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Synthesis, biological evaluation and molecular modeling study of substituted 1,2,4-triazole-3-acetic acid derivatives

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ABSTRACT

*A series of 1,2,4-triazole-3-acetamides **3a-b**, 1-acylated-1,2,4-triazole-3- acetamides **4a-b**, ethyl 5-(2-substitutedacetamido)-1H-1,2,4-triazole-3-acetates **6, 7**, **8**, ethyl 1- substituted (carbamoyl and thiocarbamoyl)-5-amino-1H-1,2,4-triazole-3-acetates **9a-j** and ethyl 5-(3(4-chlorophenyl)ureido-1H-1,2,4-triazol-3-acetate **10** were synthesized. The obtained compounds were evaluated for their anti-inflammatory. Most of the tested compounds exhibited significant anti-inflammatory activities with compounds **9a-j** were better than indomethacin. None of the tested compounds showed significant antitumor activity. Finally docking of selected compounds was performed to COX-2 and COX-1 enzymes in order to rationalize the obtained anti-inflammatory results and to predict the selectivity of the synthesized compounds.*

Keywords: Heteroarylacetic acids, 1,2,4-triazoles, anti-inflammatory, docking, COX-2, COX-1.

INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic class for treating inflammations, pain and fever. They produce their effect through inhibition of cyclooxygenase enzymes (COX-1 and COX-2). The GIT side effects associated with non selective NSAIDs [1] encouraged many groups to search for new drugs. Modification of the carboxylic acid moiety of aryl/heteroaryl acetic acid NSAIDs is one of the strategies used to attenuate the local GIT irritation [2-4]. Although COX-2 inhibitors are lacking the GIT irritation, some may be associated with cardiovascular side effects [5]; consequently there is still a strong demand for new anti-inflammatory drugs with better therapeutic profile. We previously reported that 1-acyl derivatives of ethyl 5-amino-1*H*-1,2,4- triazole-3-acetate illustrated comparable anti-inflammatory activity with indomethacin [6], **Figure (1)**. Herein, we continued the derivatization of our reported 1,2,4-triazole-3-acetic acid derivatives by formation of amides, urea and thiourea

derivatives that may have a possible synergism to the anti-inflammatory effect as previously reported [7-14].

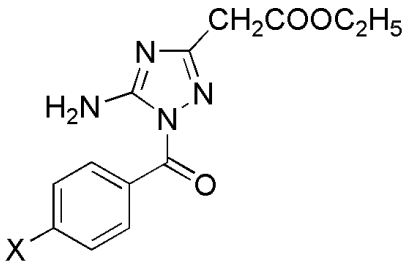


Figure (1)

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined using an electrothermal apparatus (Stuart Scientific, England) and were uncorrected. IR spectra were recorded as KBr disk using Thermo Nicolet-6700FT-IR, the data are given in υ_{max} (cm^{-1}). ^{1}H-NMR (60 MHz) spectra were carried out on Varian EM-360L, 60 MHz, (Varian, palo Alto, CA, USA) using DMSO-d_6 as a solvent and the chemical shifts are given in δ (ppm). HR-MS-Spectra were performed with Abteilung mass spectrophotometer F02- 217, Bielefeld University, Germany. Elemental analyses were performed on "Analytischer Funktionstest vario EL Fab.-Nr. 11982027". All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 GF_{245} percolated sheets 20x20 cm, layer thickness 0.2 mm (E-Merck, Germany) and were visualized by UV-lamp at wave length (λ) 254 nm. All chemicals and solvents were of reagent grade, and the latter were distilled and dried before use. Compounds **1, 2** [6], **3a, b** [15] were synthesized according to the literature procedure.

2.1.1) General method for synthesis of 1-acylated-5-amino-1*H*-1,2,4-triazole-3-acetamides 4a-d:

A solution of the substituted benzoyl chloride (0.01 mole) in dioxan (5 ml) was added dropwise to either of compounds **3a** or **3b** (0.01 mole), pyridine (1.2 ml, 0.015 mole) and dioxan (10 ml) with constant stirring at 0-5°C. The reaction mixture was stirred for 30 min at this temperature, then for 4 h at room temperature. The mixture was then poured onto water (50 ml) and the formed precipitate was filtered, washed with water and crystallized from methanol, **Scheme (1)**

2-[5-Amino-1-(4-chlorobenzoyl)-1*H*-1,2,4-triazol-3-yl]-N-benzylacetamide 4a.

Yield 86.1%; mp 314-316°C. IR (KBr, cm^{-1}):3434, 3302, 3108 (NH, NH_2), 1689,1646 (C=O acylated ring, C=O amide). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 3.5 (s, 2H, CH_2CO); 4.2 (d, 2H, NH*CH_2*Ph), 7.2 (s, 5H, C_6H_5), 7.5 (s, 2H, NH_2)*, 7.7 (d, 2H, Ar-H); 8.0 (d, 2H, Ar-H), 8.4 (t, 1H, CONH)*.Anal. Calcd for $C_{18}H_{16}ClN_5O_2$. H_2O; N, 18.08. Found: N, 17.7.

2-[5-Amino-1-(4-chlorobenzoyl)-1*H*-1,2,4-triazol-3-yl]-N-(2-phenyl-ethyl)acetamide 4b.

Yield 89.5%; mp 318°C. IR (KBr, cm^{-1}):3432,3302, 3075 (NH, NH_2), 1697,1646 (C=O acylated ring, C=O amide). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 2.8 (m,4H,*CH_2CH_2*Ph); 3.2 (d,2H, CH_2CO); 7.1 (s, 5H, C_6H_5); 7.1 (s, 2H, NH_2)*; 7.6 (s,1H, CH_2*NH*CO)*, 7.7 (d, 2H, Ar-H); 8 (d,

2H, Ar-H). Anal. Calcd for $C_{19}H_{18}ClN_5O_2$; C, 59.45; H, 4.73; N, 18.25. Found: C, 59.7; H, 4.7; N, 18.05.

2-[5-Amino-1-(4-methoxybenzoyl)-1*H*-1,2,4-triazol-3-yl]-N-benzyl-acetamide (4c).
Yield 91.3%; mp 198-200°C. IR (KBr, cm^{-1}):3423, 3299,3103 (NH, NH_2), 1695,1650 (C=O acylated ring, C=O amide). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 3.2 (s, 2H, CH_2Ph); 3.6 (s, 2H, CH_2CO); 3.8 (s, 3H, OCH_3); 7(d, 2H, Ar-H); 7.1(s, 5H, C_6H_5);7.5(s, 2H,NH_2)*;7.8 (d, 2H, Ar-H); 8.2 (t, 1H, NHCO)*. Anal. Calcd for $C_{19}H_{19}N_5O_3$; C, 62.46; H, 5.24; N, 19.17. Found: C, 62.01; H, 5.39; N, 18.52. (m/z) $[M+H]^+$365.1477. Calcd for $[C_{19}H_{19}N_5O_3]^+$: 365.1487

2-[5-Amino-1-(4-methoxybenzoyl)-1*H*-1,2,4-triazol-3-yl]-N-(2-phenylethyl)acetamide 4d.
Yield 93.5%; mp 224 °C. IR (KBr, cm^{-1}):3419, 3294, 3087, (NH, NH_2), 1695,1650 (C=O acylated ring, C=O amide). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 3.2 (m, 2H, CH_2CH_2Ph); 3.4 (s, 2H, CH_2CO); 3.8 (s, 3H, OCH_3); 4.2 (m, 2H, $NHCH_2CH_2$Ph); 7(d, 2H, Ar-H); 7.2(s, 5H, C_6H_5); 7.6 (s, 2H, NH_2)*; 8.1(d, 2H, Ar-H); 8.5(t, 1H, CH_2NHCO)* Anal. Calcd for $C_{20}H_{21}N_5O_3$; C, 63.3; H, 5.58; N, 18.46. Found: C, 63.01; H, 5.39; N, 18.52. (m/z) $[M+H]^+$ 379.1644. Calcd for $[C_{20}H_{21}N_5O_3]^+$: 379.1649

2.1.2) Synthesis of ethyl 5-(2-chloroacetamido)-1H-1,2,4-triazole-3-acetate 5:
Chloroacetyl chloride (0.7 ml, 0.0057 mole) was added gradually to a solution of **2** (1 g, 0.0057 mole) in dioxan (5 ml). The mixture was heated in water bath at 80°C for 2 h. Then the mixture was poured onto water (25 ml) and the formed ppt. was filtered, washed with water and dried. Crystallization from ethanol give **5** in 73.8 % yield, mp. 234- 236°C, **Scheme 2.** IR (KBr, cm^{-1}):3300, 3275(NH), 1728,11685(C=O ester, C=O amide). ^{1}H-NMR (60MHz, DMSO-d_6, δppm): 1.1 (t,3H, CH_2CH_3); 3.6 (s, 2H, CH_2CO); 4(q, 2H, CH_2CH_3); 4.4(s,2H,$ClCH_2CO$); 12.2(br.s,1H,NHCO)*. Anal. Calcd for $C_8H_{11}ClN_4O_4$; C, 38.96; H,5.4; N, 22.72. Found: C, 38.85; H, 5.33; N, 23.00. (m/z) $[M+H]^+$ 246.0514. Calcd for$[C_8H_{11}ClN_4O_4]^+$: 246.0519

2.1.3) General method for synthesis of ethyl [5-(substituted)-acetyl amino-1H-1,2, 4-triazol-3-yl]acetate 6,7:
Appropriate amount of thiol compounds (0.11 g, 0.001 mole) was added to mixture of anhydrous K_2CO_3 (0.25 g, 0.0018 mole) in ethanol then warmed for 15 min, and compound **5** (0.25 g, 0.001 mole) was added dropwise and refluxed for 1hr, The mixture was then poured onto water (25 ml) and the formed ppt was filtered, washed with water and crystallized from appropriate solvent, **Scheme (2)**

Ethyl [5-([(2-furylmethyl)thio]acetamido)-1*H*-1,2,4-triazol-3-yl]acetate 6.
Yield 78.1 %; mp 171-173 °C. IR (KBr, cm^{-}1):3063, 2924 (NH), 1720,1704 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t,3H,CH_2CH_3); 3.6 (s, 2H, CH_2CO); 3.7 (s, 2H,SCH_2CO); 3.9 (s,2H, -CH_2S); 4.2 (q,2H,CH_2CH_3); 6.3 (d, 2H, Furyl), 7.5(d,1H, furyl), 11.7(br.s,1H,NHCO), 13.5 (br.s,1H, N1-H). (m/z) $[M+H]^+$ 324.0895. Calcd for $[C_{13}H_{16}N_4O_4S]^+$: 324.0892

Ethyl [5-([(3-cyano-5,6-dimethylpyridin-2-yl)thio]acetamido)-1*H*-1,2,4-triazol-3-yl]acetate 7.
Yield 85%; mp 260°C. IR (KBr, cm^{-1}):3475, 3249 (NH), 1720,1635 (C=O), 2215 (CN). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t,3H,CH_2CH_3); 2.3 (s,6H,2CH_3); 2.5 (s,1H,CH); 3.6 (s,2H,

CH_2CO) 4.1 (q, 2H, *CH_2*CH_3); 4.4 (s, 2H, CH_2S); 7.1 (s, 2H,CH, NH)*.(m/z) $[M+H]^+$ 374.1140. Calcd for $[C_{16}H_{18}N_6O_3S]^+$: 374.1161

2.1.4) Synthesis of ethyl 5-(2-morpholinoacetamido)-1*H*-1,2,4-triazole-3-acetate 8:
Excess amount of morpholine (0.005 mole) was added to compound **5**(0.29 g, 0.001 mole) and refluxed for 10 min. Ethanol was added and then continue reflux for 1h, the formed ppt was filtered and crystallized from ethanol. Yield 75 %, m.p. 178°C, **Scheme (2),** IR: (KBr, cm^{-1}):3072, 2923 (NH), 1734,1653 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.2 (t, 3H, CH_2*CH_3*); 2.6(m, 4H, morpholine 2(CH_2)N); 3.2(s, 2H, CH_2CO); 3.6(m, 6H, morpholine 2(CH_2)O, CH_2CONH); 4.2(q, 2H, *CH_2*CH_3); 9.8(br.s, 1H, NHCO)*. Anal. Calcd for $C_{12}H_{19}N_5O_4$; C, 48.48; H, 6.44; N, 23.56. Found: C, 48.57; H, 6.08; N, 23.28. (m/z) $[M+H]^+$ 297.1423. Calcd for $[C_{12}H_{19}N_5O_4]^+$: 297.1437

2.1.5) General method for synthesis of ethyl 2-(1-substituted-carbamoyl and thiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl) acetate 9a-j:
Appropriate amount of isocyanate or isothiocyanate derivatives (0.006 mole) was added to a solution of compound **2** (1 g, 0.006 mole) in ethanol and stirred 2h at room temperature. The formed precipitate was filtered off and then crystallized from the appropriate solvent, **Scheme (3)**

Ethyl 2-(1-(methylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl)acetate 9a.
Yield 53%; mp 170 °C. IR (KBr, cm^{-1}):3329, 3198 (NH), 1728 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.5 (t, 3H, CH_2*CH_3*); 3.5 (s,3H,NH*CH_3*); 3.6(s, 2H, CH_2CO); 4.5 (q, 2H,*CH_2*CH_3); 8.4 (s, 2H, NH_2)*; 9.8 (s,1H,NH)*. Anal. Calcd for $C_8H_{13}N_5O_2S$; C, 39.49; H,5.39; N, 28.78. Found: C, 39.54; H, 5.43; N, 29.30.

Ethyl 2-(1-(ethylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl)acetate 9b.
Yield 52.6%; mp 140-142°C. IR (KBr, cm^{-1}):3419, 3353, 3320 (NH,NH_2), 1723 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t, 3H, CH_2*CH_3*); 3.2 (m, 7H, CH_2CO and *CH_3CH_2*NH) ; 3.9 (q,2H, *CH_2*CH_3); 5.7 (s, 2H, NH_2)*; 12.2 (br.s,1H, N1-H) *. Anal. Calcd for $C_9H_{15}N_5O_2S$ C, 42.01; H, 5.88; N, 27.22. Found: C, 41.90; H, 5.99; N, 27.80.

Ethyl 2-(1-(allylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl)acetate 9c.
Yield 52%; mp 124-126°C. IR(KBr, cm^{-1}):3322, 3200 (NH,NH_2), 1724 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.2 (t, 3H, CH_2*CH_3*); 4.1 (m, 4H, *CH_2*CH_3, CH_2CO); 5.3 (m, 5H, CH_2=CH-CH_2), 8.3 (s, 2H, NH_2)*; 10 (br.s, $NHCH_2$). Anal. Calcd for $C_{10}H_{15}N_5O_2S$; C, 44.60; H, 5.60; N, 26.00. Found: C, 44.81; H, 5.43; N, 26.70.

Ethyl 2-(1-(phenylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl) acetate 9d.
Yield 59.6%; mp 180°C. IR (KBr, cm^{-1}):3314, 3178 (NH,NH_2), 1731 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.2 (t, 3H, CH_2*CH_3*); 4.2 (s, 2H, COCH2); 4.5 (q, 2H, *CH_2*CH_3); 8 (s, 2H, NH_2)*, 8.5 (m, 5H, C_6H_5); 13 (s, 1H, NH)*. Anal. Calcd for C13H15N5O2S; N, 22.94. Found: C, 50.26; N, 22.74.

Ethyl 2-(1-(4-chlorophenylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl)acetate 9e.
Yield 54.6%; mp 173-175°C. IR (KBr, cm^{-1}):3419, 3353, 3253 (NH,NH_2), 1722 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t, 3H, CH_2CH_3); 4.2 (s, 2H, $COCH_2$); 4 (q, 2H, CH_2CH_3); 7.1-7.7 (m, 4H, C_6H_4); 7.9 (S, 2H, NH_2)*; 10.2 (s, 1H, NH)*; 12.2 (s, 1H, SH). Anal. Calcd for $C_{13}H_{14}ClN_5O_2S$; C, 45.95; H, 4.15; N, 20.61. Found: C, 45.60; H, 4.11; N, 20.90.

Ethyl 2-(1-(2-chlorophenylthiocarbamoyl)-5-amino-1*H*-1,2,4-triazol-3-yl)acetate 9f.
Yield 52 %; mp 162-163°C. IR (KBr, cm^{-1}):3328, 3273, 3241 (NH,NH_2), 1736 (C=O ester). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t,3H, CH_2CH_3); 3.6 (s, 2H, $COCH_2$); 4.1 (q, 2H, CH_2CH_3); 7.5 (m, 4H, C_6H_4); 7.8 (s, 2H, NH_2)*; 10.6 (s, 1H, NH)*. Anal. Calcd for $C_{13}H_{14}ClN_5O_2S$ C, 45.95; H, 4.15; N, 20.61. Found: C, 45.71; H, 4.25; N, 20.08.

Ethyl 2-(1-(phenylcarbamoyl)-5-amino-1*H*-1,2,4-triazole-3-yl)acetate 9g.
Yield 69%; mp 144-146°C. IR (KBr, cm^{-1}):3439, 3349, 3258 (NH,NH_2), 1727, 1645 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.2 (t, 3H, CH_2CH_3); 4 (s, 2H, $COCH_2$), 4.5 (q, 2H, CH_2CH_3); 7.9 (s, 2H, NH_2)*, 8.1-8.5 (m, 5H, C_6H_5); 9.8 (s, 1H, NH)*. Anal. Calcd for $C_{13}H_{15}N_5O_3$; C, 53.97; H, 5.23; N, 24.20. Found: C, 53.77; H, 5.05; N, 24.10.

Ethyl 2-(1-(4-chlorophenylcarbamoyl)-5-amino-1*H*-1,2,4-triazole-3-yl) acetate 9h.
Yield 73%; mp 172°C. IR: (KBr, cm^{-1}):3419, 3354, 3219 (NH,NH_2), 1735, 1658 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.2 (t, 3H, CH_2CH_3); 3.5 (s, 2H, $COCH_2$); 4 (q, 2H, CH_2CH_3); 7.6 (d, 2H, NH_2)* ;7.1-7.3 (d, 2H, Ar-H); 7.4-7.6 (d, 2H, Ar-H); 8.5 (s, 1H, NH)*. Anal. Calcd for $C_{13}H_{14}ClN_5O_3$ C, 48.23; H, 4.36; N, 21.63. Found: C, 48.02; H, 4.24; N, 21.36.

Ethyl 2-(1-(2-chlorophenylcarbamoyl)-5-amino-1*H*-1,2,4-triazole-3-yl) acetate 9i.
Yield 70%; mp 174°C. IR: (KBr, cm^{-1}):3448, 3334, 3289 (NH,NH_2), 1733, 1650 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1 (t, 3H, CH_2CH_3); 4.6 (s, 2H, $COCH_2$); 4.1 (q, 2H, CH_2CH_3); 6.6 (s, 2H, NH_2)*; 7-8.4 (m, 4H,C_6H_4); 9.3 (s, 1H, NH)*. Anal. Calcd for $C_{13}H_{14}ClN_5O_3$ C, 48.23; H, 4.36 Found: C, 47.89; H, 4.25.

Ethyl 2-(1-(cyclohexylcarbamoyl)-5-amino-1*H*-1,2,4-triazole-3-yl) acetate 9j.
Yield 66%; mp 138-140°C. IR: (KBr, cm^{-1}):3422, 3374, 3332 (NH,NH_2), 1712, 1647 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.5 (m, 14H, C_6H_{11} and CH_2CH_3); 4 (s, 2H, $COCH_2$); 4.5 (q, 2H, CH_2CH_3); 7.2 (d, 1H, NH)*, 7.5 (s, 2H, NH_2)*. Anal. Calcd for $C_{13}H_{21}ClN_5O_3$; C, 52.87; H, 7.71; N, 23.71. Found: C, 52.57; H, 6.96; N, 23.43.

2.1.6) Synthesis of ethyl 5-(3(4-chlorophenyl)ureido-1*H*-1,2,4-triazol-3-acetate 10:
Compound **9h** was heated till just melting then left at room temp, crystallized from DMF/H_2O. Yield 70%; mp >300°C. IR: (KBr, cm^{-1}):3291, 2982 (NH,NH_2), 1720, 1683 (C=O). ^{1}H-NMR (60 MHz, DMSO-d_6, δ ppm): 1.1(t, 3H, CH_2CH_3); 3.7 (s, 2H, CH_2CO); 4 (q, 2H, CH_2CH_3); 7.3 (dd, 4H, C_6H_4); 9.2(s, 1H, NHCO); 10(br.s, 1H, Ph*NH*CO). Anal. Calcd for C13H14ClN5O3 ; C, 48.23; H, 4.36; N, 21.63. Found: C, 47.98; H, 4.28; N, 21.55.

2.2. Anti-inflammatory Activity
Adult male albino rats of average weight (100g ± 10%) were divided into groups, each of six rats. Each group was treated with a suspension of the tested compound or the reference drug

orally by gastric tubes at dose level of 50 mg/kg. The control animal group, on the other hand, was treated with the vehicle, CMC. After 30 minutes, 0.1 ml of freshly prepared carrageenan solution (1% in normal saline) was injected into the sub planar region of the right hind paw of each rat. The thickness of rat paw was measured at different time intervals (0.5h, 1h, 2h, 3h) after administration of the test samples. The difference between the thicknesses of two paws (right and left) was taken as a measure of edema.

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

V_R= Average right paw displacement volume
V_L= Average leftt paw displacement volume

2.3. Antitumor Activity

Antitumor activity was evaluated for **3a, b, 4a-d, 9a, 9e, 9h** according to NCI in vitro protocols [16], they were screened against a panel consisting of 60 human tumor cell lines, derived from nine cancer types (leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer.

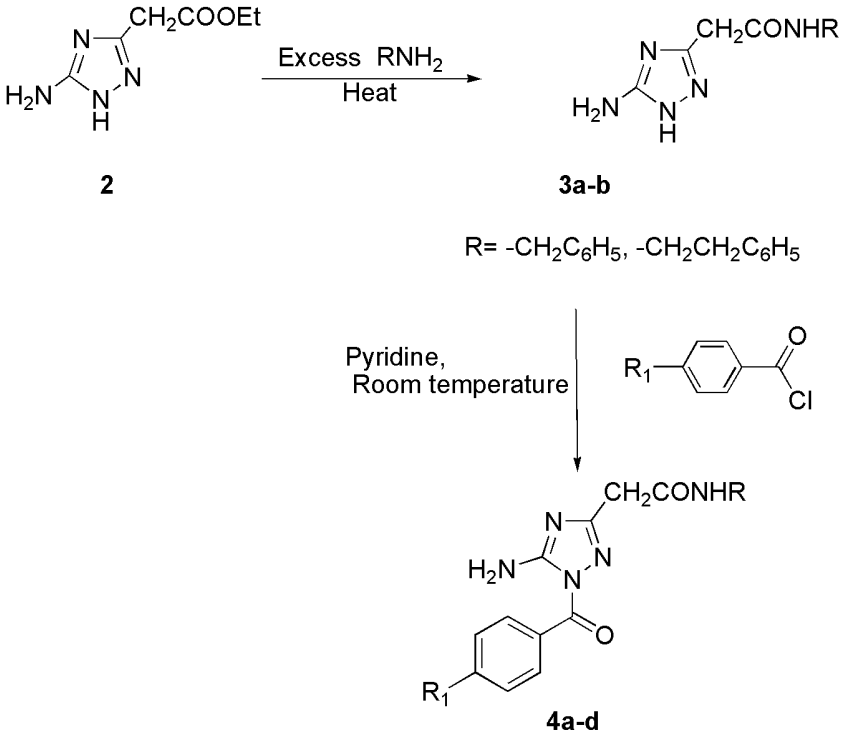
2.5. Molecular modeling

All the molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2009.06; Chemical Computing Group, Canada) as the computational software [17]. All the minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol^{-1} $Å^{-1}$ with MMFF94X force-field and the partial charges were automatically calculated. The X-ray crystallographic structure of murine COX-2 complexed with indomethacin (PDB ID: 4COX) and COX-1 complexed with flubiprofen (PDB ID: 1CQE) was obtained from the protein data bank. The enzyme was prepared for docking studies where: i) Ligand molecule was removed from the enzyme active site. ii) Hydrogen atoms were added to the structure with their standard geometry. iii) 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. iv) The obtained model was then used in predicting the ligand-enzyme interactions at the active site.

RESULTS AND DISCUSSION

3.1. Chemistry

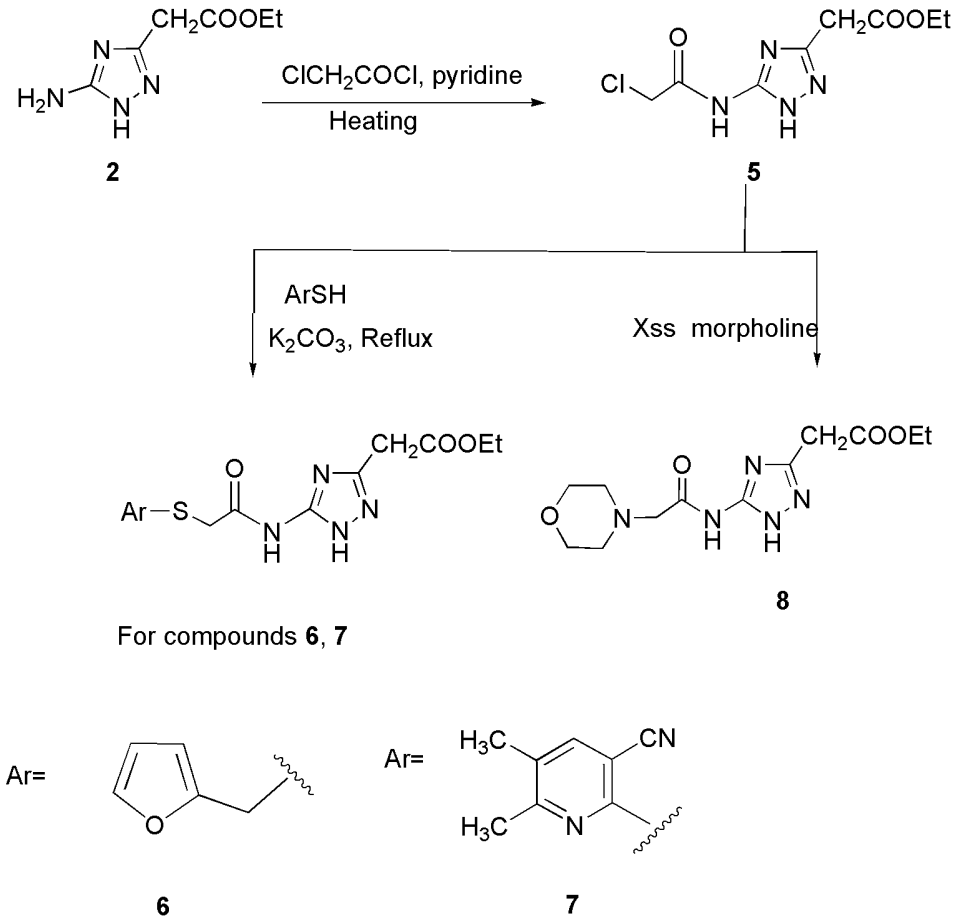
The starting 5-amino-1*H*-1,2,4-triazole-3-acetic acid **1** and it's ethyl ester **2** were prepared according to literature procedure [18]. The 5-amino-1*H*-1,2,4- triazole-3-acetamides **3a, b** were prepared by reacting the ester **2** with the appropriate amine [15] as shown in **Scheme (1)**. Acylation of amides **3a, b** with acid chlorides at room temperature yielded a ring acylated products, 1-acylated 5-amino-1*H*-1,2,4-triazole- 3-acetamides **4a-d** rather than the exocyclic amino-acylated derivatives in accordance with our reported results [6], **Scheme (1)**. In the IR spectra of **4a-d**, the free exocylic NH_2 group appeared as forked band at frequency range 3434-3294 cm^{-1} and in ^{1}H-NMR spectra this amine protons appeared as singlet between 7.5 and 7.8 ppm, with disappearance of the ring NH proton.



4a; R= -$CH_2C_6H_5$, R_1=(4-Cl), **4b**; R= -$CH_2CH_2C_6H_5$,R_1=(4-Cl)
4c; R= -$CH_2C_6H_5$,R_1= (4-OCH_3), **4d**; R= -$CH_2CH_2C_6H_5$,R_1= (4-OCH_3)

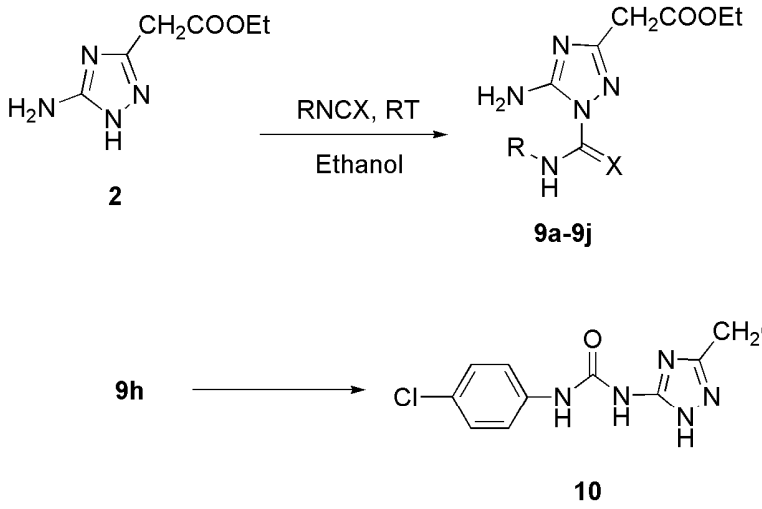
Scheme (1)

On the other hand, when equimolar amount of chloroacetyl chloride reacted with **2** at room temperature, the acylated exocyclic amino product was obtained exclusively, **Scheme (2)**. It has been reported that acylation occurs at the exocyclic amino group when using acid chlorides containing strong electron withdrawing groups [19]. The structure of the produced ethyl 5-chloroacetamido-1*H*-1,2,4-triazole-3-acetate **5** was confirmed by elemental and spectral data. In the IR spectrum of **5**, both the exocyclic and the ring NH group appeared in the ranges 3300, 3275 cm^{-1}, the ester and amide carbonyl group appeared at frequencies 1728 and 1685 cm^{-1}, respectively. The ^{1}H-NMR spectrum of **5** showed singlet signal of CH_2CO at 3.6 ppm, a broad downfield shifted signal of exocyclic NHCO at 12.2 ppm confirming the proposed structure. Reaction of **5** with thiol-containing compounds or morpholine afforded ethyl [5-(substituted)-acetamido-1*H*-1,2,4-triazol-3-yl]acetates **6**, **7**, **8**, **Scheme (2).** The structures of **6, 7, 8** were confirmed by elemental and spectral data. The IR spectra of **6**, **7**, **8** illustrates the NH groups frequencies at 3063, 2923 cm^{-1}, ester and amide carbonyl group frequencies at 1720, 1635 cm^{-1}, respectively.



Scheme (2)

The urea and thiourea derivatives **9a-j** were prepared by treatment of **2** with appropriate isocyanate or isothiocyanate derivatives. The reaction occurred at the annular NH group as previously reported [20], **Scheme (3).** The structures of **9a-j** were confirmed by elemental and spectral data. In the IR spectra of **9a-j**, the exocyclic NH_2 group appeared as forked band at frequency range 3419-3200 cm^{-1}, the frequency of C=O of the ester group appeared at range 1722-1736 cm^{-1}. In the ^{1}H-NMR spectra of **9a-j**, the free exocyclic NH_2 band appeared as a singlet at 5.7-8.2 ppm. Interestingly, compound **9h** underwent thermal rearrangement forming ethyl 5-(3(4- chlorophenyl)ureido-1*H*-1,2,4-triazol-3-acetate **10, Scheme (3).** In the IR spectra of **10**, the forked band of the exocyclic NH_2 group disappeared and the NH groups appeared at frequencies 3291, 2982 cm^{-1} , the C=O of the ester and the amide groups appeared at 1720, 1682 cm^{-1}, respectively. In the ^{1}H-NMR spectra of **10**, the singlet of the exocyclic NH_2 group disappeared, and a new band of the annular NH group appeared as broad band at 10 ppm.



For compounds **9a-f** X=S

9a; R= CH_3, **9b**; R= CH_3CH_2-, **9c**; R= CH_2=$CHCH_2$-, **9d**; R= C_6H_5-
9e; R= (4-Cl)C_6H_4-, **9f**; R=(2-Cl)C_6H_4-

For compounds **9g-j** X=O

9g; R= C_6H_5- **9h**; R= (4-Cl)C_6H_4-, **9i**; R=(2-Cl)C_6H_4-, **9j**; R=C_6H_{11}-

Scheme (3)

3.2 Biological Evaluation

The preliminary anti-inflammatory activity of compounds **4a-d, 6, 7, 8, 9d-j, 10** was evaluated using carrageenan-induced rat paw edema in comparison to indomethacin and celecoxib as reference drugs [21]. The results shown in **Table 1** illustrated that the urea or thiourea derivatives **9a-j, 10** were more potent than 1,2,4-triazole-3- acetamides **4a-d**. Generally, **9a-j** were more potent than indomethacin after 0.5h, whereas **9f, 9i** exhibited higher potency than celecoxib after 0.5h. However, after 1h, only **9f, 9g, 10** showed better activities than indomethacin. Within the urea or thiourea series **9a-j,** the 2-chlorophenyl derivatives **9i, 9f** were found more potent than their corresponding 4 chlorophenyl counterparts **9h, 9e**. The series of 5-(substituted-acetamido-1*H*-1,2,4-triazoles **6, 7, 8,** compound **7** was more active than **6, 8.** A general pattern of the anti inflammatory activity can be formulated as: Ring acylated esters [from reference 6] >5-substituted urea-derivative **10** > 1-thiourea derivatives **9a-f** > 1-urea derivatives **9g-j.**

3.4. Antitumor Activity:

Recent reports showed some NSAIDs drugs may have tumor suppression activity [22], therefore compounds **3a, b, 4-d, 9a, 9e, 9h** were tested for their antitumor activity according to the NCI 60 cell line protocol [16]. However, tested compounds showed no antitumor activity against the NCI 60 cell lines, only **3a, 4d, 9h** showed 30% growth inhibition against renal cancer cell lines.

Table (1): Anti-inflammatory activity of 1,2,4-triazole-3-acetic acid derivatives 4a-4d, 6,7, 8, 9d-9j, 10 at dose level 50 mg/kg.

Compd. No.	Anti-inflammatory activity (50 mg/kg, oral dose) % inhibition				
	0.5 h	1 h	2 h	3 h	4 h
Control	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Indomethacin	**2.94**	**17.5**	**33.1**	**42.3**	**49.1**
Celecoxib	**11.76**	**26.4**	**44.1**	**52.9**	**52.9**
4a	4*	10.2	16.3*	18.3**	20.4*
4b	2.1*	12.7	25.5*	29.7*	34*
4c	-	2.6	6.6*	6.6**	9.3**
4d	-	6.4	12.8	12.8**	23**
6	-	2.6	2.6**	7.8**	7.8**
7	-	9.3	20	20*	33.3*
8	-	7.8	7.8*	13.1**	13.1**
9d	5.84	12.8	16.7	17.1**	21.5*
9e	7.82**	8.3	13.9	15.2*	24*
9f	17.5**	21.3*	22.9	25.2*	28.9
9g	10.05	17.8	22	31	37.8
9h	3.16*	5.09	9.2	15.7**	17*
9i	14.2*	14.2	19.3	19.8**	30*
9j	7.5*	12	20	24.6*	28.4*
10	2.1	19.1	25.5	40	40*

*Significant difference at P< 0.05, ** Significant difference at P< 0.01, - : no inhibition*

3.5. Molecular Modeling Study

Molecular docking of **10** to COX-2 and COX-1 was carried out in order to rationalize the obtained anti-inflammatory results, understand the various interactions between the ligand and enzymes active sites and to predict its selectivity. Docking studies were performed by MOE (Molecular Operating Environment) using (PDB ID: 4COX) and (PDB ID: 1CQE) for COX-2 and COX-1, respectively. We performed 100 docking iterations for each ligand and the top-scoring configuration of each of the ligand-enzyme complexes was selected on energetic grounds. As shown in Figure 2, docking of the acetic acid analogue of **10** into COX-2 active site, revealed that several molecular interactions were considered to be responsible for the observed affinity. The ligand was oriented so that the carboxylate moiety was in the vicinity of Arg120 residue forming ionic interaction with the guanidinium side chain (distance = 2.54 A˚). A hydrogen bond interaction between the ligand carbonyl group and the side chain of Ser530 (distance = 2.37A˚), a hydrogen bond interaction between the ligand carboxylate group and the side chain of Tyr 355 (distance = 2.55 A˚). Furthermore, the *p*-chlorophenyl moiety of the ligand was located in the vicinity of the aromatic pocket Leu384, Phe518, Tyr38 5and Met 522. The docking top ranking score was -12.365 kcal/mole.

On the other hand docking of acetic acid analogue of **10** into COX-1 active site, revealed similar molecular interactions considered responsible for the observed affinity. As shown in **Figure (3)**,

the ligand was oriented so that the carboxylate moiety in the vicinity of Arg120 residue forming an ionic bond interaction with the guanidinium side chain (distance 2.37 A˚). A hydrogen bond interaction between the ligand carboxylate moiety with the OH group of Tyr355 (distance = 2.49A˚). The *p*-chlorophenyl moiety of the ligand was located in the vicinity of the aromatic pocket Leu352, Leu384, Phe381, Tyr385, Ser 530, and Trp387. The docking score was -11.668 kcal/mole. From the above mentioned data, we concluded that **10** have no preference to either COX-1 or COX-2 and therefore was considered non-selective inhibitor.

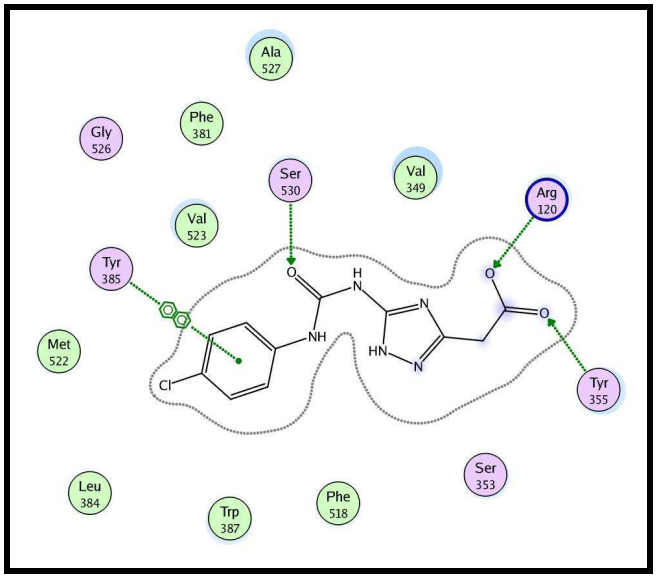


Figure (2): 2D representation of docking pose of acetic acid analogue of 10 in the active site of murine COX-2 viewed using molecular operating environment (MOE) module.

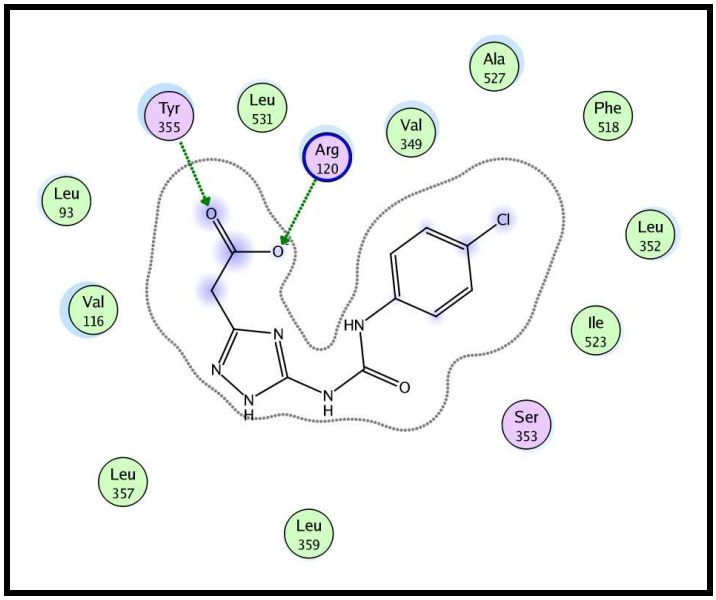


Figure (3): 2D representation of docking pose of acetic acid analogue of 10 in the active site of COX-1 viewed using molecular operating environment (MOE) module.

CONCLUSION

Herein, we reported the synthesis of different 1,2,4-triazole-3-acetic acid derivatives. The synthesized compounds were tested for potential anti-inflammatory, antitumor and antimicrobial activity. The *in vivo* evaluation of anti-inflammatory activity showed that **10** exhibited maximum inhibition of edema. Furthermore, molecular modeling studies suggested that **10** docking patterns similar to non-selective COX inhibitors. None of the tested compounds showed significant antitumor activity.

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