two

protons, amide NH at δ 8.20-10.93, N3-H and N7-H at δ 12.00-13.54 which were exchangeable with D_2O, in addition to the introduced aromatic moiety protons at δ 6.77-8.00 ppm. Some of them were further confirmed by MS (see experimental part), for example, MS of compound (**26**) revealed the molecular ion peak M^+ at *m/z* 459.30 (2.56 %) corresponding to its relative molecular mass (459.06) and a base peak at *m/z* 127 (100 %). It also showed the M^++2 peak at *m/z* 461 (1.7 %).

Preparation of 1-substituted-8-[2-(4-substituted phenyl)-2-oxoethyl-sulfanyl]-3,7-dihydro-1*H*-purine-2,6-diones (**37-50**) was achieved by reaction of compounds (**7** or **8**) respectively with equimolar amounts of *p*-(un)substituted phenacyl bromide (**30-36**) in the presence of aqueous ethanolic NaOH 1%.



7,8 **37-50**

Comp. No.	X	R^1	Comp. No.	X	R^1
1, 3, 5, 7	H	-	2, 4, 6, 8	F	-
37	H	H	44	F	H
38	H	Br	45	F	Br
39	H	Cl	46	F	Cl
40	H	F	47	F	F
41	H	CH_3	48	F	CH_3
42	H	OCH_3	49	F	OCH_3
43	H	NO_2	50	F	NO_2

Reagents and conditions (i) p-R^2-$C_6H_4COCH_2Br$/1% aqu. NaOH/EtOH

Scheme 2: Synthetic route of compounds 9a-g-10a-g

Chemical structures of compounds (**37-50**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analyses. IR spectra of the new compounds (**37-50**) are characterized by presence of characteristic bands of at 3350-3495 cm^{-1} (N-H) and at 1600-1710 cm^{-1} (C=O) groups. Moreover, compounds (**37-50**) showed the characteristic bands at 3000–3100 cm^{-1} (Ar-H stretching) in addition to its bending at 688-845 cm^{-1}. ^{1}H NMR spectra of compounds (**37-50**) showed the S-CH_2 protons resonated as singlet at δ 4.87-5.33 integrating for two protons, N1-CH_2 protons as singlet at δ 4.97-5.67 integrating for two protons, N3-H and N7-H at δ 11.89-13.87 which were exchangeable with D_2O, in addition to the aromatic protons at δ 6.62 -8.40 ppm.

Also, the structure of representative target compounds (**38**, **42** and **45**); was confirmed by MS (see experimental part). For example, MS for compound MS of compound (**42**) revealed the molecular ion peak M^+ at *m/z* 422 (24.47 %) corresponding to its relative molecular mass and a base peak at *m/z* 123 (100 %).

Moreover, ^{13}CNMR of compound (**16**) showed the CH_3 appeared at δ 20.33 ppm, it also showed two signals at δ 36.29, 42.81 ppm belonging to N1-CH_2 and S-CH_2. Also, the aromatic carbons appeared at δ 119.15, 126.79, 127.10, 128.16, 129.06, 132.45, 136.15 and 137.76 ppm. In addition to the three C=O groups which appeared at δ 150.76, 154.05, 165.29 ppm. Furthermore, compounds (**23** and **43**) were further confirmed by ESI-HRMS (*m/z*) as they showed 440.1197 (M^++1) (calcd. 440.1187) and 438.0878 (M^++1) (calcd. 438.0867) respectively.

3.2. Anti-inflammatory activity:

The newly synthesized compounds (**16-29** and **37-50**) in addition to the intermediates (**7** and **8**) were tested *in-vivo* for their anti-inflammatory effects. Results revealed that all compounds showed a gradual increase of anti-inflammatory activity up to its maximum at 3 h except compounds (**25**, **26**, **27** and **48**) as they showed maximum activity at 4 h interval, **table 1**. In some cases, the anti-inflammatory activity declined after 2 h such as compounds

(**23** and **47**). Test compounds (**16-29** and **37-50**) showed 52-114% and 32-107% of the anti-inflammatory activity of indomethacin after 3 and 4 h respectively. After 5 h, they showed 21–80% edema inhibition of the anti-inflammatory activity of indomethacin, this means that these compounds showed rapid onset of anti-inflammatory activity. Compounds (**16**, **19**, **23**, **24**, **25**, **38**, **41**, **44**, **46** and **47**) were the most active showing 73-100 % anti-inflammatory activity of indomethacin after 3h. Compound (**18**) was more active than indomethacin giving 114% anti-inflammatory activity of indomethacin after 3 h, **table 1 & Fig 1**.

Table 1: Percentage of edema inhibition of compounds 7, 8, 16-29, 37-50 and indomethacin on carrageenan induced paw edema in rats

Compd. No.	Percentage of edema inhibition ± SE					
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	--	--	--	--	--	--
Indomethacin	33.18 ± 0.29*	66.59 ± 0.29***	78.21 ± 0.29***	78.68 ± 0.14***	78.68 ± 0.29***	79.12 ± 0.12***
7	38.97 ± 0.14***	50.11 ± 0.14***	67.54 ± 0.29***	84.22 ± 0.14***	52.24 ± 0.14***	37.58 ± 0.29***
8	33.63 ± 0.46***	50.33 ± 0.14***	56.86 ± 0.14***	57.78 ± 0.14***	41.79 ± 0.14***	43.01 ± 0.14***
16	11.14 ± 0.14	33.41 ± 0.29***	51.19 ± 0.14***	62.89 ± 0.14***	38.91 ± 0.07***	21.92 ± 0.14*
17	22.05 ± 0.29*	44.32 ± 0.29***	50.98 ± 0.14***	52.03 ± 0.14***	44.03 ± 0.07***	16.49 ± 0.14
18	33.63 ± 0.29***	55.90 ± 0.29***	80.40 ± 0.57***	89.76 ± 0.29***	84.43 ± 0.14***	58.66 ± 0.29***
19	22.49 ± 0.29*	50.33 ± 0.14***	56.86 ± 0.29***	57.78 ± 0.58***	44.46 ± 0.51***	11.69 ± 0.14
20	22.27 ± 0.29*	33.41 ± 0.29***	45.75 ± 0.29***	57. 57 ± 0.29***	38.91 ± 0.07***	15. 45 ± 0.04
21	27.84 ± 0.14**	33.41 ± 0.29***	45.75 ± 0.29***	46.91 ± 0.29***	41.58 ± 0.14***	14.09 ± 0.07
22	16.93 ± 0.14	25.28 ± 0.36*	29.63 ± 0.43**	41.79 ± 0.43***	25.79 ± 0.29**	3.86 ± 0.51
23	44.32 ± 0.58***	66.59 ± 0.14***	83.66 ± 0.14***	78.68 ± 0.14***	68.02 ± 0.29***	63.46 ± 0.43***
24	33.41 ± 0.22**	38.97 ± 0.14***	67.54 ± 0.29***	73.56 ± 0.14***	52.24 ± 0.14***	48.02 ± 0.29***
25	33.18 ± 0.29***	44.32 ± 0.29***	50.98 ± 0.14***	62.69 ± 0.14***	62.69 ± 0.14***	42.59 ± 0.14***
26	11.13 ± 0.29	27.84 ± 0.14**	40.30 ± 0.14***	52.24 ± 0.14***	52.24 ± 0.14***	48.07 ± 0.29***
27	27.84 ± 0.14**	27.84 ± 0.14**	40.30 ± 0.14***	41.58 ± 0.14***	41.58 ± 0.14***	27.14 ± 0.29**
28	16.48 ± 0.14	16.48 ± 0.14	34.64 ± 0.29***	46.69 ± 0.29***	36.03 ± 0.29***	26.93 ± 0.29**
29	11.36 ± 0.29	39.19 ± 0.14***	48.80 ± 0.51***	63.11 ± 0.51***	47.12 ± 0.29***	32.57 ± 0.43***
37	22.49 ± 0.29**	33.63 ± 0.29***	40.52 ± 0.14***	41.79 ± 0.09***	36.46 ± 0.29***	32.57 ± 0.14***
38	22.27 ± 0.14**	33.41 ± 0.40***	51.19 ± 0.14***	62.89 ± 0.14***	52.24 ± 0.14***	32.36 ± 0.26***
39	10.91 ± 0.29	16.48 ± 0.14	40.09 ± 0.14***	41.36 ± 0.14***	25.37 ± 0.29***	21.71 ± 0.14**
40	22.05 ± 0.29**	33.18 ± 0.29***	48.37 ± 0.07***	52.03 ± 0.14***	30.70 ± 0.14***	21.71 ± 0.03**
41	28.06 ± 0.14***	50.33 ± 0.14***	51.42 ± 0.14***	63.11 ± 0.14***	41.79± 0.43***	43.01 ± 0.14***
42	16.70 ± 0.14*	27.84 ± 0.14***	40.31 ± 0.14***	46.91 ± 0.29***	30.92 ± 0.14***	27.14 ± 0.29***
43	28.06 ± 0.14***	28.06 ± 0.14***	40.52 ± 0.09***	57.78 ± 0.29***	20.47 ± 0.14**	16.91 ± 0.29*
44	28.06 ± 0.14***	55.90 ± 0.58***	62.31 ± 0.14***	62.31 ± 0.14***	52.45 ± 0.14***	43.01 ± 0.14***
45	27.62 ± 0.03***	44.32 ± 0.29***	50.98 ± 0.14***	68.02 ± 0.17***	41.36 ± 0.14***	32.15 ± 0.14***
46	27.84 ± 0.26***	55.68 ± 0.12***	61.25 ± 0.14***	73.56 ± 0.14***	62.89 ± 0.14***	42.79 ± 0.14***
47	16.70 ± 0.14*	38.97 ± 0.14***	67.54 ± 0.29***	62.89 ± 0.14***	57.57 ± 0.14***	42.79 ± 0.09***
48	22.05 ± 0.14**	33.18 ± 0.23***	50.98 ± 0.14***	53.30 ± 0.43***	62.69 ± 0.29***	58.24 ± 0.29***
49	27.62 ± 0.14***	33.18 ± 0.29***	50.98 ± 0.20***	57.36 ± 0.17***	52.03 ± 0.12***	47.81 ± 0.12***
50	11.14 ± 0.12	27.84 ± 0.14***	56.64 ± 0.17***	68.28 ± 0.29***	62.89 ± 0.14***	42.79 ± 0.14***

*Significant difference at * P < 0.05 versus control value; ** P < 0.01 versus control value; *** P < 0.001 versus control value (Student's t test)*

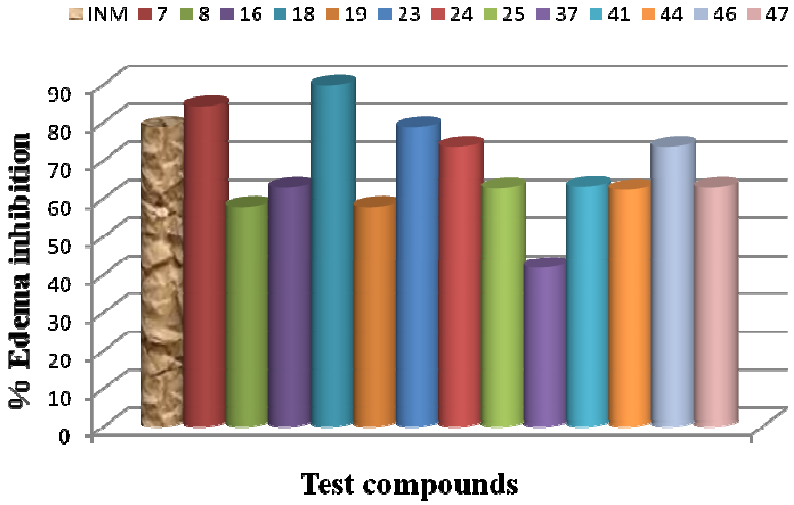


Fig. 1: Inhibirory effects of the most potent compounds 7, 8, 16, 18, 19, 23, 24, 25, 37, 41, 44, 46, 47 and indomethacin on carrageenan induced paw edema in rats after 3h

Compound (**7**) (X= H), showed 107% and 66% of the anti-inflammatory activity of indomethacin after 3 and 4 h respectively while compound (**8**) (X= F), showed 73% and 53% of the anti-inflammatory activity of indomethacin after 3 and 4 h respectively, **table 1** & **Fig 2**.

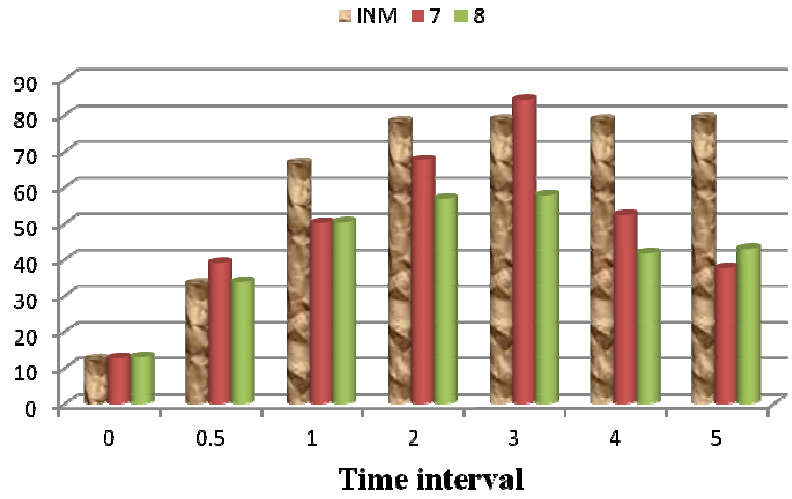


Fig.2: Inhibirory effects of compounds 7,8 and indomethacin on carrageenan induced paw edema in rats

Comparing results of anti-inflammatory activity of the thione, compound (**7**) and its anilide derivatives (**16-22**) (X= H) revealed that compound (**7**) showed 107% at 3 h interval of the anti-inflammatory activity of indomethacin, while, compounds (**16-22**) exhibited 53-114%. Results also revealed that compounds (**16**) (R^1= *p*- CH_3-Ph), (**18**) (R^1= *p*-Br-Ph) and (**19**) (R^1= *p*-Cl-Ph) were the most potent compounds in this series, showing 80%, 114% and 73% at 3 h interval of the anti-inflammatory activity of indomethacin respectively. Comparing the anti-inflammatory activity of the thione, compound (**7**), and its phenacyl bromide derivatives (**37-43**) (X= H) revealed that compounds (**37-43**) exhibited 52-80% of the anti-inflammatory activity of indomethacin at 3 h interval. Compounds (**38**) (R^2= Br) and (**41**) (R^2= CH_3) were the most potent in this series, showing 79% and 80% of the anti-inflammatory activity of indomethacin respectively at 3 h interval. This decrease in the anti-inflammatory activity of phenacyl derivatives, compounds (**37-43**) in comparison to the anilide derivatives, compounds (**16-22**) may be due to the lack of amidic linkage and this in agreement with molecular docking study.

Results of the anti-inflammatory activity of compounds (**8**), and its anilide derivatives (**23-29**) (X= F) revealed that compound (**8**) showed 73 % at 3 h interval of the anti-inflammatory activity of indomethacin. Compounds (**23-29**) showed at 3 h interval 53-100% of the anti-inflammatory activity of indomethacin. Results also revealed that compounds (**23**) (R^1= *p*-CH_3-Ph), (**24**) (R^1= *p*-$COCH_3$-Ph) and (**25**) (R^1= *p*-Br-Ph) were the most potent compounds in this series, showing 100%, 93% and 79% respectively of the anti-inflammatory activity of indomethacin at 3 h interval. Moreover, comparing the anti-inflammatory results for compound (**8**) and its phenacyl bromide derivatives (**44-50**) (X= F) revealed that they showed 73-93% of the anti-inflammatory activity of indomethacin at 3 h. Also results revealed that compounds (**44**, **46** and **47**) were the most potent compounds in this series, showing 79%, 93% and 80% respectively of the anti-inflammatory activity of indomethacin at 3 h interval.

From these observations, it could be concluded that: 1. The anilide derivatives, compounds (**16-29**) were more potent than the phenacyl derivatives, compounds (**37-50**) indicating that amide NH group is crucial for the anti-inflammatory activity and this in agreement with molecular docking study. 2. Regarding the substitution at the phenyl ring, it seems to be important, that the substituted derivatives showed higher activity if compared with the nonsubstituted derivatives. 3. Regarding the effect of N1-substitution, it is difficult to detect which is more favorable, the benzyl group or the *o*-fluorobenzyl group. In some cases, the *o*-F benzyl group seems to be more favorable than benzyl group substituents. This observation could be concluded when comparing compounds (**37-43**) with compounds (**44-50**) which showed 20-85% and 33-87% respectively of the anti-inflammatory activity of indomethacin.

3.3. Molecular Modeling Study

Molecular dockings studies to COX-2 of the synthesized compounds (**7**, **8**, **16-29** and **37-50**) were performed in order to rationalize the obtained anti-inflammatory results and to help in understanding the potential interactions between the ligand and enzyme active site.The results revealed that most of the compounds bind to Cyclooxygenase-2 enzyme through hydrogen bond to the residue Arg 120 similar to that of indomethacin. In addition, most of those compounds were oriented horizontally in the hydrophobic cage of receptor wherein, it is interacted with residues Val 349, Leu 352, Tyr 355, Leu 531 and Glu 524. These residues may be involved in flexible alignment of compounds in the hydrophobic cage of enzyme.

Docking analysis reveals that compounds (**16-29**) showed better fitting and binding into the active site pocket than compounds (**37-50**). This may be attributed to the presence of the anilide moiety in the first series which help in the positioning of those compounds in the receptor. Compounds (**16-29**) showed two good hydrogen bonding, first through the anilide carbonyl to the Arg 120 and the second through carbonyl moiety at the position 2 of the purine ring to Ser 530. The first hydrogen bond scoring is from 22.3-25.4% and with a distance of 2.31-2.52 Å and the second hydrogen bond scoring is from 12.4-15.4% and with a distance of 2.84-3.07 Å, **Fig 3**.

On the other hand, compounds (**37-50**) showed one hydrogen bonding only between the Arg 120 and the carbonyl moiety at the position 2 of the purine ring; the scoring for this bond is 23-24% and the distance is 2.35-2.37 Å, **Fig 3**.

Introduction of the flouro group into series (**23-29**) and (**44-50**) from series (**16-22**) and (**37-43**) respectively, while results in a little increase in the ClogP of the fluoro compounds, however it does not affect much on the binding of the different compounds in the cyclooxygenase-2 binding site.

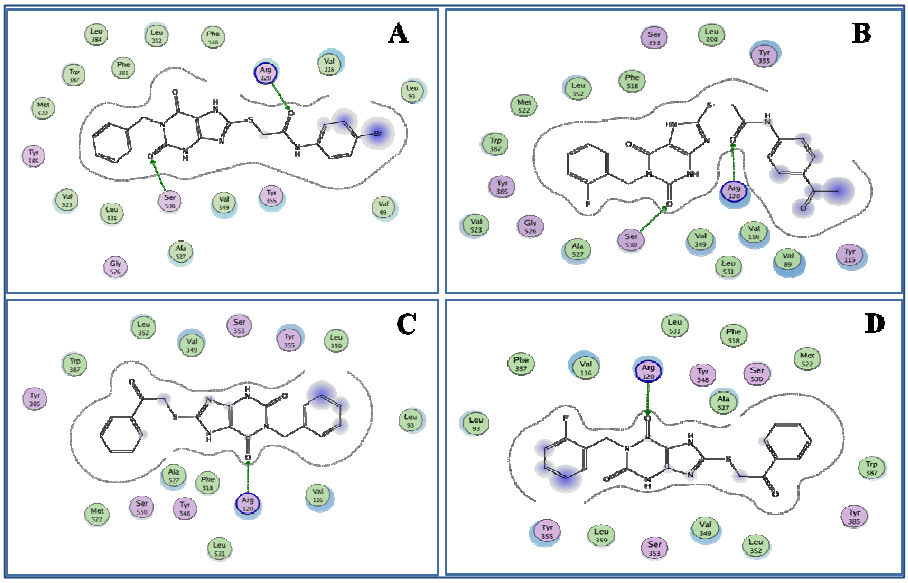


Fig. 3: A. Compound (18) Ligand Interaction to Cyclooxygenase-2, B. Compound (23) Ligand Interaction to Cyclooxygenase-2, C. Compound (37) Ligand Interaction to Cyclooxygenase-2 and D. Compound (44) Ligand Interaction to Cyclooxygenase-2

CONCLUSION

In the present work, the synthesis of *N*-Substituted aryl-2-chloroacetamides **9-15** and phenacyl bromide derivatives **30-36** is reported. The proposed structures of the new compounds were confirmed by spectroscopic and elemental analyses. Anti-inflammatory data have shown that the synthesized compounds have well to excellent activity compared to that of indomethacin.

Acknowledgments
The authors extend their gratitude to Dr. Ahmed S. Aboraia, Lecturer of Medicinal Chemistry, Medicinal Chemistry Dept., Faculty of Pharmacy, Assiut University, Egypt, for making the required alterations to the molecular modeling discussion. Also, we are grateful to the Department of medicinal Chemistry for allowing its facility to performing the docking and alignment studies. Great thanks are presented to Prof. Dr. Ahmed Osman, Department of Pharmacology, Faculty of Medicine, Assiut University, for his kind supervision of the anti-inflammatory activity.

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