RESEARCH ARTICLE

Synthesis and Antimicrobial Evaluation of Sulfonylhydrazide Derivatives of Etodolac

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ABSTRACT

A new series of Etodolac derivatives, aryl sulfonyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetohydrazide derivatives (3a,3b and 3c) have been synthesized from etodolac hydrazide by using deferent sulfonyl chlorides in dichloromethane and triethyl amine. The anti-microbial effect of the new derivatives has been assessed in vitro against gram-negative, gram-positive bacteria and fungi activity. Compound (3c) showed the highest anti-microbial activities against *Klebsiella pneumoniae* and *Candida albicans* compared to other compounds while compounds (3a) and (3b) were more effective against Gram-positive bacteria than gram-negative bacteria. The structures of all final target sulfonyl-hydrazide derivatives gwere successfully synthesized and confirmed based on their spectral and analytical data. Target compounds were identified and characterized by their melting point, Thin Layer Chromatography, attenuated total reflectance-Fourier transform infrared (ATR-FTIR), and ^{1}H NMR.

Keywords: Anti-microbial activity, Etodolac, Hydrazine hydrate, Sulfonyl chlorides.

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INTRODUCTION

There is still an increasing need for a synthesis of compounds that show promising activity as therapeutic agents with lower toxicity. However, the increasing resistance of pathogens towards the available antibiotics is still a most important cause of death. Sulfonamides are a class of drugs that include the sulfonamide chemical functional group (R-SO2-NH2).[1,2] They are a member of the earliest anti-microbial class of chemicals with broad anti-microbial activity and are efficient against pathogenic strains of gram-negative and gram-positive bacteria. They are used to treat a wide range of bacterial infections, like respiratory tract infections, skin infections, malaria etc.[3] Sulfonamides also have a wide range of pharmacological activities, like antiviral, antioxidant, anticarbonic anhydrase, diuretic, hypoglycemic, antithyroid, anti-inflammatory, antiglaucoma, antineoplastic, etc.[4,5] Etodolac (ET) is a member of a large family of the non-steroidal anti-inflammatory drugs (NSAIDs) with anti-inflammatory, antipyretic, and analgesic properties that have been approved by the food and drug administration in January 1991.[6,7] In general, NSAIDs show a wide range of anti-microbial trend. However, they also exert a potent decrease in adherence, biofilm formation, and other pathogenicity factors in addition to the capacity to elevate or reduce antibiotic susceptibility. Changes in an NSAID structure might cause an increment in its antibacterial action and further investigations could lead efficient anti-microbial compounds.[8] ET is an inhibitor of cyclooxygenase-2 NSAID, a drug category that has previously proved anti-microbial and anti-cancer activities in previous studies.[9-11] In the present work we synthesized Etodolac sulfonylhydrazide derivatives through reaction of Etodolac hydrazide with three different sulfonyl chlorides to obtain the final compounds. The reaction pathway was illustrated in Scheme l.

MATERIALS AND METHODS

All the chemicals utilized in the synthesis were of the highest analytical quality. The melting points of the final synthesized derivatives were determined (uncorrected). All melting points reported in this article were determined using the Thomas Hoover Apparatus. The aluminum precoated silica gel 60 F254 sheets (Merck) were utilized for thin layer chromatography (TLC) and R_f values of the intermediates and final products which showed single round spots appeared after exposing the chromatograms to iodine vapor indicating the purity and the completion of the reactions. Infrared spectra were determined in a KBr disc using a Shimadzu fourier transform infrared (FTIR) spectrophotometer. ^{1}HNMR spectra were performed on BRUKER model Ultrashield spectrophotometer (300 MHz) and DMSO-d6 was used as a solvent.

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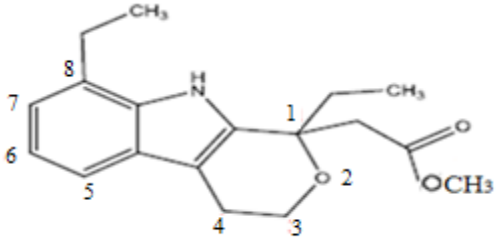
Synthesis of etodolac methyl ester [methyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-b] indol-1-yl) acetate] (compound l)[12]

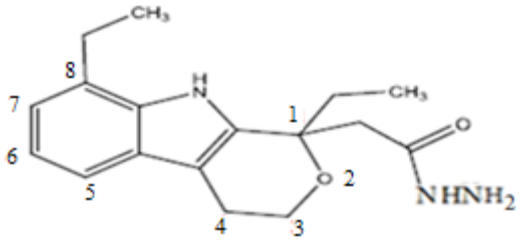
A mixture of ET (0.03 moles, 8.62g) and methanol (60 mL) was stirred in a 250 mL round bottom flask till the clear solution was achieved. The resultant solution was cooled to 0°C using an ice bath, and with continuous stirring, 4.5 mL of concentrated H_2SO_4 was added drop by drop; the mixture was refluxed at 70°C for five hours with magnetic stirring. The reaction's progress was checked by the TLC and a mixture of petroleum ether: acetone (l:l) was used as an eluent, the resultant mixture was allowed to cool to room temperature, then it was thrown over 90 mL of cold distilled water, after that, a 5% sodium bicarbonate solution ($NaHCO_3$) was added to neutralize the excess acid. A yellowish precipitate (PPT) of ET methyl ester was produced. The PPT was obtained via filtration, then washed with chilled distilled water and dried. After that, it was recrystallized from ethanol. Yellowish powder, yield = 65 %, m.p. (128–129°C). Rf = 0.78, IR (KBr disc), (v cm^{-1}): 3379 (NH) str. indole, 3060 Aromatic (C-H) str., 2967 (C-H) asymm. str. of CH3 and CH2, 2875 (C-H) symm. str. of CH_3 and CH_2, 1719: (C=O) str. ester, 1238 (C-O-C) str. ether. ^{1}H NMR: (DMSOd6; 300 MHz; δ=ppm): 0.62 (3H, t, -of CH_2-CH_3 at carbon 1), 1.26 (3H, t, of -CH_2-CH_3 at carbon 8), 1.9 (2H, q, of -CH_2-CH_3 at carbon 1), 2.5-3.04 (6H, m, of -CH_2-CH_3 at carbon 8, -CH2-$COOCH_3$ at carbon 1and -CH_2 at carbon 4), 3.56 (3H, s, of -$COOCH_3$), 3.80 (2H, dd, of -CH_2 at carbon 3), 6.79- 7.00 (2H, m, Ar-H5, H6), 7.23 (1H, d, Ar-H7), 10.48 (s, 1H, Indole, N-H).



(1)

Synthesis of ET hydrazide (2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b] indol-1yl) aceto-hydrazide) (compound 2)[13]

A solution of compound l (0.02 moles, 6 g) in 70 mL of absolute ethanol was mixed with hydrazine hydrate 98% (0.04 moles, 2 mL), then, the obtained mixture was refluxed at 80°C for 6 hours. At the end of the reflux time, the mixture was left to be cooled down to room temperature, then cold distilled water was added to the mixture, a white precipitate was formed which was left overnight. The obtained white PPT was filtered, rinsed with cold distilled water several times, left to dry, and recrystallized from ethanol. Yield 86%, m.p. (187–189°C). R_f = 0.40 (Acetone : Petroleum ether (l:l). IR (KBr disc), (v cm^{-1}): 3354, 3315 (N-H) str. indole and hydrazide, 3060 (CH) str. of aromatic ring, 2970 (C-H) asymm. str. of CH_3 and CH_2, 2875 (C-H) symm. str. of CH_3, and CH_2,1655 (C=O) str. amide, 1620 (NH) bend., 1240 (C-O-C) str.of ether. ^{1}H NMR: 0.61 (3H, t,of-CH2-CH3 at carbon1), 1.25 (3H, t, of-CH2-CH3 at carbon8), 2.04 (2H, q,of-CH2-CH3 at carbon1), 2.61-2.9 (6H, m, CH2-CONHNH2, -CH2-CH3 at carbon8, and -CH2 at carbon4), 3.95 (2H, dd,-CH2 at carnon3), 4.25 (2H, bs, NH-NH2), 6.80-6.98 (2H, m,Ar-H5, H6), 7.22 (d, 1H, Ar-H7), 8.92 (s,1H, NH-NH2), 10.54 (s, 1H, Indole N-H).



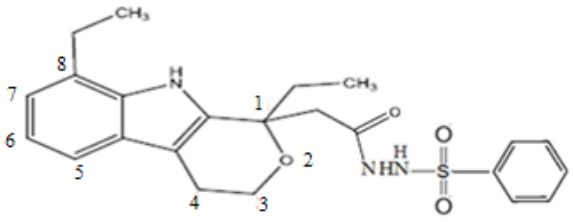
(2)

Synthesis of aryl Sulfonyl (2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b] indol-1yl) aceto-hydrazide derivatives (compound 3a,3b and 3c)[14]

Compound 2 (0.65 g, 0.004 mole) in 20 mL dichloromethane with benzene-sulfonyl chloride (0.7g, 0.004 mol), p- toluenesulfonyl chloride (0.76g, 0.004mol), and 4-chloro-benzenesulfonyl chloride (0.84 g, 0.004 mol) respectively in the presence of triethylamine (0.01mol,1.4mL) were stirred overnight at room temperature. In a separatory funnel, the mixture was emptied and rinsed with 100 mL distilled water. The organic layer was dried in the presence of anhydrous sodium sulfate, and a rotary evaporator was used to remove the solvent, to give products.

(3a) N'-(2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b] indol-1-yl) acetyl) benzenesulfonohydrazide

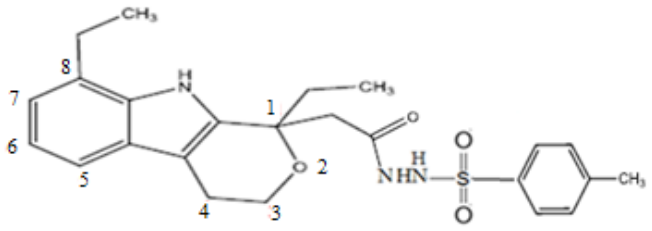
Yellowish-white powder, Yield = 61%, m.p. (190-192 °C). Rf = 0.60 (Ethyl acetate 4: n-Hexane 6). IR (KBr disc), (v cm^{-1}): 3383, 3271 (NH) str.of indole and amide, 3059 Aromatic (C-H) str., 2935 (C-H) asymm. str. of CH3 and CH2, 2877 (CH) symm. str. of CH3 and CH2, 1670 (C=O) str. of amide, 1556-1446 Ar. (C=C) str., 1335asym., 1165sym. of (S=O) str, 1215 (C-O-C) str.of ether. ^{1}H NMR: 0.68 (t,3H,of-CH2-CH3 at carbon1), 1.28 (3H, t,of-CH_2-CH_3 at carbon8), 2.15 (2H, q,of-CH2-CH3 at carbon1), 2.66 (2H, q,of-CH2-CH3 at carbon8), 2.77-3.03 (4H, m, of-CH2CONH at carbon1, and -CH_2 at carbon4), 4.00 (dd,2H, -CH_2 at carbon3), 6.83-7.94 (8H, m, ofAr-H and Ar-H), 9.81 (1H, bs, -CO-NH), 10.08, (1H, bs, -SO_2-NH),10.49 (1H, s, Indole N-H).



(3a)

(3b) N'-(2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b] indol-1-yl) acetyl)-4-methylbenzene-sulfonohydrazide

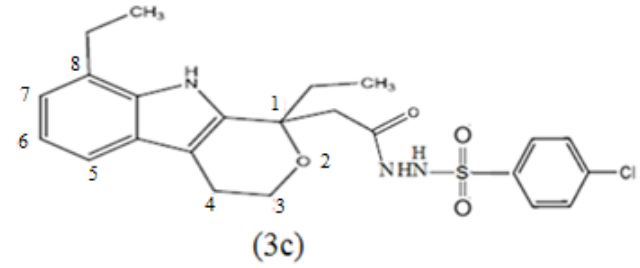
Off-white powder, Yield = 59%, m.p. (205-207 ºC). Rf = 0.62 (Ethyl acetate 4: n-Hexane 6). IR (KBr disc), (v cm^{-1}): 3390, 3294: (NH) str.of indole and amide, 3055: (C-H) str. of aromatic ring, 2967 (C-H) asymm. str. of CH3 and CH2, 2850 (CH) symm. str. of CH_3 and CH_2, 1666: (C=O) str. of amide, 1597-1450 Ar. (C=C) str., 1338 asym., 1165sym. (S=O) str., 1230 (C-O-C) str. of ether. ^{1}H NMR: 0.68 (3H, t,-CH_2-CH_3 at carbon1), 1.25 (3H, t,of-CH_2-CH_3 at carbon8), 2.11 (2H, q,of-CH_2-CH_3 at carbon1), 2.22 (s, 3H, p-CH_3), 2.71 (2H, q,-CH_2-CH_3 at carbon$_8$), 2.82-3.02 (4H, m,-CH_2CONH at carbon1, and -CH_2 at carbon4), 4.01 (2H, dd,-CH_2 at carbon$_3$), 6.86-7.93 (m,7H,of Ar-H and Ar-H$^-$), 9.82 (1H, bs, -CO-NH), 10.04, (l H, bs, - SO_2-NH),10.51 (l H, s, Indole N-H).



(3b)

(3c) 4-chloro-N'-(2-(1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-b]indol-1-yl) acetyl) benzene-sulfonohydrazide

Fait yellow powder, Yield = 66%, m.p. (217-219 ºC). Rf = 0.68 (Ethyl acetate 4: n-Hexane 6). IR (KBr disc), (v cm^{-1}) 3321, 3259: (NH) str.of indole and amide, 3047: (C-H) str.of aromatic ring, 2958 (C-H) asymm. str.of CH_3 and CH_2, 2870 (CH) symm. str. of CH_3 and CH_2, 1639 (C=O) str. amide, 1585-1465 Ar. (C=C) str., 1354 asym., 1142 sym. (S=O) str., 1220 (C-O-C) str.of ether. ^{1}H NMR: 0.67 (3H, t,of-CH2-CH3 at carbon1), 1.25 (3H, t,of-CH_2-CH_3 at carbon8), 2.12 (2H, q,of-CH_2-CH_3 at carbon1), 2.63 (2H, q,-CH_2-CH_3 at carbon8), 2.79-2.92 (4H, m, -CH2CONH at carbon1, and -CH_2 at carbon4), 3.99 (2H, dd,-CH_2 at carbon$_3$), 6.84-7.95 (m,7H, Ar-H and Ar-H$^-$), 9.91 (1H, bs, -CO-NH), 10.24, (1H, bs, - SO2-NH),10.49 (1H, s, Indole N-H).



(3c)

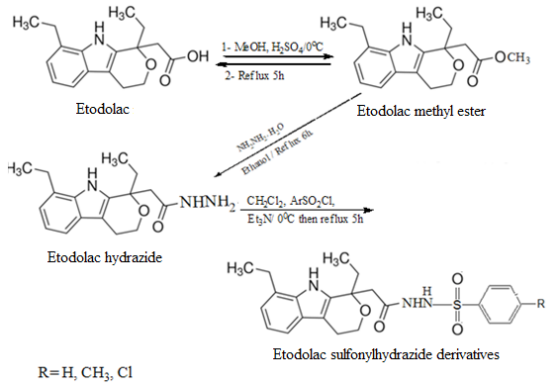
Antimicrobial Activity[15]

The target derivatives were studied for their anti-microbial activity against "gram-positive bacteria (*Staphylococcus aureus*, *beta-hemolytic-Streptococcus pyogenes*), gram-negative bacteria (*klebsiella pneumonia)* & fungi (*Candida albicans*)" by disc diffusion method. Bacterial culture medium was nutrient agar; blood agar was used for *S. pyogenes*, while Sabouraud dextrose was used for the fungal growth agar medium. DMSO was used to dissolve final compounds at concentration of 100 µg/mL. The reference antibiotics were Ciprofloxacin and Miconozole, whereas the control was DMSO. The inhibition zones were detected at the end of an incubation period of 24 hours at 35°C for bacteria and 5 days at 28°C for Fungi.

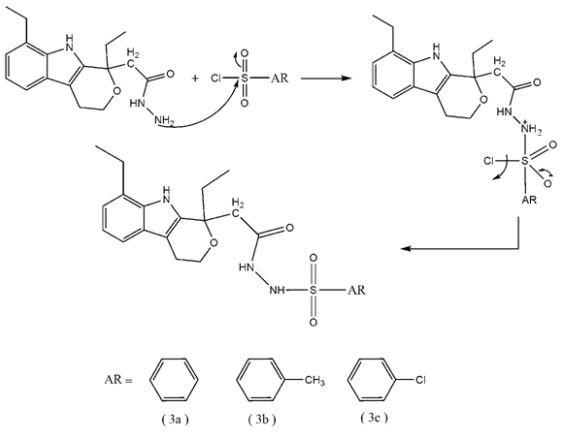
RESULTS AND DISCUSSION

Chemistry

The general synthetic procedure of the Etodolac sulfonyl-hydrazide derivatives (3a, 3b and 3c) is summarized in Scheme 1. Etodolac methyl ester compound (1) was prepared by reacting of Etodolac with methanol with the aid of concentrated H_2SO_4. Compound (2) was synthesized by the reaction of Etodolac methyl ester with hydrazine hydrate. The synthesis of the final Etodolac sulfonyl-hydrazide derivatives includes the reaction of Etodolac hydrazide with benzenesulfonyl chloride, para-toluenesulfonyl chloride & 4-chlorobenzene-sulfonyl chloride, respectively, in dichloromethane and triethylamine as a base. The final reaction is initiated by the amine's nucleophilic attack on the sulfonylchloride's sulfur atom, resulting in the liberation of HCl, as shown in the (Scheme 2).



Scheme 1: The Synthesis of target compounds (3a, 3b, and 3c)



Scheme 2: Mechanism of sulfonamide formation

Table 1: Anti-microbial testing data (inhibition zone in mm) for final compounds

		S. aureus	*S. pyogenes*	*K. pneumonia*	*Candida albicans*
Compound NO	*Conc. µg/mL*	*Zone of inhibition (mm)*			
3a	10^2	12	10	9	10
3b	10^2	12	11	8	11
3c	10^2	10	9	11	13
Ciprfloxacin	10^2	22	23	20	
Miconazole	10^2				22

The structures of these derivatives have been confirmed by infrared absorption spectra by the disappearance of asymmetric and symmetric absorption bands for amine group of compound (2) and the appearance of new absorption peak in the final prepared compounds between (3390–3259) cm^{-1} belong to (NH) group of indole and amide. The presence of (C=O) stretching of amides was confirmed by the peaks at the range the peaks confirmed 1670–1639 cm^{-1} as well as the appearance of sulfone group at the range of 1350–1335 cm^{-1}. The ^{1}H-NMR spectra showed signals in the regions 6.84–7.95 ppm and 9.81–10.50 ppm attributed to aromatic and NH protons.

Anti-microbial Activity

The newly synthesized derivatives (3a, 3b, and 3d) were tested for their anti-microbial activity. From the result in table 1, Compounds 3a and 3b had a moderate level of activity against both *S. aureus* and *S. pyogenes* and low activity against *K. pneumonia*, while compound 3c demonstrated good activity against *K. pneumonia* and a low level of activity against *S. aureus* and *S. pyogenes* at concentration 100 µg/mL when compared to Ciprofloxacin. All compounds displayed a moderate level of activity against *Candida albicans* when compared to Miconazole.

CONCLUSION

The synthesis of these proposed compounds was successfully achieved by following the stated procedures as previously described. The results obtained from this investigation indicated that the strategy adopted for the synthesis of the designed derivatives was successful since the conformity of synthesized compounds was obtained based on data from physical and chemical analyses, which included (TLC, melting point, FTIR, and ^{1}HMNR). These compounds show good anti-microbial activity comparable with marketable compounds.

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