

Synthesis, Identification and Preliminary Pharmacological Evaluation of New Hydrazone and 1, 3, 4-oxadiazole Derivatives of Ketorolac

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Abstract

New series of hydrazones (3a–b) and 1, 3, 4-oxadiazole S-alkylated derivatives (5a–b) of ketorolac (NSAID) were synthesized and their structures were identified by using ATR-FT-IR and ¹HNMR spectroscopic analytical techniques. The synthesized compounds were evaluated in vitro for their antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and fungal species (*Candida albicans*). After the approval of the ethical committee in the college of pharmacy, University of Baghdad, In vivo screening for their anti-inflammatory activity was done using albino rats as an animal model that were subjected to white egg-induced acute inflammation. Results have shown that the target compounds were successfully synthesized, and it was discovered that they have mild to moderate activity against E.Coil and S.aureus while no effect has shown on C.albicans. In vivo studies showed they were effective in lowering paw edema thickness and had an anti-inflammatory effect similar to that of the standard (ketorolac)

Keywords: NSAIDS, Ketorolac, Hydrazones, 1,3,4-Oxadiazole.

تخليق وتشخيص وتقييم اولي لفعالية مشتقات جديدة لكيتورولاك

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الخلاصة

تم تحضير سلسلة جديدة من مشتقات هيدرازون للكييتورولاك (3أ-ب) و مشتقات من نواة 1 و 3 و 4-اوكساديازول المشتقة من الكيتورولاك (5أ-ب) وتم تشخيص المركبات المحضرة باستخدام مطياف الاشعة تحت الحمراء وجهاز الرنين النووي المغناطيسي. المركبات المحضرة ، تم تقييم فعاليتها المضادة للالتهاب الفطري و البكتري (للبكتريا السالبة ايكولاي والموجبة ستافلوكوكس اربوس) خارج الجسم الحي وتقييم فعاليتها المضادة للالتهاب داخل الجسم الحي من خلال تحفيز التهاب حاد في الجرذان باستخدام بياض البيض. تم تصنيع المركبات المستهدفة بنجاح ، وقد وجد انها لا تمتلك فعالية ضد فطريات كانديدا البكانز وفعالية بسيطة الى متوسطة ضد بكتريا ايكولاي وستافلوكوكس اربوس. وتم اكتشاف انها تمتلك الفعالية في تقليل سماكة وذمة المخالب ولها تأثير مضاد للالتهابات مشابه للتاثير الماده القياسية الكيتورولاك . الكلمات المفتاحية: مضاد الالتهاب غير الستيرويدي، هايدرازونو مركبات حلقيه غير متجانسة، 1،3،4-اوكساديازول.

Introduction

Ketorolac is one of the non-selective non-steroidal anti-inflammatory drugs (NSAIDs) ^(1,2), which have analgesic, and antipyretic, in addition to their ability to reduce inflammation. These medications block the Cyclooxygenases (COXs) enzymes, which control the rate of production of prostanoids such thromboxanes and prostaglandins. COX-2 inhibitors, sometimes referred to as coxibs, selectively inhibit COX-2 enzymes as opposed to nonselective NSAIDs, which inhibit both COX-1 and COX-2 ^(3,4). Many bacterial species have developed stronger resistance in recent years, which may be because of improper and inappropriate antibiotic usage.

Several compounds with notable effects encouraged scientists to create new chemicals in this field. It has been discovered that previously produced compounds having a hydrazide-hydrazone structure have strong antibacterial activity ⁽⁵⁾. Hydrazones have an azomethine (-NHN=CH) group. These compounds are extensively researched due to their simple synthesis and a vast range of pharmacological potential, leading to the development of drugs with improved activity and reduced toxicity profiles ⁽⁶⁾. Differently substituted hydrazones have been produced and proven to be active against

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various pharmacological targets using various synthesis procedures and adequate SAR analyses. They are known to have a variety of biological properties, including antibacterial, anti-inflammatory, anti-cancer, and anti-malarial properties. These findings have served as a guide for the creation of new hydrazones⁽⁷⁾.

Organic chemical compounds with a ring-like structure that contain one or more heteroatoms mostly nitrogen, sulfur or oxygen are known as heterocyclic compounds or heterocycles. Although they have a general structure that is similar to that of cyclic organic compounds, which only contain carbon atoms, heterocycles have unique physicochemical characteristics from all carbon ring analogs due to the substitution of one or more carbon atoms with heteroatoms. Heterocycles have a variety of applications, including antibacterial, anti-inflammatory, and antioxidant properties^(8,9). Oxadiazole, a heterocyclic five-membered molecule with the typical formula of $(C_2H_2ON_2)$, is formed by replacing two (-CH=) of a furan ring with two nitrogen atoms. There are numerous oxadiazole isomers depending on the position of the nitrogen atom in the ring structure⁽¹⁰⁾. 1, 3, 4-oxadiazole is a more important pharmacologically active isomer than the other oxadiazole isomers⁽¹¹⁻¹³⁾. This work aimed to synthesizing new hydrazones and 1, 3, 4-oxadiazole derivatives, derived from Ketorolac and evaluate their pharmacological activities.

Materials and Methods

All the solvents and chemicals used in the synthesis were analytical grade, acquired from private sources, and used without further purification. The TLC method was employed to verify the reaction's success and the product's purity. The capillary tube and the electronic melting point apparatus were used to measure melting points (uncorrected) (Stuart SMP30). The infrared spectra were obtained at the University of Baghdad/ College of Pharmacy using an ATR FT-IR spectrophotometer from Shimadzu, Japan.). ¹H NMR spectra were captured using a BRUKER model Ultra Shield 400 MHz spectrophotometer with DMSO-d₆ as the solvent.

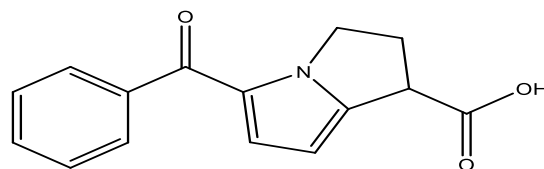


Figure 1. structure of ketorolac (parent nucleus)

Synthesis of ketorolac methyl ester (methyl -5-benzoyl-2,3-dihydro -1H-pyrrolizine -1-carboxylate) (compound 1)⁽¹⁴⁾

Ketorolac powder approximately (0.0235 moles, 6gm) in a 250 ml round flask, then 20 ml of methanol was added with stirring to produce clear yellow solution. An ice bath was used to cool the obtained mixture to 0 °C, and then 5 ml of concentrated sulfuric acid (H₂SO₄) was added drop by drop while stirring continuously. The mixture was then set on reflux for 6 h with stirring. Following the conclusion of the reaction, which was checked by using TLC (Ethyl acetate: N-Hexane (6:4)) as solvent system, the mixture was cooled to room temperature. Then, the extra acid was neutralized by adding 5% w/v of sodium bicarbonate solution. The precipitate was filtered, collected, washed with water, dried, and crystallized from absolute ethanol. An orange to red crystal of ketorolac methyl ester was produced, yield = 66%, M.P. (91 °C). IR, (ν cm⁻¹): 3051: Aromatic (C-H) Str. 2951, 2900: (C-H) Asym. Str. of CH₃ and CH₂ .2839, 2815: (C-H) Sym. Str. of CH₃ and CH₂, 1732: (C=O) Str. of ester, 1172: (C-O-C) Str. of ether. ¹H NMR: (400 MHz, DMSO-d₆ =ppm): 3.7 (3H, s, COOCH₃), 2.77 (2H, m, C2 of pyrrolidine), 4.23 (1H, d, at C1 of pyrrolidine), 4.31-4.43 (2H, dd, at C3 pyrrolidine), 6.1-6.78 (2H, d, at C6, C7 of pyrrole), 7.52-7.6-7.67 (5H, m, Aromatic H).

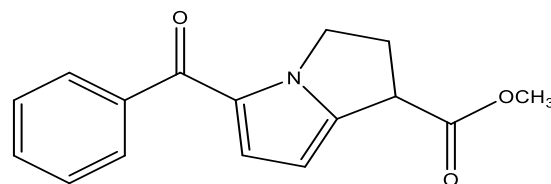


Figure 2. methyl -5-benzoyl-2,3-dihydro -1H-pyrrolizine -1-carboxylate (compound 1)

Synthesis of ketorolac hydrazone (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazone) (compound 2)⁽¹⁵⁾

Ketorolac methyl ester (compound 1) (0.006 moles, 1.7g) was added to absolute ethanol (35ml) mixing until dissolved and become a clear dark orange solution then, A sufficient amount of approximately (0.06 moles, 3 ml) of hydrazine hydrate 80% was added, and the mixture was left with stirring at room temperature until it transformed into a turbid yellow suspension 6h after which it was refluxed for 12 h. Then mixture was stirred at room temperature for about 8 h. Then the mixture was then poured on to crush ice, creating a precipitate. The precipitate that resulted was filtered, repeatedly rinsed with cold water, dried, and reconstituted from absolute ethanol. Faint yellow powder, yield = 61.1%, m.p. (158-160 °C). IR (ν cm⁻¹): 3336, 3298: (NH) Str. Of hydrazide, 3062: Aromatic (C-H) Str. 2970: (C-H) Asym. Str. of CH₂. 2875: (C-H) Sym. Str. of CH₃, 1643: (C=O) str. of amide, 1604: (NH) bend .1573, 1573, 1492(C=C) str. of aromatic. ¹H NMR: 11.53 (H, s, -NH-NH₂), 3.88 (2H, S, NH-NH₂), 2.93,2.77 (2H, m, at C2 of pyrrolidine), 4.8 (1H, m, at C1 of pyrrolidine), 4.4 (2H, m, at C3 of pyrrolidine), 6.75,7(2H, d, at C6 and C7 of pyrrole), 7.75-7.58 (5H, m, aromatic H)

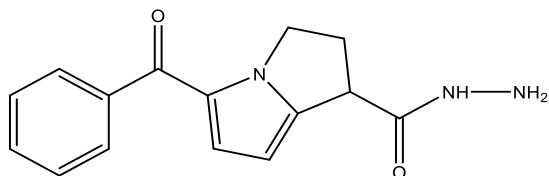


Figure 3. 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazone (compound 2)

Synthesis of aryl hydrazones (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazone) (Compounds 3a-b)⁽¹⁶⁾

[salicylaldehyde (0.005 mol, 0.61g)] and [*p*-nitro-benzaldehyde (0.005 mole, 0.75g)] were separately dissolved in absolute ethanol and then were added a few drops of glacial acetic acid with continuous stirring. Each of the mentioned aldehydes was added separately to a stirred solution of compound 2 (0.005 mol, 1.35g), which was dissolved in absolute ethanol (20mL). Every reaction mixture was then refluxed for 12-18 hours. Cold water was added to the mixture when the reaction was complete (as determined by TLC). To obtain the desired products, the precipitate that had formed was collected, dried, and recrystallized with ethanol as the solvent.

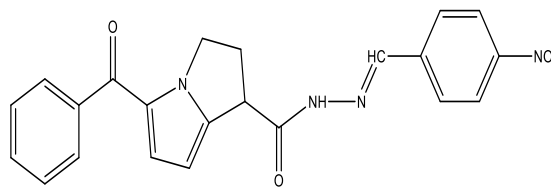


Figure 4. 5-benzoyl-N'-(4-nitrobenzylidene)-1,2-dihydro-1H-pyrrolizine-1-carbohydrazone (Compound 3a)

Light yellow powder, yield = 88%, m.p. (230 °C). IR (ν cm⁻¹): 3186: (NH) Str. Of hydrazone, 3082: Aromatic (C-H) Str. 2966: (C-H) Asym. Str. of CH₂ 2866: (C-H) Sym. Str. of CH₂, 1674: (C=O) Str. of amide, 1600: (C=N) Str. of imine. 1523, 1342 (N-O) Asym and Sym Str. respectively .1581, 1554 (C=C) Str. of Aromatic. ¹H NMR: 11.99, 12.15 (H, s, -NH-N=CH), 8.39 (1H, s, C-H), 2.81, 2.95 (2H, m, at C2 of pyrrolidine), 4.44 (2H, m, at C3 of pyrrolidine), 4.98 (1H, m, at C1 of pyrrolidine), 6.7, 5.93 (2H, d, at C6 and C7 of pyrrole), 7.53-8.31 (9H, m, Aromatic H)

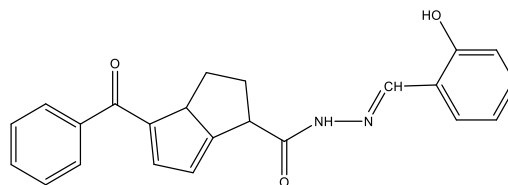


Figure 5. 5-benzoyl-N'-(2-hydroxybenzylidene)-2,3-dihydro-1H-pyrrolizine-1-carbohydrazone (Compound 3b)

Yellow powder, yield = 79%, m.p. (220 °C). IR (ν cm⁻¹): 3213: (NH) Str. of hydrazone, 3066: Aromatic (C-H) Str., 2962: (C-H) Asym. Str. of CH₂ .2835: (C-H) Sym. Str. of CH₂, 1674: (C=O) Str. of amide, 1604: (C=N) Str. of imine. 1570, 1527 (C=C) Str. of Aromatic. ¹H NMR: 11.53, 12.03 (H, S, -NH-N=C), 11.01 (1H, S, OH), 8.48 (1H, S, N=C-H), 2.74, 2.93 (2H, m, at C2 of pyrrolidine), 4.8 (1H, m, at C1 of pyrrolidine), 4.4 (2H, m, at C3 of pyrrolidine), 5.93, 6.74 (2H, d, at C7, C6 of pyrrole), 6.83-7.75 (9H, m, aromatic H).

Synthesis of 5(5-benzoyl-2, 3-dihydro-1H-pyrrolizin-1-yl)-1,3,4-oxadiazole-2-thiol. (compound 4)⁽¹⁷⁻¹⁸⁾

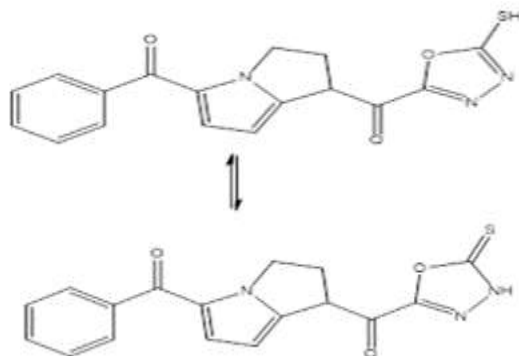


Figure 6. compound (4)

A ketorolac hydrazide (compound 2) (0.0037mole, 1g) was dissolved in 15 ml of 50 w/w% of aqueous ethanol, then KOH (0.008 mole, 0.47g) was added to the mixture slowly with continued stirring for 15 minutes at 0°C. Then a volume of CS₂ equivalent to (0.007mole, 0.53g) added to the mixture gradually. Finally, the mixture reflux for 22h until all H₂S gas evaporate monitoring by lead strips. At the end of reaction which follows by TLC, the solution was placed into a beaker with 30 ml of crushed ice water, and concentrated HCl was added until the pH was acidic (2-3). The resulting crude ppt. was filtered, dried, and base-acid purified.

Light gray, yield = 71%, m.p. (128-130°C). IR (ν cm⁻¹): 3132: (N-H) Str. of thioamide (oxadiazole), 3086: aromatic ring (C-H) Str., 3059: (C-H) Asym. Str. of CH₂; 2927 (C-H) Sym. Str. of CH₂; 1597(C=N) Str. of imine of oxadiazole; 1045 (C=S) Str. of oxadiazole; 1485, 1566 (C=C) Str. of aromatic. ¹H NMR : 14.58 (1H, S, S-H), 4.45 (1H, m, at C1 of pyrrolidine), 4.78 (2H, m, at C2 of pyrrolidine), 2.84, 3.01 (2H, m, at C3 of pyrrolidine), 6.18, 6.83(2H, d, at C7 C6 of pyrrole), 7.54-7.79 (5H, m, aromatic H).

Synthesis of 2{[5-(5-benzoyl-2,3-dihydro-1H-pyrrolizine - 1- yl)-1, 3 , 4 - oxadiazole - 2 - yl] sulfanyl } -1- (4methoxyphenyl) ethan-1-one (compound 5a)⁽¹⁹⁾

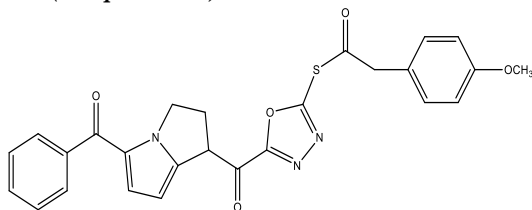


Figure 7. 2{[5-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-yl)-1,3,4-oxadiazole-2-yl]sulfanyl}-1 (4methoxyphenyl) ethan-1-one (compound 5a)

Compound 4 (0.002 mole, 0.62 g) with NaOH (0.002mole, 0.08g) was stirred in aqueous solution for 5 min. After dissolved and form a clear solution, ethanolic solution of 4-Methoxyphenacylbromide (0.002mole, 0.45g) was added, with reflux for 30 min. At the end of reaction which follows by TLC. The 4-methoxyphenacylbromide gave solid precipitate that was obtained by filtration and recrystallized from ethanol.

White powder, yield = 72%, m.p. (138-140°C). IR (ν cm⁻¹): 3059: aromatic ring(C-H) Str., 3012,2912: (C-H) Asym. Str. Of CH₂ and CH₃.2850,2835: (C-H) Sym. Str. Of CH₃ and CH₃.1666: (C=O) Str. Of carbonyl.1577: (C=N) Str. Of imine of oxadiazole.1481,1501 (C=C) Str. Of aromatic. ¹H NMR: 5.10 (2H, S, CH₂), 4.9 (1H, m, at C1 of pyrrolidine), 4.46 (2H, m, at C2 of pyrrolidine), 2.86,3.04 (2H, m, at C3 of pyrrolidine), 6.12 ,6.80(1H,d, at C7 C6 of pyrrole),7.10-8.05 (9H,m,aromatic H).

Synthesis of 1-(4-bromo-phenyl)-2-[[5-(2,3-dihydro-1pyrazolyl] sulfanyl] ethan-1-one (compound 5b)

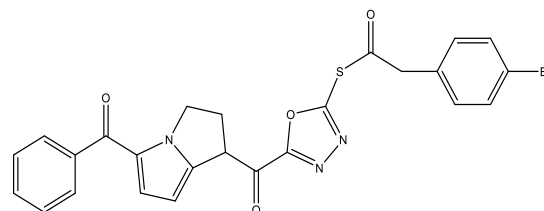
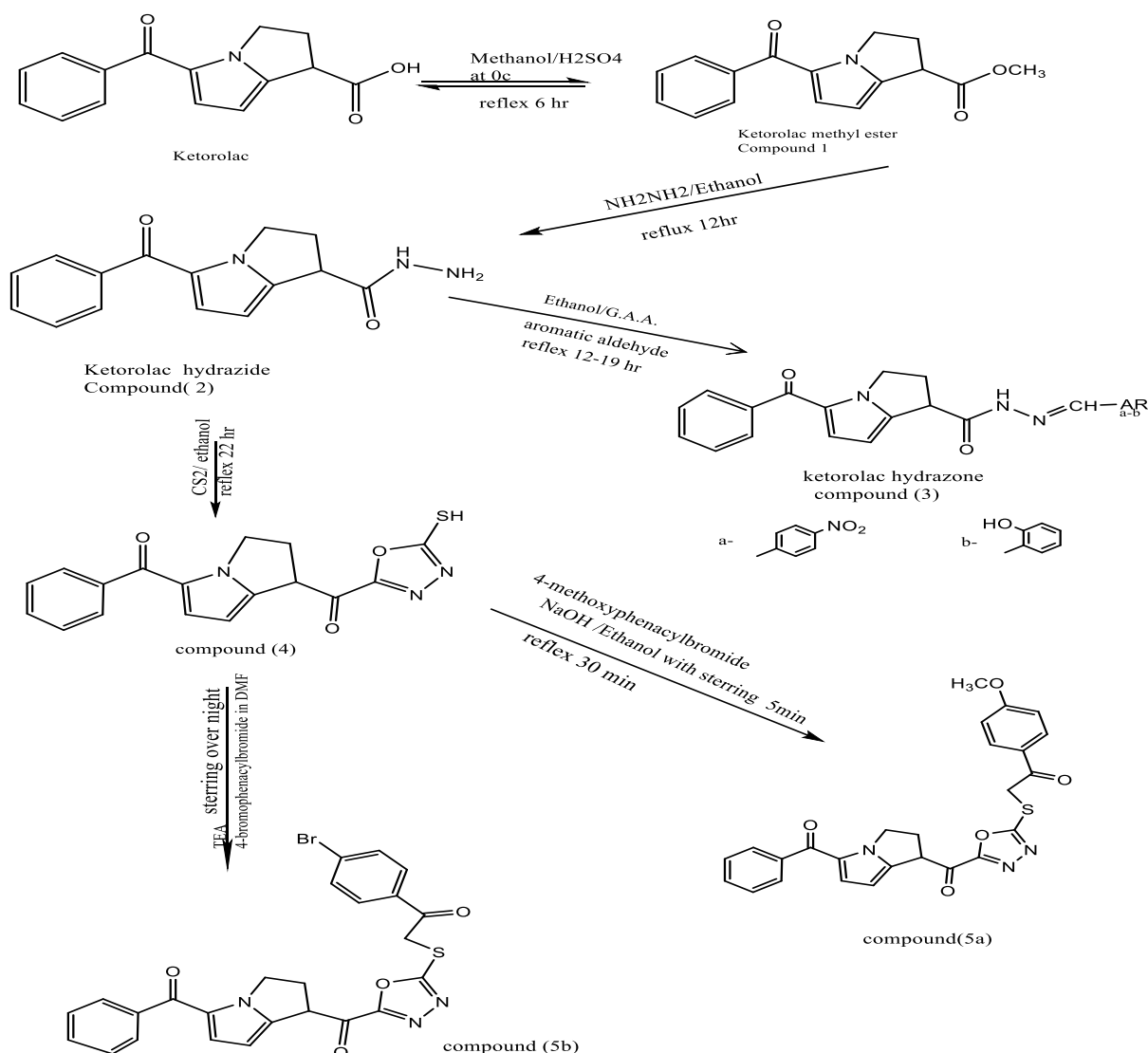


Figure 8. 1-(4-bromo-phenyl)-2-[[5-(2,3-dihydro-1pyrazolyl] sulfanyl] ethan-1-one (compound 5b)

Compound 4 (0.002 mole, 0.62 g) was added with equimolar of triethylamine (0.002mole ,0.2 ml) to 10 ml distill water with stirring until it dissolved then added 4-bromophenacylbromide dissolved in DMF slowly with continuous stirring overnight. The formed solid precipitate collects from solution, wash with cold water several times, and dried. Gray powder, yield = 75%, m.p. (159-160 °C). IR (ν cm⁻¹): 3059: Aromatic ring(C-H) Str., 2912: (C-H) Asym. Str. of CH₂ .2890: (C-H) Sym. Str. of CH₂.1678: (C=O) Str. of carbonyl.1585: (C=N) Str. of imine of oxadiazole.1485, 1573 (C=C) Str. of aromatic. ¹H NMR: 5.10 (2H, S, CH₂), 4.92 (1H, m, at C1 of pyrrolidine), 4.46 (2H, m, of CH₂ at C2 of pyrrolidine), 2.86,3.04 (2H, m, of CH₂ at C3 of pyrrolidine), 6.11,6.80(2H,d, of CH at C7 andC6 of pyrrole), 7.54-8.00 (9H,m,aromatic H).



Scheme 1. Synthesis of the titled compounds.

Preliminary pharmacological research

1-Antimicrobial Activity^(20,21)

By using the disc diffusion method, the target derivatives' anti-microbial efficacy was investigated *in vitro* against "gram-positive bacteria (*Staphylococcus aureus*), gram-negative bacteria (*E.Coil*), and fungus (*Candida albicans*). The bacterial suspension consisting of 10⁸ CFU/ml of bacteria and 10⁸ CFU/ml of yeasts were used to perform the assay. The procedure used for the assay was done by taking (100) μ L of suspension and spread over nutrient agar (NA). Gram-positive and Gram-negative bacteria were using Tryptone Soya Agar (TSA), whereas fungi were cultured using Sabouraud dextrose Agar (SDA). Synthesized compounds were dissolved in DMSO at a concentration of 1g/mL. Amoxicillin and Fluconazole served as the reference antibiotics, while DMSO (dimethyl sulfoxide) served as the control. At

the conclusion of a 24-hour period of incubation at 37°C, the inhibitory zones were measured in mm.

2. Evaluating anti-inflammatory activity⁽²²⁾

A study to evaluate anti-inflammatory activity was done, that tested the final synthesized compounds (3a-b,5a-b) *in vivo* by using egg-white-induced rat paw edema in order to compare them to ketorolac anti-inflammatory activity. The newly synthesized compounds were screened for their anti-inflammatory efficacy based on the reduction in paw thickness.

Laboratory animal albino rats (200 \pm 10gm) were obtained from the animal house of the Iraqi Center for Cancer and Medical Genetics Research and remained under standardized conditions for two weeks. The rats were divided into six groups (6 animals in each group). The first group is known as the control and was treated with solvent

(10% DMSO). The second group known as a reference and was treated with ketorolac dissolved in DMSO at 2mg/kg⁽²³⁾ and the rest was treated with synthesized compounds (3a-b and 5a-b) dissolved in DMSO. The given dose of ketorolac was 2mg/kg with an equivalent weight of the synthesized compounds. The following procedure was started by treating animals with compounds dissolved in DMSO intra-peritoneal at zero time, then after 30 min, inducing acute inflammation by subcutaneous injection of undiluted egg-white to the right of the paw of a hand and measuring the sign of inflammation which was the formation of edema at the site of subcutaneous injection by using Varina. All the results of the biological evaluation were expressed as mean \pm SEM which analyzed for statistical significance by using T-Test for mean value to compare between them, then compare between ketorolac and syntheses compounds in their ability to reduce edema using ANOVA (Two factors without Replication) was used to compare between different groups. P-value < 0.05 was assumed significant.

Results and Discussion

Chemistry

Scheme 1 illustrates the synthetic approaches used to create the final target compounds (1-5). In these, compound (1) ketorolac methyl ester was created by reacting the parent nucleus (ketorolac) with methanol in the presence of concentrated H₂SO₄ as a catalyst.

Compound (1) and hydrazine hydrate (NH₂NH₂.H₂O) were refluxed together with stirring to create compound (2), ketorolac hydrazide. Refluxing an ethanolic suspension of compound (2) with different aromatic aldehydes in the presence of drops of glacial acetic acid results in the formation of ketorolac hydrazone compounds (3a-b).

Refluxing an ethanolic suspension of compound (2) with CS₂ in the presence of ethanolic KOH results in the cyclization process, which is necessary for the synthesis of compound (4) having the 1, 3, 4-oxadiazole-2-thiol moiety. Compound (5) is S-alkylated with various alkyl halides (a-b) in the presence of ethanolic KOH and NaOH.

The following new synthesized compounds were characterized and identified by using the FTIR and ¹HNMR spectrum studies. The target's infrared spectrum Compounds (1-5) captured the distinctive absorption bands of the functional groups each molecule contains. The ketorolac methyl ester compound (1) revealed the existence of a

distinctive strong, sharp band at 1732 cm⁻¹, which is indicative of ester (C=O) stretching. The (C=O) stretching band of the ester at 1732 cm⁻¹ was vanished in the ketorolac hydrazide compound (2), and a new band that indicated the synthesis of the hydrazide amide (C=O) group was appeared at 1643 cm⁻¹. Additionally, two distinctive absorption bands at 3336 and 3298 cm⁻¹ were developed, these bands were caused by the primary amine N-H stretching of hydrazide (asymmetric and symmetric absorption bands). Due to (C=O) amide stretching, both aryl hydrazone derivatives (3a-b) displayed distinctive absorption bands at (1674) cm⁻¹ in their IR spectra. Along with the creation of new bands between 1600 and 1604 cm⁻¹ both caused by the imine's (C=N) stretching. Additionally, due to the (N-H) stretching of the amide, a second absorption band that emerged in the region of (3186-3213) cm⁻¹ also appeared. The asymmetric and symmetric stretching bands of NO₂, produced distinctive adsorption bands at 1523 and 1342 cm⁻¹, respectively for compound 3a.

The formation of 2-mercapto oxadiazole derivatives was supported by the appearance of two new bands at 1597 and 1054 cm⁻¹ assigned for (C=N) and (C=S) stretching, respectively, along with the disappearance of the (C=O) stretching band of the hydrazide compound (2), which was evidenced by the synthesis of the 1,3,4-oxadiazole-2-thiol compound (5). In general, the development of S-alkylated derivatives was verified by the FT-observation IRs of the (C=S) stretching band's absence. The development of the (C=O) stretching band at 1666 and 1678 cm⁻¹ for compound 5a and compound 5b. The suggested derivatives' structures were supported by the ¹HNMR spectra. Compound (1) showed a singlet peak for ester protons (OCH₃) at 3.7 ppm. One singlet peak at 3.88 ppm that was caused by the NH- NH₂ protons and two singlet peaks at 11.69, 11.53 ppm caused by NH-NH₂ protons, appeared in compound (2). Due to the exist of iminol form of hydrazide (compound 2), the molecule may exist in the form of *E* or *Z* isomers⁽²⁴⁾. The acyl hydrazones (3a-b) ¹HNMR spectra displayed the distinctive signals for hydrazone synthesis, which were indicated by the elimination of singlets for the NH-NH hydrazide protons at 3.88 and appear of two sets to two separated singlets which correspond for both the protons (-N=CH-), (-CONH-), (8.2-8.46) ppm and (11.9, 12.15) for compound 3a and (11.53, 12.03) ppm for compound 3b, respectively, that existed in this location. The presence of hydrazones as *E/Z*

geometric isomers around the C=N double bond may be the cause of the amide protons showing up as two singlets. The ^1H NMR spectra for chemical 4 revealed a singlet peak at (14.58) ppm that represents the (-SH) proton of thione with the absence of NH-NH₂ of hydrazide protons at 3.88 and 11.53 ppm that illustrating the ring configuration of 1,

3,4 oxadiazole. The singlet proton of thione at (14.58) ppm in the S-alkylated derivatives (5a-b ^1H NMR)'s spectra vanished, and a singlet peak attributable to the protons of methylene (S-CH₂) appeared at (5.10) ppm. The other protons all appeared in the expected areas.

Table 1. Antimicrobial potency of the generated compounds *in vitro* measured in mm.

Compound	Concentration 1000 $\mu\text{g/ml}$	<i>S.aureus</i>	<i>E.coil</i>	<i>Candida albicans</i>
3a	=	8	12	-
3b	=	9	6	-
5a	=	7	-	-
5b	=	10.03	8	-
Amoxicillin	=	49.2	-	-
Fluconazole	=	-	-	35
DMSO	-	-	-	-

(-) = No activity, slightly active (Inhibition zone in between 5-10 mm), moderately active (Inhibition zone in between 10-15 mm) and highly active (Inhibition zone more than 15 mm).

Table 2. Comparison between the effect of (ketorolac) reference and the (DMSO) control on paw edema thickness

Time min	Paw thickness in mm \pm SEM		
	Control (DMSO)	Reference (ketorolac)	<i>P-Value</i>
0	5.7133 \pm .07172	5.6950 \pm .04992	0.4190
30	6.4417 \pm .05833	6.3917 \pm .05833	0.2789
60	7.4583 \pm .06112	7.3333 \pm .06412	0.0942
120	8.9667 \pm .9804	8.6750 \pm .13889	0.0584
180	9.39 \pm .07895	9.1833 \pm .07491*	.0433
240	8.725 \pm .099	8.2167 \pm .1256*	0.005
300	7.791 \pm .097	6.667 \pm .100*	0.000057

Data is presented as the mean \pm SEM of paw thickness in mm. (*): Significant difference with respect to control group ($P < 0.05$). Ketorolac and dimethyl sulfoxide were injected intra-peritoneally at time (0). The infusion of undiluted egg white takes place at time (30). (Induction of paw edema).

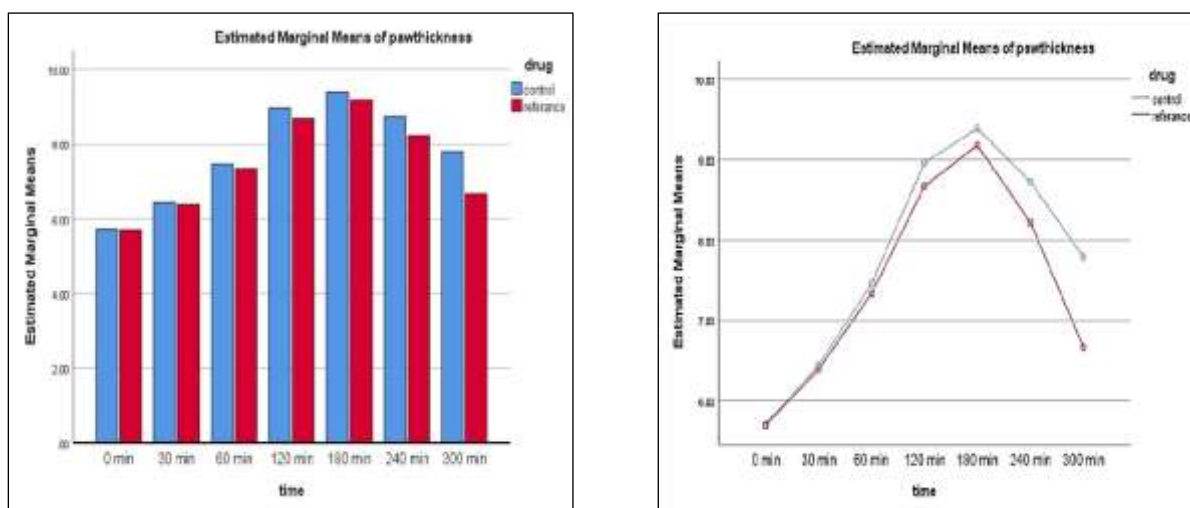


Figure 1. Effect of ketorolac (reference) and dimethyl sulfoxide (control) on egg-white induced paw edema in rats.

Table 3. Effect of control (DSMO), reference (Ketorolac) and synthesized compounds on paw edema induced by white egg.

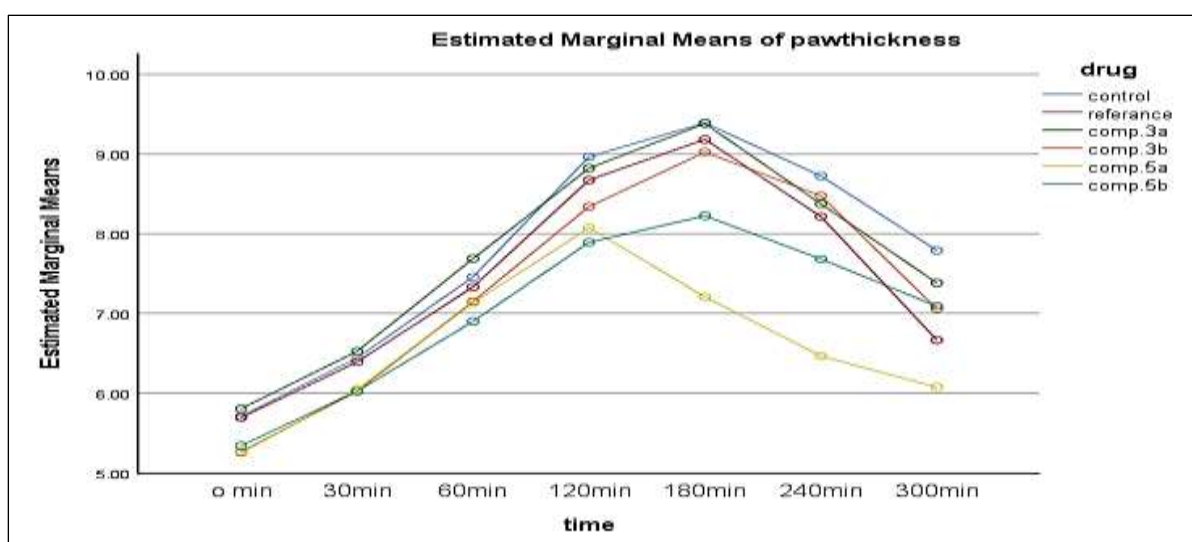
Paw thickness in mm							
Mean \pm SEM							
COM	0 min	30 min	60 min	120 min	180 min	240 min	300 min
Control	5.71 \pm 0.07	6.44 \pm 0.05	7.45 \pm 0.06	8.96 \pm 0.09	9.39 \pm 0.07	8.72 \pm 0.1	7.79 \pm 0.09
Standard	5.69 \pm 0.05	6.39 \pm 0.05	7.33 \pm 0.06	8.67 \pm 0.13	9.18 \pm 0.07*	8.37 \pm 0.12*	6.66 \pm 0.10*
3a	5.80 \pm 0.036	6.52 \pm 0.048	7.68 \pm 0.06	8.82 \pm 0.06	9.38 \pm 0.084	8.37 \pm 0.12*	6.66 \pm 0.10*
3b	5.26 \pm 0.036	6.02 \pm 0.048	7.15 \pm 0.102	8.34 \pm 0.11*	9.02 \pm 0.11*	8.47 \pm 0.20*	7.05 \pm 0.12*
5a	5.25 \pm 0.05	6.05 \pm 0.05	7.13 \pm 0.099*	8.07 \pm 0.53*	7.20 \pm 0.08*	6.46 \pm 0.07*	6.07 \pm 0.09*
5b	5.34 \pm 0.024	6.02 \pm 0.062	6.9 \pm 0.174	7.89 \pm 0.15*	8.22 \pm 0.15*	7.68 \pm 0.15*	7.09 \pm 0.12*

Paw thickness measured in mm and expressed as Mean \pm SEM

0 min is the time of IP. Inject animals with DMSO, ketorolac and synthesized compound.

30min is the time of SC injection with undiluted white – egg

(*): Significant difference with respect to control group (P<0.05).

**Figure 2. Effect of control (DMSO), reference (Ketorolac) and synthesized compounds on paw edema induced by white egg.**

Conclusion

The successful synthesis of new ketorolac derivatives was accomplished. ¹HNMR and ART-FT-IR spectroscopy were used to determine their chemical structure. The antimicrobial activity of these compounds was also evaluated, and all synthetic compounds do not affect *C.albicans*. They have mild to moderate activity against *E.Coil* and *S.aureus*. Additionally, using the egg-white-produced edema technique, the target compound's anti-inflammatory activity was also assessed. The synthetic compound (3b) exhibits effects that are comparable to standard at 120, 180, and 240 min, but less than the reference (ketorolac) at 300min, while compound (5b) exhibits significantly higher activity than standard in reduction paw thickness at 120, 180, and 240 min, but is less effective than standard at 300 min.

The molecule (5a) exhibits effects that are superior to ketorolac in decreasing rat paw edema at 120, 180, 240, and 300min.

Compound 3a showed a significant difference from control at 240 and 300 min in reduction paw thickness. *In vivo* anti-inflammatory studies exhibit that the synthesis of new derivatives of a non-steroidal anti-inflammatory ketorolac drug maintains its anti-inflammatory potency.

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Conflicts of Interest

The authors declare that there are no conflict-of-interest issues and the work has not received any external funding

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Ethics Statements

This study was approved by the Scientific and Ethical Committees of the Collage of Pharmacy / University of Baghdad.

Author Contribution

The first author :(Aliaa) contributed to the synthesis of final compounds, the analysis of IR and H1-NMR data , the discussion of anti-inflammatory and anti-bacterial activity, the drafting of the manuscript and critical revising of the manuscript. The second author (Muthanna) reviewed the results and approved the final version of the manuscript.

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