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Synthesis, Characterization and preliminary anti-microbial evaluation of new ketorolac hydrazone Derivatives

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Abstract

Non-steroidal anti-inflammatory medicines (NSAIDs) are a commonly used category of medications that show almost the same pharmacological activity. The key role of their pharmacological activity is to inhibit prostaglandin synthesis as they suppress cyclooxygenase enzymes. Although they share the same mechanism of action, they belong to different structural groups. Ketorolac is one of the NSAIDs, possessing powerful analgesic and anti-inflammatory activity but with severe side effects that limit its use for acute pain rather than chronic use for longer. The synthesis of a new series of ketorolac hydrazone derivatives was done starting from ketorolac powder through esterification, forming ketorolac ester compound (A), then reacting with hydrazine hydrate to give compound B (hydrazide), which finally binds to different types of aromatic aldehydes like dimethylaminebenzaldehyde, 3,4dimethylbenzaldehde, and others in ethanol with a sufficient amount of glacial acetic acid to produce ketorolac hydrazone. The synthesized compounds were identified using ARTFT-IR spectroscopy and^{1}HNMR analysis and then evaluated for their microbial activity. All the synthesized compounds gave mild to moderate antibacterial activity against E.coil and S.aureus .while all compounds had no antifungal activity against C.albicans .

Keywords

NSAIDs, COX inhibitors, ketorolac, Hydrazide, Hydrazone.

NSAIDs have antipyretic (reduce fever), analgesic (kill pain), and anti-inflammatory effects at higher doses [(1)]. NSAIDs exert their action through the inhibition of both, prostaglandin-endoperoxide synthase enzymes, which are known as COX1 and COX2[(2)(3).] While the inhibition of COX2 results in the basic therapeutic activity, concomitant inhibition of COX1 results in unwanted side effects such as gastrointestinal irritation, ulceration, and bleeding [(4)]. The enzyme COX2 is only expressed in inflammatory cells, while the other enzyme COX1 is in normal cells. Both enzymes are homodimers of mainly the same amino acids. Nonetheless, three changes to the amino acids in COX-2 result in a larger and more accessible channel. When an amino acid valine is replaced with a relatively large amino acid isoleucine (Ile) residue in COX-1 at the same site in the enzyme's active area, a structural change occurs at position 523 of COX-2. This change to the COX-2 enzyme makes accessing a new side pocket possible, which is necessary for COX-2 drug selectivity [(5)]. Therefore, synthesis of NSAID derivatives may enhance drug selectivity toward COX2 and thus, reduce adverse effects, which are a result of the free carboxylic group [(6)]. Furthermore, the adverse effect of the drug can be masked by binding the carboxylic group to another moiety, thus reducing side effects and improving activity [(7)]. Ketorolac (KT) is one of the potent

NSAIDs used to treat moderate to severe pain, but its use is limited to acute pain due to its severe side effects [8]. Ketorolac is a pyrrolizine derivative of carboxylic acid [9] [(±)-5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid], as shown in Figure 1. As a nonselective inhibitor of cyclooxygenase (COX), ketorolac inhibits both COX-1 and COX-2 [9,10]

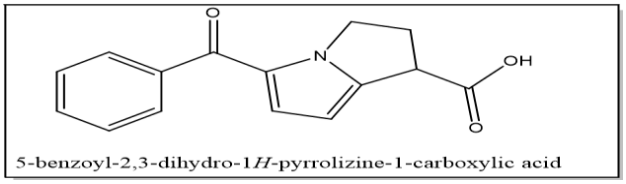


Figure (1) Structural formula of ketorolac

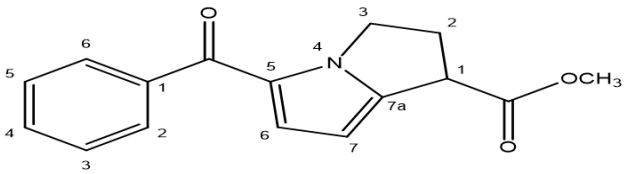
An azomethine (NH−N=CH−) linked to a carbonyl group produces Schiff base compounds. It may be produced by an acid-catalyzed reversible condensation reaction between primary amine and carbonyl molecules. [11,12]. It has also been shown to demonstrate a variety of biological functions, such as antifungal, antibacterial, antimalarial, anti-inflammatory, and antiviral activities [13]Recently, several attempts were made to synthesize and develop new non-antibiotic medications, such as Hydrazone and Hydrazide derivatives, in response to the rise in microbial resistance to routinely used drugs rapidly and effectively[14,15]. In this regard, new ketorolac derivatives having a hydrazone moiety were synthesized in an effort to create new ketorolac derivatives with enhanced activity and selectivity for COX-2, resulting in fewer GIT side effects.

Materials and Experimental Methods

ketorolac and different type of aldehyde were obtained from Company of Hyper chem. (China), Other chemicals and solvents, such as ethanol, ethyl acetate, glacial acetic acid, conc. H_2SO_4, hydrazine hydrate, and others, were purchased from private lenders and utilized without needing to be further purified. To confirm the reaction's completion and product purity, TLC technique was used. Electronic Melting Point Apparatus was used to measure melting points (Stuart SMP30). Using a ARTFT-IR Spectrophotometer, IR spectra were obtained.^{1}H-NMR spectra were acquired using a BRUKER model Ultra shield 300 MHz spectrophotometer and DMSO-d6 as the solvent.

Synthesis of ketorolac ester (methyl -5-benzoyl-2,3-dihydro -1H-pyrrolizine -1-carboxylate) (compound A)[16]

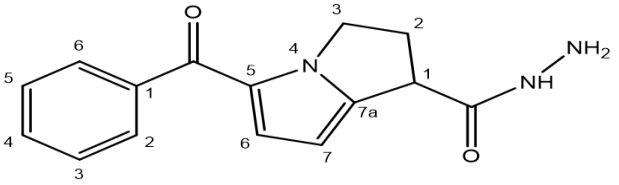
A weight of ketorolac powder approximately (6g , 0.021 moles) put in flask, then 20 ml of methanol was added to it with stirring to produce clear yellow solution. An ice bath was used to cool the obtained mixture, then drops of concentrated sulfuric acid (H_2SO_4) were added while stirring continuously. The mixture was then set on a reflux for 6 hr. with stirring. Following the conclusion of the reaction, which was checked using TLC , 75 mL of cold D.W. was added once The temperature of the combination was at room temperature. Then, the extra acid was neutralize by adding 5% w/v of saturated sodium bicarbonate solution to it. The precipitate was filtered, recovered, washed with D.W, dried, and then crystallized again from ethanol. An orange to reddish crystal of ketorolac methyl ester was produced , yield = 66%, M.P. (91C°). IR (υ cm-1): 3070: Aromatic (C-H) str. 2987, 2968: (C-H) asymm. str. of CH_3 and CH_2.2891, 2875: (C-H) symm. str. of CH_3 and CH_2, 1732: (C=O) str. of ester, 1180: (C-O-C) str. of ether.^{1}HNMR: (300 MHz, DMSOd6 □=ppm): 3.7 (3H, S, -CH3 of COOCH3), 2.77 (2H, m, -CH_2 at C2 of pyrrolidine), 4.23 (1H, d, -CH at C1 of pyrrolidine), 4.31-4.43 (2H, dd, -CH_2 at C3 pyrrolidine), 6.1- 6.78 (2H, d, at C6,C7 of pyrrole), 7.52 - 7.6 - 7.67 (5H, m, Aromatic H).



Synthesis of ketorolac hydrazide (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide) (compound B)[17]

A weight of ketorolac methyl ester (compound A) (0.006 moles,1.7g) added to absolute ethanol (35ml) mixing together until dissolved and become a clear dark orange solution then, A sufficient amount of approximately 0.06 moles (3 ml) of hydrazine hydrate was added, and the mixture was stirring over one night at room temperature until it transformed into a turbid yellow suspension (6 h), after which it was refluxed for 24 hr. The mixture was stirred at room temperature following the reflux time (r.t.). The mixture was then mixed with crushed ice water, creating an off-white precipitate that was left to stand overnight. The precipitate that resulted was filtered, repeatedly rinsed with cold D.W, dried, and reconstituted from ethanol. Faint yellow puffy powder, yield = 61.1%, m.p. (158-160 C°).). IR (υ cm-1): 3336, 3294: (NH) str. of hydrazide, 3062: Aromatic (C-H) str. 2987: (C-H) asymm. str. of CH_2.2888: (C-H) symm. str. of CH_2,1647: (C=O) str. of amide, 1604: (NH) bend .1527,1492,1466(C=C) str. of Aromatic. ^{1}H 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, of CH at C1 of pyrrolidine),4.4 (2H,m, of CH_2 at C3 of pyrrolidine),7(1H,d, of CH at C2 of pyrrole), 6.75(1H,d, C7 of pyrrole), 7.75-7.58 (5H,m,aromatic H) .



Synthesis of aryl hydrazones (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide) derivatives (k1 − k3)[18]

Each of the following aldehydes was dissolved in ethanol and then added a few drops of glacial acetic acid. (Scheme 1):

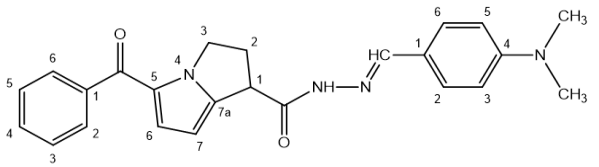
(1) [dimethylamine benzaldehyde (0.005 moles, 0.745 g)].

(2) [3,4 dimethyl benzaldehyde (0.005 moles, 0.674g)].

(3) [4-hydroxy-3,5-dimethoxybenzaldehyde(0.005moles,0.91g)]. Each of the mentioned aldehydes was put with absolute ethanol and a few drops of glacial acetic acid in a round-bottomed flask with magnetic stirrer combinations and added separately to a stirred solution of compound B (0.005 mol, 1.35g), which was dissolved in absolute ethanol (20 mL). Every reaction mixture was then refluxed for 12−18 hours. 50 mL of ice-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 refracted with ethanol as the solvent.

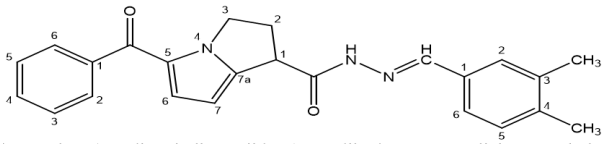
(k1)(E)-5-benzoyl-N-(4-(dimethylamino)benzylidene)-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide.

Yellow powder, Yield =77 %, m.p=230 (C°). IR (υ cm-№): 3271: (NH) str. of hydrazone, 3082: Aromatic (C-H) str.,2958,2920: (C-H) asymm. str. of CH_3 and CH_2. 2875,2860: (C-H) symm. str. of CH_3 and CH_2, 1666: (C=O) str. of amide.1600: (C=N) str., 1527,1492: Ar. (C=C) str.№HNMR: 2.28(6H,s, 2- CH_3 at N), 2.77,2.94 (2H, m, -CH_2 of C2 of pyrrolidine), 4.4 (2H,m, -CH_2 of C3 of pyrrolidine), 4.9 (1H, m, -CH at C1 of pyrrolidine),5.92 (1H, d, -CH at C6 of pyrrole), 6.75 (1H, d, CH at C7 of pyrrole), 7.21, 7.50-7.75 (9H, 3m, Ar-H), 8.02 (1H, ss, N=CH), 11.55, 11.72 (1H, ss, -CO-NH).



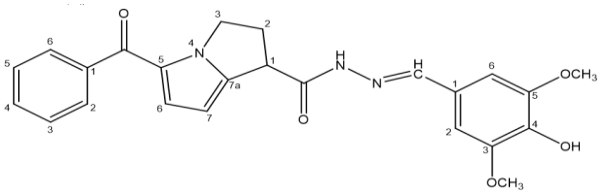
(k2)(E)-5-benzoyl-N-(3,4-dimethylbenzylidene)-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide.

Pale yellow powder, Yield=79%, m.p. (220-222 C°). IR (υ cm-№): 3249: NH str. of hydrazone, 3089: Aromatic (C-H) str., 2939: (C-H) asymm. str. of CH_3, 2866: (C-H) symm. str. of CH_3, 1660: (C=O) str. of amide, 1573: (C=N) str., 1527,1492,1454: Ar. (C=C) str.№H NMR: 2.28 (6H, S, -CH_3 at C3and C4), 2.8,2.94(2H, m, -CH_2at C2 of pyrrolidine), 4.44 (2H, m, CH_2atC3 of pyrrolidine), 4.92 (H, m, CH atC1 of pyrrolidine), 5.93(1H, d, -CH atC7 of pyrrole), 6.77 (1H, d, - CH at C6 of pyrrole), 7.25,7.46,7.54,7.63, 7.76 (9H, m, Ar-H), 8.04(1H, ss, N=CH), 11.59, 11.76 (1H, ss, -CO-NH).



(K3)(E)-5-benzoyl-N-(4-hydroxy-3,5-dimethoxybenzyllidene)-2,3-dihydro-1H-pyrrolidine-1-carbohydrazide.

powder, Yield=81%, m.p. (165-166 C°). IR (υ cm-№): 3568: (O-H) str.3213: NH str. of hydrazone, 3062: Aromatic (C-H) str. 1670: (C=O) str. of amide, 1585: (C=N) str., 1562,1512,1492: Ar. (C=C) str.№H NMR: 3.86 (6H, S, -CH_3 at C3and C4), 2.7,2.94(2H, m, -CH_3at C2 of pyrrolidine), 4.4,4.3 (2H, m, CH_2atC3 of pyrrolidine), 4.96 (H, m, CH atC1 of pyrrolidine), 6.11(1H, d, -CH atC7 of pyrrole), 6.7 (1H, d, - CH at C6 of pyrrole), 7.25,7.46,7.54,7.63, 7.76 (9H, m, Ar-H), 8.17(1H, ss, N=CH), 11.5, 11.7 (1H, ss, -CO-NH).



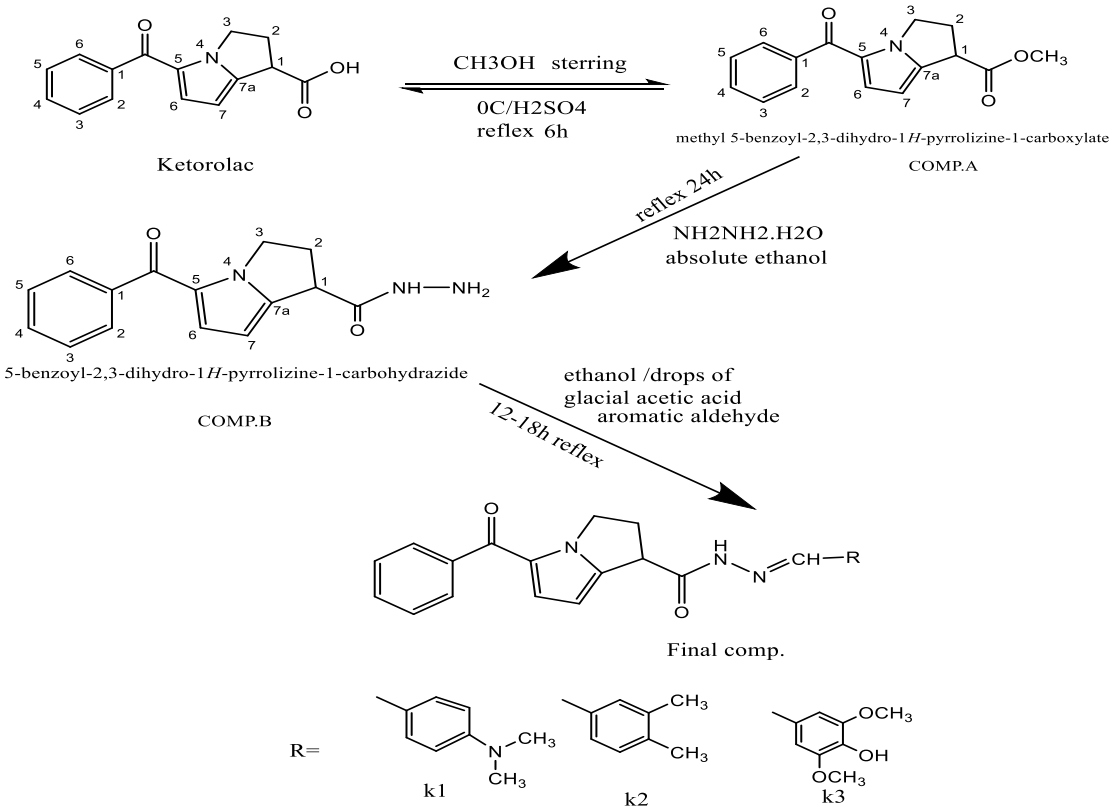
Results and Discussion

Chemistry

In Scheme 1, the general synthesis process for the ketorolac hydrazone derivatives (K1−K3) is outlined. By reacting ketorolac with methanol while using few drops of H_2SO_4, the chemical (A) known as ketorolac

methyl ester was created. Ketorolac methyl ester and hydrazine hydrate were combined to create compound (B). The final ketorolac hydrazone derivatives are created by reacting ketorolac hydrazide with various aromatic aldehydes while using ethanol and glacial acetic acid as catalysts.

FT-IR has been used to determine the structures of these compounds. The infrared absorption spectra of the derivatives were determined by the appearance of (C=O) carbonyl stretching of ester at 1732 cm-1 for compound (A), which then shifted nearly to (1647)cm-1due to amide formation and the appearance of two new bands of asymmetric and symmetric stretching of N-H (3336-3294)cm-1for hydrazide. The disappearance of the absorption bands for the amine group of compound (B) and the formation of a new absorption band between 1597 and 1600 cm-1 in the eventually generated compounds connected to the (C=N) group of imine. The1H-NMR spectra displayed signals about 11.51-11.76 ppm, 7.9-8.05 ppm, which were ascribed to N-H protons and N=CH.



Scheme (1) the synthesis of target compound(k1-k4)

Antimicrobial Activity [19]

By using the Disc Diffusion method ,the antimicrobial activity of the target derivatives was evaluated in vitro against "gram-positive bacteria (S.aureus), gram-negative bacteria (E.coil), and fungus" (C. albicans).The bacterial suspension consist of 10^8 CFU/ml of bacteria and 10^6CFU/ml of yeasts was using to perform the assay .the procedure used for assay was done by taken (100) µL of suspension and spread over nutrient ager (NA). Gram positive and Gram negative bacteria were cultured using (TSA), whereas fungi was cultured using Sabouraud dextrose ager (SDA). Compounds that were synthesised were dissolved in DMSO at a concentration of 1000mg/mL. Amoxicillin and Fluconazole served as the reference antibiotics, while DMSO served as the control and solvent. After 24 hours of incubation at 37°C, the inhibitory zones were measured in millimetres.

Table 1: the anti-microbial evaluation of target compounds (K1-K4)

Compound	S.aureus	E.coil	C.albicans
K1	12	7	-
K2	9	8	-
K3	9	4	-
Amoxicillin	45	-	-
Fluconazole	-	-	23
DMSO	-	-	-

Inhibition zone measured in mm, inhibition zone (0-5mm) = no active, (5-10mm) = slightly active, (10-

15mm)=moderate active , (more than 15mm)= high active.

Conclusion

Following the previously mentioned processes allowed for the effective synthesis of the proposed compounds in the current investigation. The results of this investigation indicated that the procedure employed to produce the derivatives was successful, since the conformity of the resulting compounds was assessed using data from physical and chemical analyses, which included spectroscopic analysis (TLC, melting point, FTIR, and¹HNMR). These compounds possess low to moderate antimicrobial activity, equivalent to commercially available substances..

In general all tested compounds showed a slightly to moderate activity against gram positive and gram negative bacteria unlike the parent compound ketorolac. These tested compounds exert significant antibacterial activity in comparison to DMSO as control group. In comparison to standard compound (amoxcillin), tested compound exerted lower effect against Staphylococcus aureus and E coil.

The antibacterial activity shows that the compounds K1 has a moderate activity against S. aureus and slighly activity against E. coli. And K2 gave also a slightly activity against S. aureus and E. coli. The K3 gave slight activity against S. aureus only.

The final compounds were examined to determine their antifungal activity using the well diffusion method. clotrimazole, a standard antifungal drug, was utilized, and DMSO was selected as a solvent and control.

As shown in the above table, all synthesized derivatives have no effect against Candida albicans based on the size of inhibition zone of the reference drug .

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