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Synthesis, Characterization and Preliminary Pharmacological Evaluation of Triazolothiadiazoles Derived from some NSAIDs and Thiocarbohydrazide

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Abstract:

The synthesis of new NSAIDs with improved efficacy and selectivity towards COX2, which encouraged by the various biological activities of 1,2,4-triazoles and 1,3,4-thiadiazoles. In this experiment, the production of 1,2,4-triazolothiadiazoles derivatives from Ibuprofen, Naproxen and Indomethacin. We have enhanced anti-inflammatory and analgesic activities by conventional method and microwave-assisted technique, and then compare the time consuming by reaction and yield percent of the product in both way, besides evaluation of anti-inflammatory action of the target compounds by pharmacological test with predictable selectivity towards COX-2 enzyme. Synthesis of the target compounds (P1a-3b, N1a-3b and I1a-3b) has been successfully accomplished by checking purity, characterization, also identification of the synthetic compounds which detected by estimation of physical properties, FT-IR and ^{1}H-NMR spectroscopy. In vivo potent anti-inflammatory activity of the ending compounds is evaluating in rats utilizing egg-white prompted edema model of inflammation. The experienced compounds (P1a-3b, N1a-3b and I1a-3b) and the reference drugs (Ibuprofen, Naproxen and Indomethacin) produced significant reduction in paw edema in compare to the effect of control group. Wholly tested compounds produced considerable decrease of paw edema in contrast to control group. However, compounds (P3b, N3b and I1b) have considerable more paw edema declining than Ibuprofen, Naproxen and Indomethacin. Intermediate and target compounds are synthesis by microwave method have better result by time and yield in compare with conventional way. The synthesized compounds (Pa1-3b and N1a-3b) may exhibit expected selectivity towards COX-2 enzyme properly due to their large size than its parent Ibuprofen, Naproxen.

Key words: anti-inflammatory activity, microwave method, Ibuprofen, Naproxen, Indomethacin, triazolothiadiazole

تصنيع وتشخيص وتثمين دوائي لمركبات ترايازولوثياديازول المشتقة من ادوية مضادات الالتهاب غير الستيرويدية وثايوكاربوهيدرازايد

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الخلاصة:

صناعة مضادات التهاب جديدة ذات فعالية أكبر والتفضيلية أكثر تجاه تثبيط COX 2. في هذه التجربة تم انتاج مشتقات ١،٢،٤ تريازولوثياديازول مع تحسين خصائصها العلاجية المضادة للالتهابات والتسكين بواسطة الطريقة التقليدية وطريقة اشعة الميكروويف وقارنا بين الطريقتين بالوقت المستهلك بالتفاعل وكمية الناتج وكذلك ثمنّا الناتج من التفاعل دوائيا وتفضيليا لتثبيط أنزيم COX2. ان تصنيع المركبات المستهدفة (P1a-3b، N1a-3b، I1a-3b) قد انجز بنجاح وتم التأكد من نقاوة المركبات المصنعة وذلك بقياس الخواص الفزيائية (درجة الانصهار ومعامل التعويق) وقياس أطياف الاشعة تحت الحمراء واطياف التردد النووي المغناطيسي. تم تقييم تأثير مضادات الالتهاب للمركبات النهائية في الجسم الحي (الجرذان) باستخدام زلال البيض لاستحداث وذمة تحت الجلد كنموذج للالتهاب. ان المركبات المختبرة (P1a-3b, N1a-3b and I1a-3b) والدواء المقارن الايبوبروفين والنبروكسين والاندوميثاسين اظهر انخفاض مؤثر للوذمة مقارنة مع

البروبيل كلايكول كمجموعة ضابطة. على كل حال فان المركبات (P3b، N3b، I1b) اظهرت بشكل كبير وملحوظ خفض للوذمة أكثر من المركب المقارن. المركبات الوسطية والمستهدفة التي صنعت بطريقة اشعة الميكروويف كانت الأسرع والاكثر ناتج. مركبات (P1a-3b، N1a-3b) بسبب كبر حجمها مقارنة بالمركب الأصلي المشتقة منه أظهرت تفضيلية انتقائية اكثر تجاه تثبيط انزيم COX2.

الكلمات الرئيسية: الفعالية المضادة للالتهاب، طريقة اشعة الميكروويف، ايبوبروفين، نبروكسين، اندوميثاسين، ترايازولوثياديازول

Introduction:

Non-steroidal anti-inflammatory pills (NSAIDs) need, been normally utilized within human medication to decrease ache and inflammation [1]. It is well understood that NSAIDs share a common pharmacologic method of action via the inhibition of cyclooxygenase (COX) enzymes [2]. The primary clinical use of NSAIDs is in the treatment of musculoskeletal disorders, migraines, dental, postoperative pain and dysmenorrhea [3].

The most bothersome NSAIDs' adverse effects are the consequence inhibition of platelet, inhibition the production of prostaglandin that required for the normal functions of gastrointestinal and the kidney, cardio toxicity & hepatotoxicity plus drug induced asthmatic responses [4]. (2RS)-1[4-(2-methylpropyl) phenyl] propionic acid is Ibuprofen, which had been announced in (1969) as the primary member of propionic acid derivatives [5]. It can be available like a mixture of diastereoisomeric that includes half in which pharmacologically active as (S (+) enantiomer and the other half mass is (R(-) ibuprofen [6]. Naproxen belongs to derivative of propionic acid associated with the aryl acetic acid class of NSAIDs. It's chemical name (S)-6-methoxya-methyl-2-naphthaleneacetic acid [7]. Some recent reports also disclosed that among all NSAIDs, only naproxen has been found to be safe in terms of cardiovascular toxicity [8]. The analgesic and anti-inflammatory effects of Ibuprofen and Naproxen are thought to arise from the inhibition of COX-2 rather than COX-1[9]. The 2-(1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl) acetic acid is Indomethacin that is a nonselective inhibitor of (COX 1 & COX 2) enzymes [10]. Indomethacin such as Ibuprofen or Naproxen is a drug fighting the inflammation with the exceptional, its merely drug, which work to terminate hemicranias continua (HC) [11]. Microwave-assisted synthesis is set to alteration organic chemistry; the technology is mostly applicable to syntheses in therapeutic and combinatorial chemistry and contrasted with conventional methods offers improved speed, reproducibility and flexibility [12]. Microwave (MW) irradiation encourages better thermal administration of chemical reactions. The quick MW heat transfer permits reactions to complete very much faster contrasted with conventional heating techniques frequently resulting in expanded product yield. Besides, the results of temperature sensitive reactions from kinetic or thermodynamic pathways can be specifically tuned and confined [13]. Several five membered aromatic systems having three heteroatoms at symmetrical positions have been studied because of their interesting physiological properties [14]. Triazoles are under examination from numerous years since they are most important class of heterocyclic compounds, two tautomeric forms existed of 1,2,4-triazoles (1H &4H-1,2,4-triazole [15]. A great amount of (1,2,4-triazoles) have been integrated into wide assortment of therapeutically fascinating drug competitors having antimicrobial [16], anti-inflammatory [17], analgesic [18] and anticancer activities [19] [20]. Thiadiazol is a heterocyclic compound with five-membered, it has two nitrogen atoms besides one sulfur atom [21]. There are four isomeric types, 1,3,4-thiadiazole represent

an essential heterocyclic system because of their pharmacological activities [22], these isomeric types appeared in figure (1)

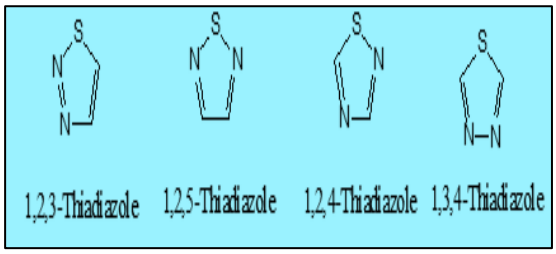


Figure (1): Thiadiazole isomeric types.

1,3,4-thiadiazole and their derivatives has extensive variety of therapeutic activities such as antimicrobial [23], diuretics [24], antiulcer [25], antimycobacterial [26], anti-inflammatory [27], anticonvulsant [28], anticancer [29], anti-leishmanial [30], and antidiabetic [31][32].

Material and methods:

All chemicals and reagents were obtained from the commercial supplier (Merck – Germany, sigma – Aldrich –Germany, BDH – England and Fluka –USA). Ibuprofen, Naproxen and Indomethacin was supplied from Shanghai**,** China**.** Melting points were determined by capillary method on Thomas Hoover apparatus (England). FT-IR spectra were recorded by using Shimadzu –Japan spectrophotometer and the determination of spectrophotometer and the determination of the spectra were performed by using KBr discs. Thin layer chromatography (TLC) was run on Kieslgel GF254 (60), Merck (Germany), to check the purity of the products as well as monitoring the progress of reactions. Compounds were revealed by reactivity by irradiation with UV light and chromatograms were eluted by Chloroform: methanol (85: 15). The ^{1}H-NMR spectra was achieved at the Jordan University, Faculty of Science and Department of Chemistry. Instrument Model: Bruker 300 MHz-Avanc III.

2.1 Chemical synthesis and physical data of synthesized compounds

2.1.1 Synthesis of the intermediate compound triazole:

Synthesis of 4-amino-3[1-(4-isobutylphenyl)ethyl]-5-mercapto-1,2,4-triazole (P1, P2, P3) from ibuprofen**,** (S)-4-amino-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thiol (N1, N2 and N3) from naproxen, also (3-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methoxy-2-methyl-1H-indol-1-yl)(4-chlorophenyl)methanone (I1, I2 and I3) from indomethacin, by three different methods: **a**- Oil bath, **b**- Fusion reflex and **c**- Microwave irradiation as illustrated in scheme (1).

a. By oil bath: An equimolar mixture of NSAID (0.01 mol) and thiocarbohydrazide (0.01 mol) taken in a 100 ml r.b. flask was heated on an oil bath till the contents melted. The reaction mixture was continuously stirred and maintained at a temperature of 165-175°C for further half an hour. Product that obtained was allowed to cool, and then treated with dilute sodium bicarbonate solution, in order to remove any unreacted acid left. The solid was filtered, washed with water, dried, and recrystallized from ethanol to obtain the pure triazoles [33]. **(P1, N1 and I1).**

C14H20N4S (P1): white powder, yield 67%, melting point: 150-153℃ [34] [35] Rf= 0.61, IR (cm^{-1}): 3372&3282 (Stretching vibration of NH_2), 2531 (stretching vibration of SH), 1634 (C=N stretching vibration.

C15H16N4OS (N1): Yellow powder, yield 61%, melting point: 71-74℃ [36], Rf= 0.50, IR (cm^{-1}): 3347& 3311 (Stretching vibration of NH_2), 2593 (stretching vibration of SH), 1629 (C=N stretching vibration.

C20H18ClN5O2S (I1): Deep orange powder, yield 72%, melting point: 223-225℃ [37] Rf= 0.37, IR (cm^{-1}): 3322& 3252 (Stretching vibration of NH_2), 2597 (stretching vibration of SH), 1654 (C=N stretching vibration).

b. By fusion reflex method an equimolar mixture of Indomethacin (0.01 mol) and thiocarbohydrazide (0.01 mol) taken in a 100 ml r.b. flask was heated for 4-5h at temperature of 165-175°C with continuous stirring. Product that obtained was allowed to cool and treated with dilute sodium bicarbonate solution, in order to remove any unreacted acid left. The solid was filtered, washed with water, dried, and recrystallized from ethanol to obtain the pure triazoles [38] **(P2, N2 and I2)**

C14H20N4S (P2): Off white powder, yield 67%, melting point: 150-152℃ [34] [35], Rf= 0.41, IR (cm^{-1}): 3356&3215 (Stretching vibration of NH_2), 2586 (stretching vibration of SH), 1658 (C=N stretching vibration.

$C_{15}H_{16}N_4OS$ (N2): Yellow powder, yield 65%, melting point: 72-75℃ [36], Rf= 0.60, IR (cm^{-1}): 3335& 3317 (Stretching vibration of NH_2), 2586 (stretching vibration of SH), 1631 (C=N stretching vibration.

$C_{20}H_{18}ClN_5O_2S$ (I2): Pale brown powder, yield 69%, melting point: 222-225℃ [37] Rf= 0.38, IR (cm^{-1}): 3295& 3234 (Stretching vibration of NH_2), 2621 (stretching vibration of SH), 1657 (C=N stretching vibration).

c. An equimolar mixture NSAID (0.01 mol) and thiocarbohydrazide (0.01 mol) were ground together to get a uniform mixture. This was zapped inside a 100 ml beaker and subjected to microwave irradiation on a microwave oven operating at 180 W for about 30-35 min. It was allowed to cool and treated with dilute sodium bicarbonate solution in order to remove any unreacted acid left. The solid was filtered, washed with water, dried, and recrystallized from ethanol to obtain the pure triazole (P3, N3 and I3) [39].

$C_{14}H_{20}N_4S$ (P3): white powder, yield 83%, melting point: 150-152℃ [34] [35], Rf= 0.56, IR (cm^{-1}): 3298& 3208 (Stretching vibration of NH_2), 2580 (stretching vibration of SH), 1633 (C=N stretching vibration.

C15H16N4OS (N3): Pale brown powder, yield 79%, melting point: 72-74℃ [36], Rf= 0.81, IR (cm^{-1}): 3317& 3251 (Stretching vibration of NH_2), 2604 (stretching vibration of SH), 1653 (C=N stretching vibration

C20H18ClN5O2S (I3): Yellow powder, yield 80%, melting point: 222-224℃ [37] Rf= 0.62, IR (cm^{-1}): 3293& 3201 (Stretching vibration of NH_2), 2583 (stretching vibration of SH), 1633 (C=N stretching vibration).

2.1.2 Synthesis of the targeted compound triazolothiadiazole:

The cyclocondensation of triazole with aromatic carboxylic acids such as benzoic acid in the presence of phosphorous oxychloride employed in the synthesis of 3-(1-(4-isobutylphenyl)ethyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (P1a, P1b, P2a, P2b, P3a and P3b) from (P1 or P2 or P3), (S)-3-(1-(6-methoxynaphthalen-2-yl)ethyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (N1a, N1b, N2a, N2b, N3a and N3b) figure (5) from (N1 or N2 or N3) and (4-chlorophenyl)(5-methoxy-2-methyl-3-((6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)-1H-indol-1-yl)methanone (1a, 1b, 2a, 2b, 3a, 3b) by both microwave and conventional method.

a. Conventional method: a mixture of triazole (0.01 mol), benzoic acid (0.01 mol), and phosphorus oxychloride (20 ml) was heated for reflux on an oil bath at temperature 170°C for 14-16h. The resulting reaction mass was poured into crushed ice with stirring. The solid thus obtained was filtered, washed with dilute sodium bicarbonate solution, followed by water, dried, and recrystallized from ethanol **(P1a, P2a, P3a, N1a, N2a, N3a, I1a, I2a and I3a)**.[20] [40] [41]

$C_{21}H_{22}N_4S$ (P1a): yellow powder, yield 39%, melting point: 122-124℃, Rf= 0.61, IR (cm^{-1}): 3055 (C-H stretching of aromatic), 1629 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.19- 1.62 (d, 9H, for CH_3 protons of ibuprofen), δ 1.83 (m, 1H, for CH proton

of ibuprofen), δ 2.51 (d, 2H, for CH_2 protons of ibuprofen), δ 3.86 (q, 1H, for CH proton of ibuprofen), 6.82-8.02 (m, 9H, for aromatic ring of ibuprofen and aromatic protons

$C_{21}H_{22}N_4S$ (**P2a**): deep yellow powder, yield 47%, melting point: 121-124℃, Rf= 0.74, IR (cm^{-1}): 3066 (C-H stretching of aromatic), 1612 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 0.89- 1.56 (d, 9H, for CH_3 protons of ibuprofen), δ 1.37 (m, 1H, for CH proton of ibuprofen), δ 2.51 (d, 2H, for CH_2 protons of ibuprofen), δ 4.46 (q, 1H, for CH proton of ibuprofen), 6.85-8.02 (m, 9H, for aromatic ring of ibuprofen and aromatic protons.

$C_{21}H_{22}N_4S$ (**P3a**): yellow powder, yield 51%, melting point: 122-125℃, Rf= 0.89, IR (cm^{-1}): 3072 (C-H stretching of aromatic), 1623 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 0.83- 1.71 (d, 9H, for CH_3 protons of ibuprofen), δ 1.81 (m, 1H, for CH proton of ibuprofen), δ 2.42 (d, 2H, for CH_2 protons of ibuprofen), δ 4.19 (q, 1H, for CH proton of ibuprofen), 6.97-8.02 (m, 9H, for aromatic ring of ibuprofen and aromatic protons.

$C_{22}H_{18}N_4OS$ (**N1a**): Brown powder, yield 49%, melting point: 144-146℃, Rf= 0.52, IR (cm^{-1}): 3055
(C-H stretching of aromatic), 1651 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.62 (d, 3H, for CH_3 protons of naproxen), δ 3.85 (s, 3H, for CH_3 protons of methoxide), δ 4.42 (q, 1H, for CH proton of naproxen), δ 6.84-8.01 (m, 11H, Multiplet, for naphthalene &aromatic protons).

$C_{22}H_{18}N_4OS$ (**N2a**): Brown powder, yield 49%, melting point: 144-146℃, Rf= 0.52, IR (cm^{-1}): 3055
(C-H stretching of aromatic), 1651 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.65 (d, 3H, for CH_3 protons of naproxen), δ 3.87 (s, 3H, for CH_3 protons of methoxide), δ 4.41 (q, 1H, for CH proton of naproxen), δ 7.27-7.90 (m, 11H, Multiplet, for naphthalene &aromatic protons).

$C_{22}H_{18}N_4OS$ (**N3a**): Brown powder, yield 49%, melting point: 144-146℃, Rf= 0.52, IR (cm^{-1}): 3055
(C-H stretching of aromatic), 1651 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.63 (d, 3H, for CH_3 protons of naproxen), δ 3.87(s, 3H, for CH_3 protons of methoxide), δ 4.41 (q, 1H, for CH proton of naproxen), δ 7.18-8.02 (m, 11H, Multiplet, for naphthalene &aromatic protons).

$C_{27}H_{20}ClN_5O_2S$ (**I1a**): Deep brown powder, yield 43%, melting point: 273-275℃, Rf= 0.51, IR (cm^{-1}): 3066 (C-H stretching of aromatic), 1616(C=N stretching vibration). 1HNMR spectra (300 MHz): δ 2.27 (s, 3H, for CH_3 protons of indomethacin), δ 3.81 (s, 3H, for CH_3 protons of methoxide), δ 3.85 (s, 2H, for CH2 proton of indomethacin), δ 6.69-7.34 (m, 3H, for indole ring of indomethacin), 7.47-8.00 (m, 9H, for aromatic ring of indomethacin and aromatic protons.

$C_{27}H_{20}ClN_5O_2S$ (**I2a**): Deep brown powder, yield 65%, melting point: 271-273℃, Rf= 0.65, IR (cm^{-1}): 3055 (C-H stretching of aromatic), 1661 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 2.28 (s, 3H, for CH_3 protons of indomethacin), δ 3.81 (s, 3H, for CH_3 protons of methoxide), δ 3.92 (s, 2H, for CH2 proton of indomethacin), δ 6.80-7.20 (m, 3H, for indole ring of indomethacin), 7.47-8.02 (m, 9H, for aromatic ring of indomethacin and aromatic protons

$C_{27}H_{20}ClN_5O_2S$ (**I3a**): Deep brown powder, yield 61%, melting point: 271-274℃, Rf= 0.63, IR (cm^{-1}): 3062 (C-H stretching of aromatic), 1620(C=N stretching vibration). 1HNMR spectra (300 MHz): δ 2.14 (s, 3H, for CH_3 protons of indomethacin), δ 3.75 (s, 3H, for CH_3 protons of methoxide), δ 3.87 (s, 2H, for CH2 proton of indomethacin), δ 6.78-7.30 (m, 3H, for indole ring of indomethacin),

7.42-8.01 (m, 9H, for aromatic ring of indomethacin and aromatic protons

microwave method**:** A mixture of triazole (0.01 mol), benzoic acids (0.01 mol), and phosphorus oxychloride (5 ml) taken in a 100 ml r.b. flask was irradiated on a microwave oven at 160 W for 4–5 min. The resulting reaction mass was poured into crushed ice with stirring. The solid thus obtained was filtered, washed with dilute sodium bicarbonate solution, followed by water, dried, and recrystallized from ethanol **(P1b, P2b, P3b, N1b, N2b, N3b, I1b, I2b and I3b)**.[39] [42].

$C_{21}H_{22}N_4S$ (**P1b**): pale yellow powder, yield 54%, melting point: 122-124℃, Rf= 0.33, IR (cm^{-1}): 3057 (C-H stretching of aromatic), 1631(C=N stretching vibration). 1HNMR spectra (300 MHz): δ 0.87- 1.63 (d, 9H, for CH_3 protons of ibuprofen), δ 1.81 (m, 1H, for CH proton of ibuprofen), δ 2.42 (d, 2H, for CH_2 protons of ibuprofen), δ 3.85 (q, 1H, for CH proton of ibuprofen), 6.85-8.01 (m, 9H, for aromatic ring of ibuprofen and aromatic protons

$C_{21}H_{22}N_4S$ (**P2b**): yellow powder, yield 63%, melting point: 122-124℃, Rf= 0.72, IR (cm^{-1}): 3055 (C-H stretching of aromatic), 1633 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.23- 1.65 (d, 9H, for CH_3 protons of ibuprofen), δ 1.85 (m, 1H, for CH proton of ibuprofen), δ 2.50 (d, 2H, for CH_2 protons of ibuprofen), δ 3.88 (q, 1H, for CH proton of ibuprofen), 7.12-8.02 (m, 9H, for aromatic ring of ibuprofen and aromatic protons.

$C_{21}H_{22}N_4S$ (**P3b**): yellow powder, yield 72%, melting point: 122-124℃, Rf= 0.73, IR (cm^{-1}): 3047 (C-H stretching of aromatic), 1648 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 0.86- 1.61 (d, 9H, for CH_3 protons of ibuprofen), δ 1.84 (m, 1H, for CH proton of ibuprofen), δ 2.49 (d, 2H, for CH_2 protons of ibuprofen), δ 4.42 (q, 1H, for CH proton of ibuprofen), 7,18-8.01 (m, 9H, for aromatic ring of ibuprofen and aromatic protons.

$C_{22}H_{18}N_4OS$ (**N1b**): yellow powder, yield 75%, melting point: 143-145℃, Rf= 0.33, IR (cm^{-1}): 3053 (C-H stretching of aromatic), 1631 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.63 (d, 3H, for CH_3 protons of naproxen), δ 3.87 (s, 3H, for CH_3 protons of methoxide), δ 4.32 (q, 1H, for CH proton of naproxen), δ 7.28-8.02 (m, 11H, Multiplet, for naphthalene &aromatic protons).

C22H18N4OS (**N2b**): yellow powder, yield 63%, melting point: 143-146℃, Rf= 0.47, IR (cm^{-1}): 3059 (C-H stretching of aromatic), 1632 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.69 (d, 3H, for CH_3 protons of naproxen), δ 3.86 (s, 3H, for CH_3 protons of methoxide), δ 4.40 (q, 1H, for CH proton of naproxen), δ 7.12-8.02 (m, 11H, Multiplet, for naphthalene &aromatic protons).

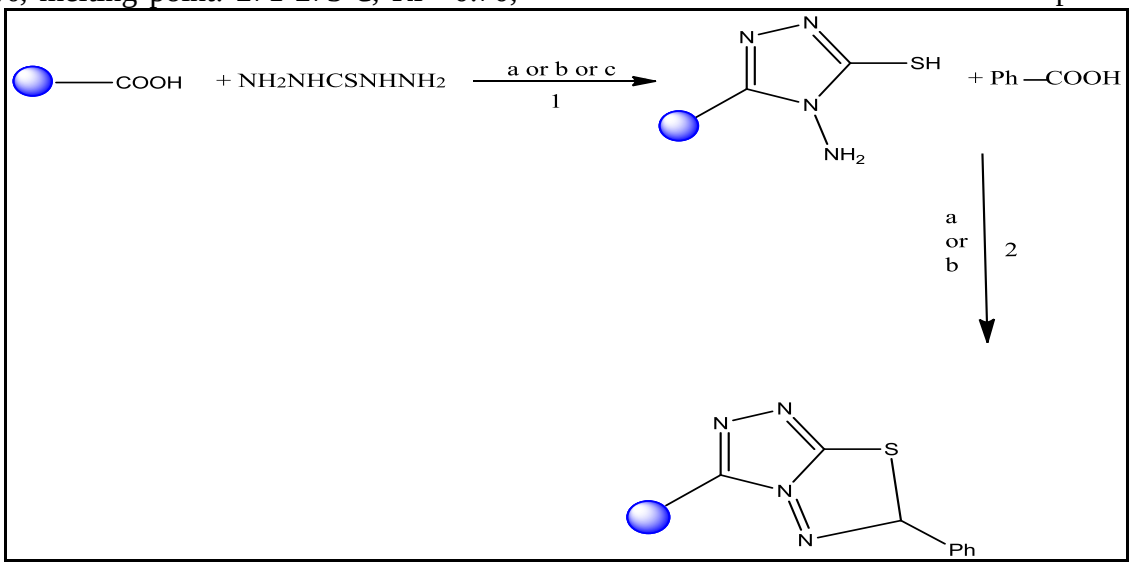
C22H18N4OS (**N3b**): pale brown powder, yield 78%, melting point: 143-145℃, Rf= 0.76, IR (cm^{-1}): 3055 (C-H stretching of aromatic), 1632 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 1.63 (d, 3H, for CH_3 protons of naproxen), δ 3.81 (s, 3H, for CH_3 protons of methoxide), δ 3.92 (q, 1H, for CH proton of naproxen), δ 7.27-8.00 (m, 11H, Multiplet, for naphthalene &aromatic protons).

$C_{27}H_{20}ClN_5O_2S$ (**1b**): yellow powder, yield 77%, melting point: 271-273℃, Rf= 0.79, IR (cm^{-1}): 3051 (C-H stretching of aromatic), 1648(C=N stretching vibration). 1HNMR spectra (300 MHz): δ 2.31 (s, 3H, for CH_3 protons of indomethacin), δ 3.81 (s, 3H, for CH_3 protons of methoxide), δ 3.95 (s, 2H, for CH2 proton of indomethacin), δ 6.71-7.25 (m, 3H, for indole ring of indomethacin), 7.46-8.00 (m, 9H, for aromatic ring of indomethacin and aromatic protons

C27H20ClN5O2S (**2b**): brown powder, yield 86%, melting point: 272-274℃, Rf= 0.87, IR (cm^{-1}): 3078 (C-H stretching of aromatic), 1656(C=N stretching vibration).

1HNMR spectra (300 MHz): δ 2.33 (s, 3H, for CH_3 protons of indomethacin), δ 3.79 (s, 3H, for CH_3 protons of methoxide), δ 3.88 (s, 2H, for CH2 proton of indomethacin), δ 6.73-7.25 (m, 3H, for indole ring of indomethacin), 7.41-8.00 (m, 9H, for aromatic ring of indomethacin and aromatic protons

$C_{27}H_{20}ClN_5O_2S$ (**3b**): brown powder, yield 88%, melting point: 271-273℃, Rf= 0.70, IR (cm^{-1}): 3059(C-H stretching of aromatic), 1647 (C=N stretching vibration). 1HNMR spectra (300 MHz): δ 2.28(s, 3H, for CH_3 protons of indomethacin), δ 3.79 (s, 3H, for CH_3 protons of methoxide), δ 3.89 (s, 2H, for CH2 proton of indomethacin), 6.74-7.25 (m, 3H, for indole ring of indomethacin), 7042-8.00 (m, 9H, for aromatic ring of indomethacin and aromatic protons).



Scheme-1: synthesis of triazolothiadiazole derivatives

COOH = NSAID (Ibuprofen or Naproxen or Indomethacin), Ph-COOH = benzoic acid **1**- Reagents and conditions: (**a**) Oil bath with stirring, at 165-175°C, (**b**) Fusion reflux with stirring 5-6 h, at 165-175°C (**c**) Microwave irradiation at 180 W, 30-35 min. **2**- Reagents and conditions: (**a**) $POCl_3$, reflux 14-16 h, at 170°C (**b**) $POCl_3$, Microwave irradiation 160 W, 4-5 min

The docking studies:

The steps of docking are binding orientations and interactions of most active compounds were analyzed using Maestro™ software package (v. 14.1, Schrödinger, LLC, New York, NY, 2011). With **Protein code**: 3LN1. **Docking steps** into the active site of COX-2 enzyme started by extracting a 3D structure of the enzyme in complex with Celecoxib drug (PDB ID: 3LN1). First, water molecules and hetero groups were removed from receptor and protein structure was refined and minimized using employs OPLS-2005 force field calculations. A grid incorporating COX-2 active sites residues was generated and used to dock optimized compounds into the enzyme. Finally, docking analysis was applied for five poses per compound and the highest scored value for each pose was displayed and described. The following residues identify the active site of COX-2 enzyme:

GLY 512, VAL 335, ALA 513, LEU 517, TYR 341, LEU 345, VAL 102, ARG 106, SER 339, ARG 499, VAL 509, HIE 75, ALA 502, PHE 504, ILE 503, GLN 178, MET 508, LEU 338, LEU 370, TYR 371, PHE 367, TRP 373, TYR 334, SER 516.

The COX-2 active site is classified into three significant regions; first, one is the hydrophobic pocket, which it's definition as TYR 341, TRP 373, PHE 504, ALA 502 and LEU 517. The second region being the entrance of the active site lined with the hydrophilic residues ARG 106, GLU 524, TYR 355, and the third is a side pocket lined by HIS 90 ARG 513 and Val523 [43], [44], [45], [46]as in Figure (2).

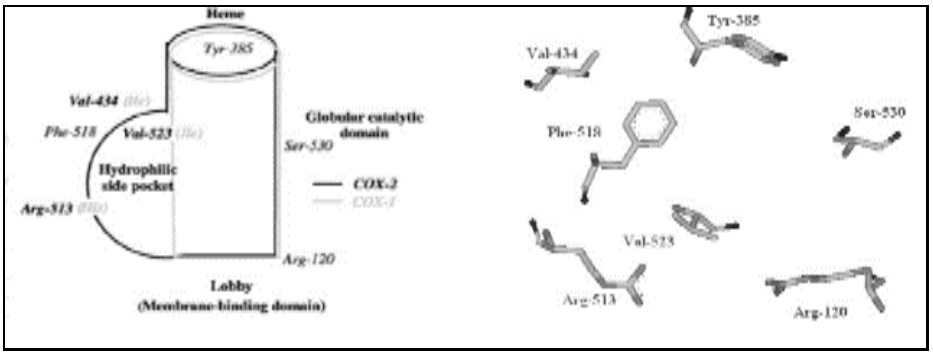


Figure-2: The COX-2 active site [43]

Anti-inflammatory action evaluation for the tested compounds:

In vivo intense anti-inflammatory activities of the desired compounds (1 P1a-3b, N1a-3b and I1a-3b) were assessed using egg-white provoked paw edema in Albino rats. The effect on the paw edema was the measure of the anti-inflammatory activity of derivatives of ibuprofen, naproxen and indomethacin. The decrease of paw thickness is the basis of screening of the anti-inflammatory activity of anew-synthesized final compounds. Albino rats of both sex weighing (170 ± 10 g) were provided by National Center for Drug Control and Research and were kept in the the animal house of the College of Pharmacy, Al-Mustansiriyah University under constant circumstances. A commercial chaw was used for feeding animals and they had free entrance to water. They were separated into different twenty-two groups (each one contains of 6 rats) as follow:

Group A: six rats served as control and treated with the vehicle (propylene glycol 50%v/v).

Group B: six rats treated with Ibuprofen as reference substance in a dose of 50 mg/kg as suspension in 50% v/v propylene glycol [47]

Group C: six rats treated with (s)-Naproxen as reference substance in a dose of 50mg/kg dissolved in Propylene glycol [48]

Group D:six rats treated withIndo-methacin as reference substance in a dose of 2mg/kg as suspension in 50% v/v propylene glycol [49]

Group triazolothiadiazole: six rats /group treated with the tested compounds ***(P1a-3b, N1a-3b and I1a-3b)*** respectively in dose that determined below, also dissolved in propylene glycol.

By utilizing the egg-white prompted edema model was examined the anti-inflammatory action of the tested compounds. Through using vernea could be calculating the paw thickness at seven times intervals: **(0, 30, 60, 120, 180, 240 and 300-min.)** next to administration of the drug. For delivering of an acute inflammation through utilizing the undiluted egg-white by subcutaneous injection (s.i) of (0.05 ml) into the left hind paw at the plantar side of the rats after the drug or vehicle administration intra peritoneal by (30 min.).

The data, which was expressing by the (**mean ± SEM**) and products were analyzing to significantly statistic for correlation among mean values by utilizing student t-test two (Sample Assuming Equal Variances). By utilizing ANOVA: two elements without repetition, the correlation among various collections could be making. Probability (P) value of below (0.05) was considering significantly.

Ibuprofen, Naproxen and Indomethacin were used as reference substances. They administered by intraperitoneal route (i.p.), Ibuprofen which is given in a dose of 2mg/kg Indomethacin which is given in a

dose of 50mg/kg, Naproxen which is given in a dose of 50mg/kg [50]. Indomethacin which is given in a dose of 50mg/kg [47], so; the doses of synthesized compounds are calculated as bellow:

Calculations for Dose Determination:

$$\frac{dose\ of\ refrence\ Compound}{refrence\ moleculare\ wihgt} = \frac{dose\ of\ tested\ compuond}{tested\ compuond\ molecular\ wight}$$ [51]

M.Wt. of Ibuprofen= 206.285 g/mol
50mg / kg / 206.285 = Dose / M.Wt. of the tested compound
M.Wt. of (s)-Naproxen = 230.26
50mg / kg / 230.26 = Dose / M.Wt. of the tested compound [50]
M.Wt. of Indomethacin= 357.79 g/mol
2mg / kg / 357.79 = Dose / M.Wt. of the tested compound [47]

Table 1: Molecular weight and dose of the compounds

Compounds	Molecular Weight	Dose mg/ kg
Ibuprofen	206.28	50
P1a, P1b, P2a, P2b, P3a, P3b	362.49	87.9
Naproxen	230.26	50
N1a, N1b, N2a, N2b, N3a, N3b	386.47	84
Indomethacin	357.79	2
I1a, I1b, I2a, I2b, I3a, I3b	514	2.9

Results

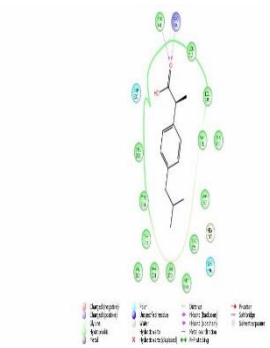
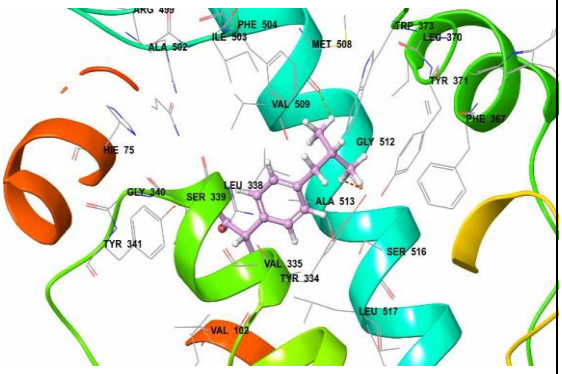
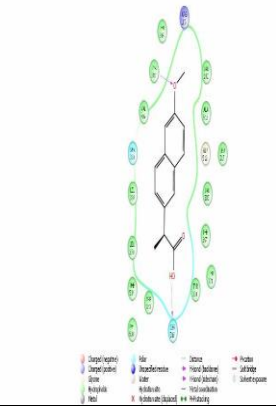
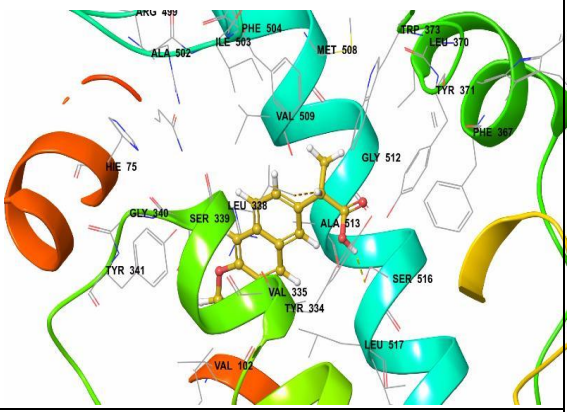
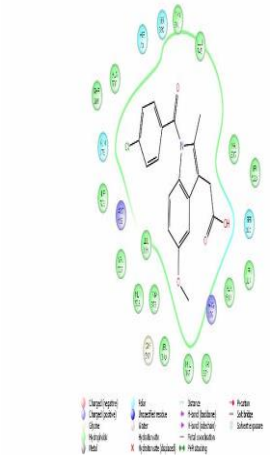
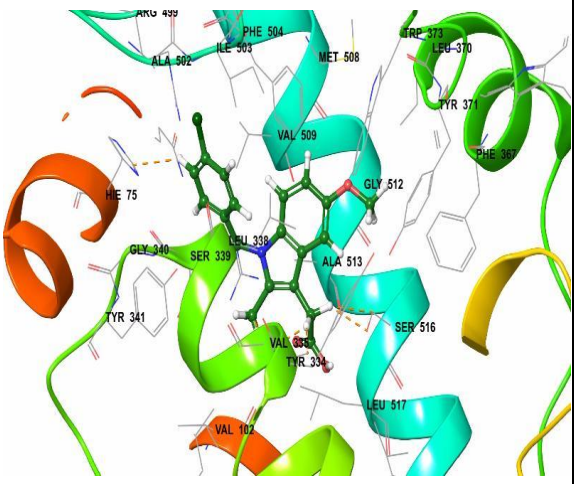
Figure (3), (4) and (5) show the effect of all tested compounds with statistically significant (P<0.05) reduction in paw edema thickness.

Table (3), (4) and (5) explains the effect of tested compounds (P1a-3b, N1a-3b and I1a-3b) in comparison to control and ibuprofen, naproxen and indomethacin.

According to docking result, the (P2) group is refer to (P1a-3b), (N2) refer to (N1a-3b), and (I2) refer to (I1a-3b). (N2 and P2) compounds contain one chiral centers. Only (N2 and P2) compounds shows a docking ability to the enzyme according to our setting with this software for this docking procedure, notice that ligand-COX-2 complex generated by docking revealed intricate interactions with a COX-2 channel, which (P2) including Pi-Pi stacking in aromatic ring of Ibuprofen with key residues TYR 341, and in

aromatic ring TYR 371, hydrophobic interactions with LEU 338, ALA 513, LEU 517, PHE 367 and VAL 335, while (N2) including Pi- Pi stacking in aromatic ring TRP 373, hydrophobic interactions with VAL 102, LEU 345, TYR 341and ALA 502, as appear in Table-1.

Table -2: target compounds bind with the active sites of COX-2 enzyme.

Comp. name	Structure	Binding site
Ibuprofen		
Naproxen		
Indomethacin		

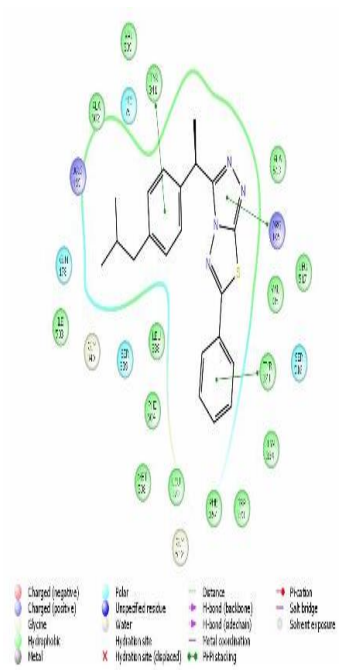
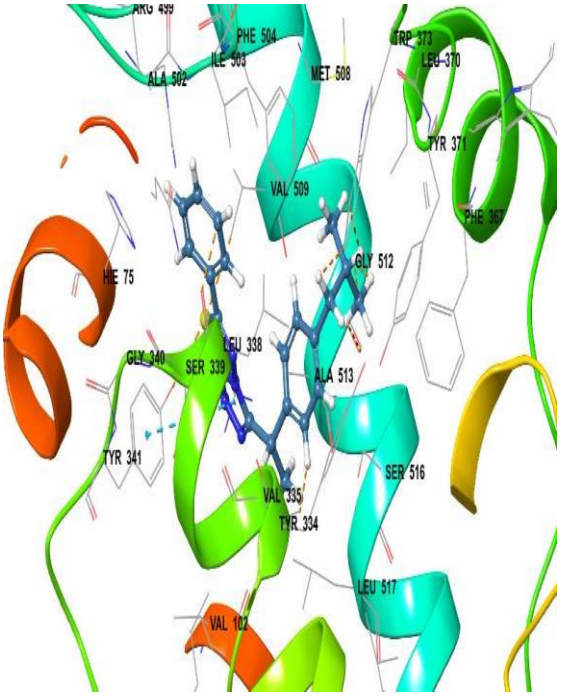
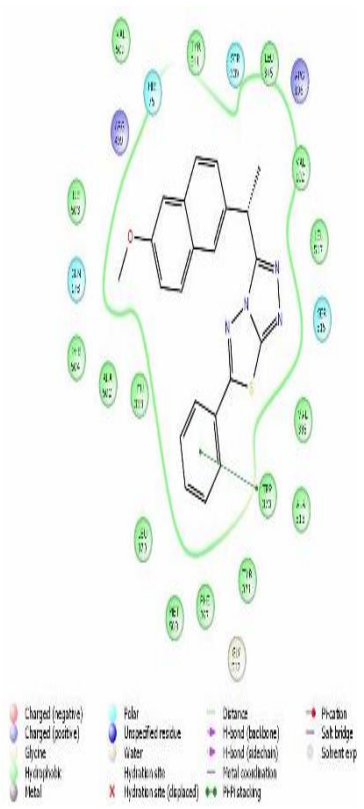
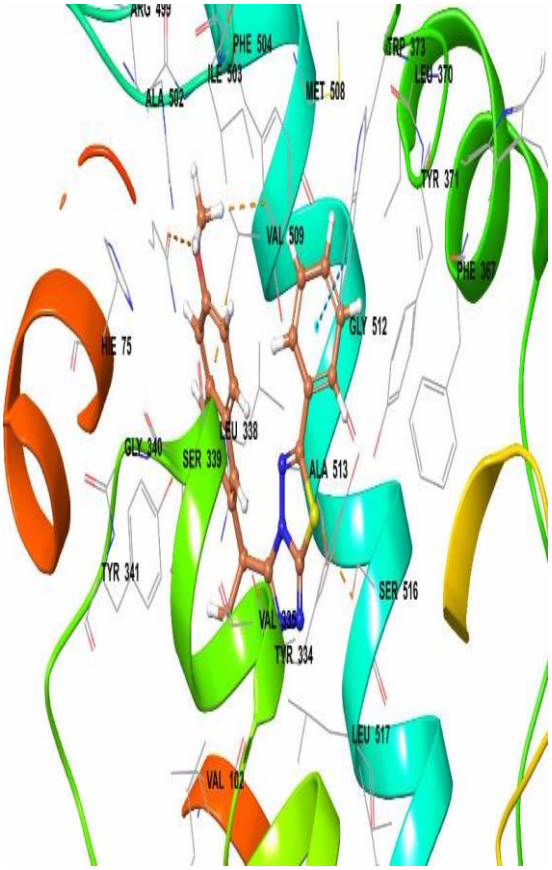
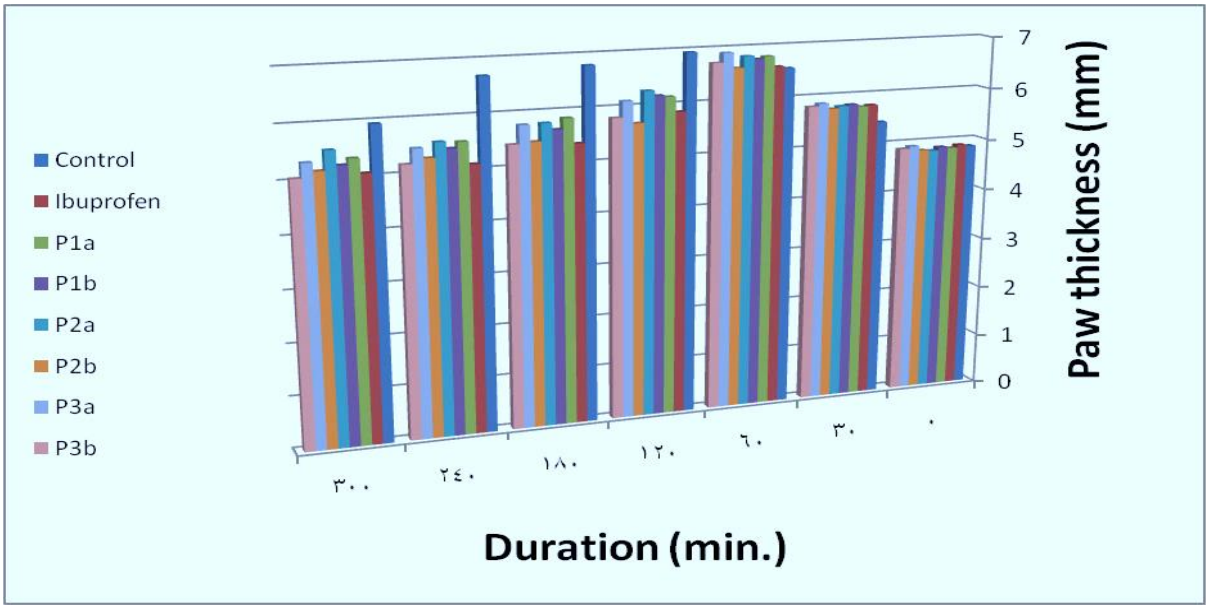
P2		
N2		

Table (3): The Anti-Inflammatory Effect of Control, Ibuprofen and Compounds (P1a-3b) on Egg-White Induced Paw Edema in Rats.

Paw thickness (mm) n=6	Compound	Time (min)						
		0	30	60	120	180	240	300
	Control	4.86±0.03	5.44±0.06	6.57±0.02	6.95±0.04	6.80±0.07	6.70±0.06	5.95±0.02
	Ibuprofen	4.89±0.04	5.78±0.02	6.61±0.05	a*5.86±0.04	a*5.38±0.02	a*5.12±0.04	b*5.08±0.03
	P1a	4.84±0.05	5.76±0.03	6.81±0.06	b*6.15±0.03	b*5.86±0.02	a*5.54±0.05	c*5.36±0.04
	P1b	4.87±0.02	5.81±0.05	6.77±0.02	b*6.18±0.02	a*5.66±0.01	a*5.43±0.04	b*5.25±0.01
	P2a	4.81±0.05	5.79±0.02	6.83±0.05	c*6.28±0.01	a*5.79±0.02	a*5.56±0.01	*5.53±0.05
	P2b	4.83±0.04	5.75±0.02	6.62±0.04	a*5.69±0.05	a*5.46±0.03	a*5.28±0.02	b*5.17±0.02
	P3a	4.92±0.05	5.86±0.08	6.91±0.05	b*6.11±0.06	a*5.78±0.01	a*5.47±0.01	c*5.33±0.03
	P3b	4.88±0.02	5.81±0.05	6.73±0.06	a*5.81±0.04	a*5.43±0.02	a*5.19±0.03	b*5.06±0.01

Non-identical superscripts (a, b&c) among different tested compounds are considered significantly different (P<0.05); *significantly different compared to control (P<0.05). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of ibuprofen, tested compounds and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema).



Figures -3: Effect of Ibuprofen, propylene glycol and tested compounds (P1a-3b) on egg-white induced paw edema in rats.

Table-4: The anti-inflammatory effect of control, Naproxen and compounds (N1a-3b) on egg-white induced paw edema in rats.

Paw thickness (mm) n=6	Compound	Time (min)						
		0	30	60	120	180	240	300
	Control	4.86±0.03	5.44±0.06	6.57±0.02	6.95±0.04	6.80±0.07	6.70±0.06	5.95±0.02
	Naproxen	4.83±0.05	5.73±0.06	6.52±0.05	a*5.83±0.05	a*5.32±0.06	a*5.09±0.06	b*5.06±0.04
	N1a	4.86±0.03	5.79±0.03	6.70±0.07	a*5.92±0.06	6.72±0.05	a*5.59±0.02	*5.49±0.01
	N1b	4.83±0.05	5.77±0.02	6.61±0.06	a*5.87±0.01	a*5.50±0.06	a*5.31±0.03	c*5.28±0.01
	N2a	4.87±0.02	5.73±0.06	6.65±0.04	a*5.89±0.02	a*5.67±0.02	a*5.52±0.05	c*5.45±0.03
	N2b	4.86±0.02	5.79±0.01	6.57±0.02	a*5.81±0.07	a*5.45±0.02	a*5.22±0.07	b*5.12±0.01
	N3a	4.82±0.05	5.75±0.03	6.59±0.02	a*5.81±0.06	a*5.43±0.03	a*5.27±0.02	b*5.19±0.04
	N3b	4.85±0.06	5.72±0.05	6.55±0.04	a*5.79±0.03	a*5.31±0.06	a*5.11±0.01	b*4.98±0.02

Non-identical superscripts (a, b&c) among different tested compounds are considered significantly different (P<0.05); *significantly different compared to control (P<0.05). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of naproxen, tested compounds and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema).

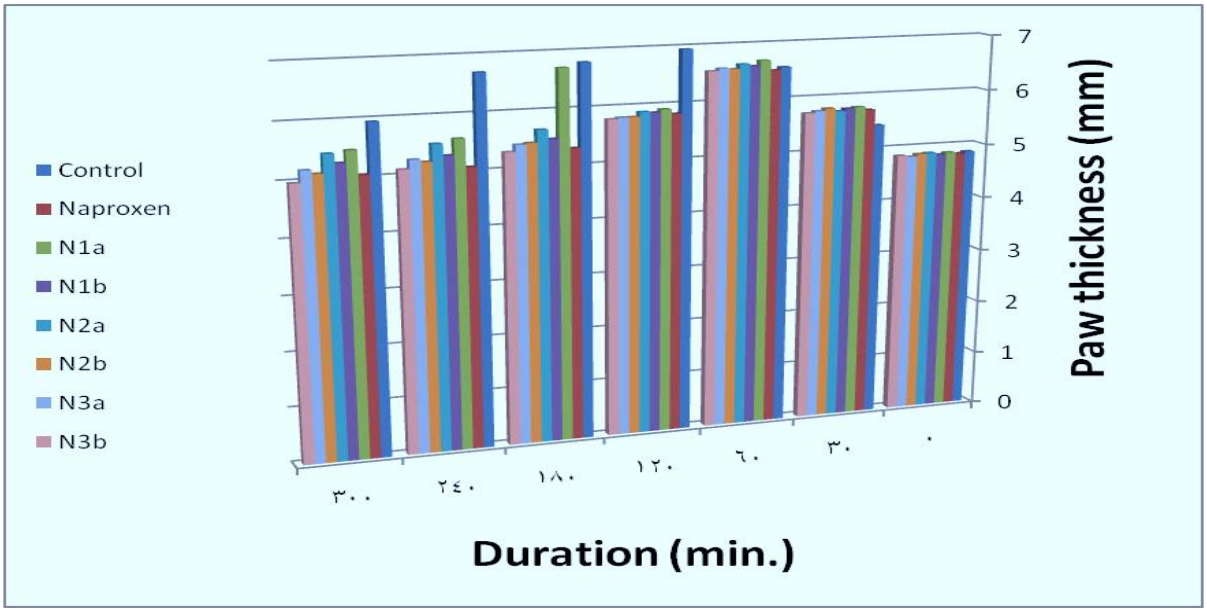


Figure-4: Effect of Naproxen, propylene glycol and tested compounds (N1a-3b) on egg-white induced paw edema in rats

Table -5: explains the anti-Inflammatory effect of control, Indomethacin and compounds (I1a-3b) on egg-white induced paw edema in rats.

Paw thickness (mm) n=6	Compound	Time (min)						
		0	30	60	120	180	240	300
	Control	4.86±0.03	5.44±0.06	6.57±0.02	6.95±0.04	6.80±0.07	6.70±0.06	5.95±0.02
	Indomethacin	4.92±0.07	5.65±0.04	6.75±0.04	6.87±0.02	b*5.81±0.06	a*5.32±0.03	b*5.10±0.05
	I1a	4.86±0.03	5.59±0.04	6.67±0.02	6.77±0.02	b*5.86±0.03	b*5.59±0.02	5.37±0.03
	I1b	4.81±0.07	5.56±0.03	6.64±0.06	6.78±0.02	a*5.61±0.05	a*5.23±0.02	b*5.08±0.04
	I2a	4.81±0.06	5.53±0.04	6.61±0.05	6.73±0.06	a*5.79±0.02	a*5.50±0.06	5.31±0.05
	I2b	4.93±0.05	5.62±0.07	6.78±0.02	6.91±0.05	a*5.79±0.05	a*5.32±0.04	b*5.19±0.02
	I3a	5.02±0.06	5.69±0.03	6.77±0.02	6.85±0.04	a*5.69±0.03	a*5.38±0.02	b*5.19±0.01
	I3b	5.06±0.04	5.75±0.03	6.84±0.07	6.94±0.02	a*5.78±0.07	a*5.31±0.03	b*5.12±0.05

Non-identical superscripts (a, b&c) among different tested compounds are considered significantly different (P<0.05); *significantly different compared to control (P<0.05). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of indomethacin, tested compounds and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema).

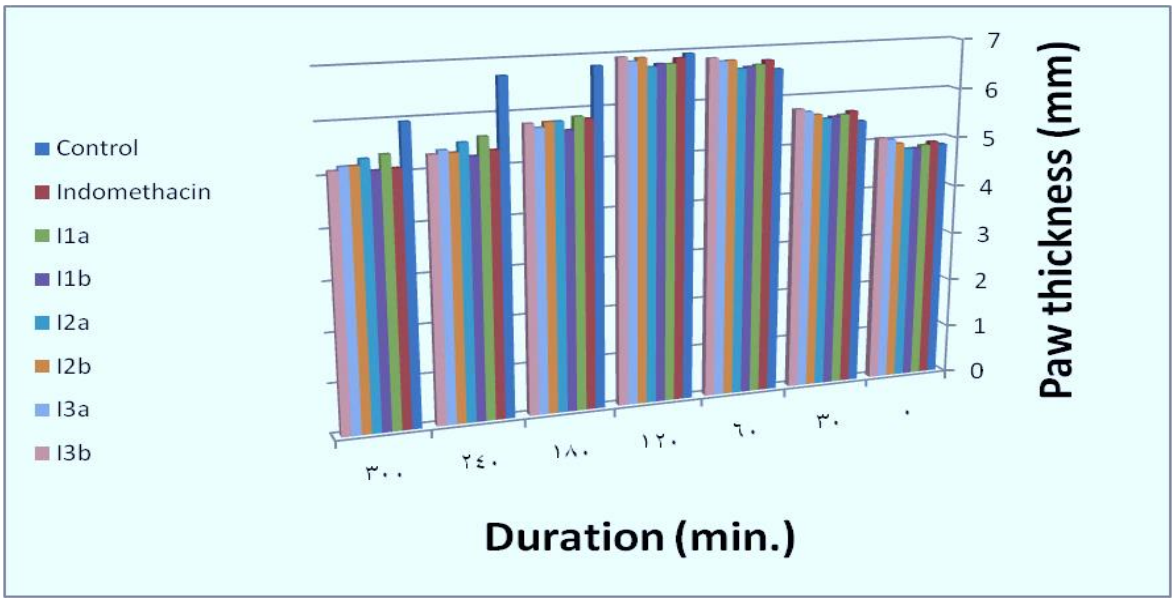


Figure-5: Effect of Indomethacin, propylene glycol and tested compounds (I1a-3b) on egg-white induced paw edema in rats

Discussion:

For acute inflammation, could be using the carrageenan-producing edema that representing as experimental animal model also, is supposed to be biphasic. The carrageenan model early phase (1–2 hr.) is mostly mediated by serotonin, histamine in addition elevation in the prostaglandins synthesis in the damaged surroundings tissue, while the late phase is sustained by release of prostaglandin beside is mediated by: bradykinin, polymorph nuclear cells, leukotrienes and prostaglandins produced by macrophages tissue [52].

Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, all due to the metabolism of AA[52].

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle (control group) and ibuprofen, naproxen and indomethacin. The tested compounds and the reference drug produced significant reduction of paw edema with respect to the effect of propylene glycol 50%v/v (control group). The effect of Ibuprofen and Indomethacin and their tested compounds started at time (120 min.), while Naproxen and its derivatives started at (60 min.) which indicate fast onset of action. The effect of tested compounds and NSAIDs that used continued until the end of experiment**.** Compound (P3b and I1b) exert significantly higher paw edema reduction than reference drug at time (120-240 min.), while (P2b, I2b and I3b) exert similar effect to them at the same time). Compound (N3b) exert significantly higher paw edema reduction than Naproxen at time (60-240 min.), while (N2b) exert similar effect to it. Compounds (P1a, P2a, I1a and I2a) produced significantly lower inhibitory effect than Ibuprofen and Indomethacin at time (120-240min.). Compounds (N1a) and (N2a) produced significantly lower inhibitory effect than Naproxen at time (60-240min.). At time (300 min.), all tested compounds show comparable effect to that of standard drugs.

The compounds synthesis by the microwave method are more efficient as anti-inflammatory agents than those synthesis by the conventional method, also, producing higher yields, probably, due to microwave irradiation enables the polarization of the molecule under irradiation causing fast reaction to happen and the uniform spreading of the heat.

According to docking score, the best binding affinity is docking score with more negative value. The positive control compounds (Indomethacin, Naproxen, and Ibuprofen) show docking score between -8.846 to -8.453. Compound (N2) in both isomers and (P2) in both isomers show docking score between -8.929 to -6.541 [53], as seen in figure (3). Compound (N2) in both isomers is better than (P2) in both isomers, which (N2) has negative docking score higher than the reference drug (Naproxen), while (P2) give similar result to Ibuprofen. Compound (I2) did not show activity because many reasons. One of the reasons is because the size of compound is too large to inter inside the active site. All compounds interaction locates similar to positive controls inside the enzyme pocket surrounded by similar amino acid.

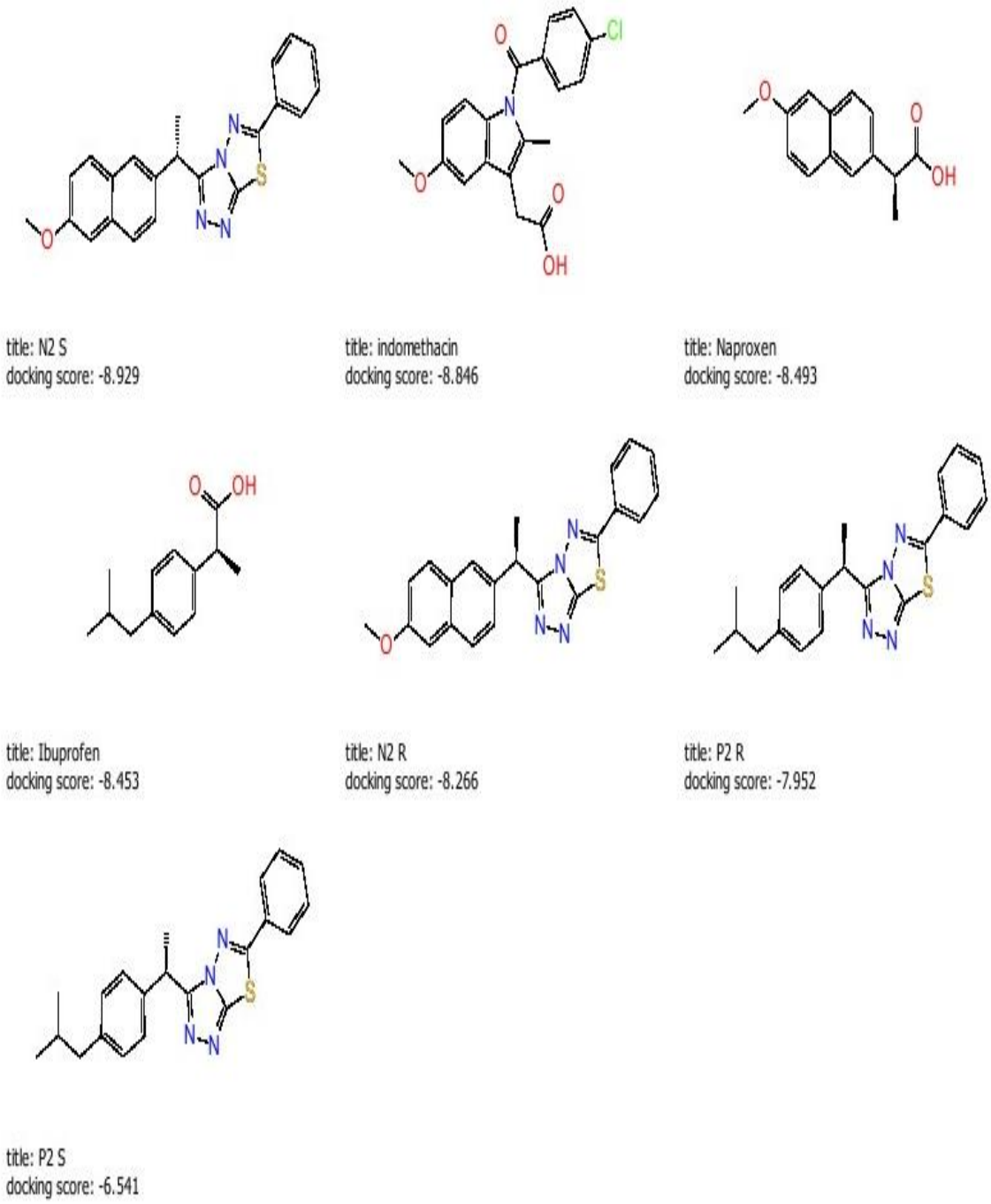


Figure -2: docking result.

Conclusion

The synthesis of the designed compounds has been successfully achieved. Characterization and identification of the synthesized compounds were confirmed by determination of physical properties (melting point and Rf value), FT-IR spectroscopy and 1H-NMR spectra.

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