SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION FOR ESTER DERIVATIVES FROM SOME NSAIDS DRUGS

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Abstract: *The current research involves the synthesis of some NSAID derivatives and evaluating their antimicrobial and antioxidant activity. Briefly, some NSAID compounds were converted into ester derivatives using the Fischer esterification method. The synthesized compounds were spectrometrically characterized via modern methods FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra. Antibacterial results showed good activity for Z1, Z2, and Z5 derivatives toward Escherichia coli compared to Ciprofloxacin. Also, compounds Z2 and Z4 exhibited good inhibition of Candida albicans compared to Fluconazole. In addition, the results of the DPPH investigation revealed good antioxidant activity for derivatives Z1 and Z5, with inhibition percentages of 91.22 and 86.51%, respectively, compared to ascorbic acid. Certainly, the results obtained have reinforced the importance of the synthesized compounds.*
Keywords: *NSAIDs drugs, Antioxidant, Fischer-esterification, Antimicrobial*

Introduction

Heterocyclic compounds are one of the important classes in the pharmaceutical industry, as they are used in many antibiotics, such as anti-cancer, antioxidant, anti-memory, antiviral, and many other diseases [1-8]. Researchers have shown great interest in studying derivatives prepared from heterocyclic compounds, and their biological effectiveness has been studied. These derivatives have raised interest in the medical field, as they are used in many medical drugs available in local markets and recognized by the World Health Organization [9]. Among these compounds are Non-steroidal drugs (NSAIDs), such as (Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, and Oxaprozin), used extensively to treat various conditions such as postoperative surgical conditions, chronic pain, rheumatoid arthritis, menstrual cramps, and osteoarthritis, and also used as analgesics and antipyretics [10-13]. At present, NSAIDs are among the most popular over-the-counter drugs across the world, constituting 5% of all the prescribed medicines [14-16]. Numerous *in vitro* studies have revealed that some common NSAID derivatives exhibit diverse biological activities such as anticancer [17], antimicrobial [18], anti-HIV [19], anti-Alzheimer [20], and other activities [21-25]. In the present work, we synthesized a new series of ester derivatives boosted with NSAIDs drugs and evaluated their biological activity.

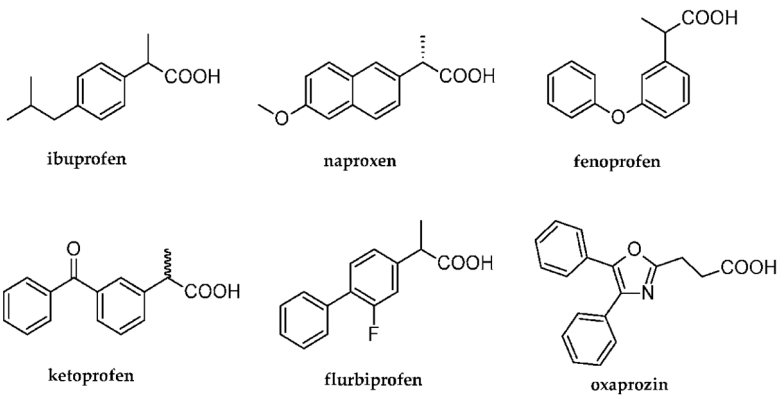


Fig. 1. Structures of non-steroidal anti-inflammatory drugs (NSAIDs)

Experimental part

The chemicals and solvents used in this study were purchased from chemical companies (Sigma-Aldrich and TCI). FT-IR spectra were recorded with an FT-IR spectrophotometer (BRUKER) and (Shimadzu). ^{1}H-NMR and ^{13}C-NMR measurements were measured on Bruker AMX instruments (400 and 100 MHz) using DMSO-d6 as a solvent and TMS as a reference. Analytical TLC was carried out on 60 F254 plates (0.2mm thick). Melting points were determined on an SMP device (Gallenkamp) and remain uncorrected.

Synthesis. ***General Procedure for the Preparation of compounds [Z1-Z5]***

In a 100 ml round-bottom flask with three necks put on a heater with a magnetic stirrer, condenser, and thermometer, 5 mmol of carboxylic acids Y1-5 were individually dissolved in 25 ml of 1-propanol with an addition of 0.5 ml of concentrated sulfuric acid. After that, the reaction mixture was refluxed for 10-14 h (check by TLC). Next, the reaction mixture was cooled to 25°C, washed with brine solution, and then extracted with chloroform. After the extraction process, the organic extract was dried and then concentrated under a vacuum to get the solid. The products obtained were purified with flash chromatography using methanol–dichloromethane (8:2).

Propyl 4-(1H-indol-3-yl)butanoate Z1. White powder; yield 71%; m.p. 190-192°C. FT-IR (KBr, cm-1): ν 3386 (N-H), 3054 (C-H), 1720 (C=O), 1564 (C=C). 1H-NMR (DMSO-d6, ppm): δ 1.17 (t, 1.17(t, 3H, CH3), 1.42 (t, 2H, CH2), 1.88-1.95 (m, 2H, CH2), 2.33 (t, 2.33(t, 2H, CH2), 2.70 (t, 2H, CH2), 4.05 (t, 4.05 (t, 2H, CH2), 6.92-7.53 (H-aromatic), 10.94 (s, 1H, NH). 13C NMR (DMSO-d6, ppm): δ 14.59(CH3), 24.98(CH2), 25.88(CH2), 27.72(CH2), 33.68(CH2-CO), 60.61(OCH2), 111.72-136.77 (C-aromatic), 173.44(C=O).

Propyl 2-(6-methoxynaphthalen-2-yl)propanoate Z2. Pale yellow powder; yield 79%; m.p. 175-177°C. FT-IR (KBr, cm-1): ν 3055 (C-H), 1724 (C=O), 1517 (C=C). ^{1}H-NMR (DMSO-d6, ppm): δ 1.02(t,3H,CH3), 1.48(d,3H,CH3), 1.80-189 (m,2H,CH2), 3.95(q,1H,CH), 3.87(s,3H,CH3), 4.01(t,2H,OCH2), 7.14-7.82 (H-aromatic). ^{13}C-NMR (DMSO-d6, ppm): δ 10.85 (CH3), 18.84 (CH3), 21.94 (CH2), 44.95 (CH), 55.61 (CH2-O), 65.83 (O-CH3), 152.65-157.65 (C-aromatic), 174.43 (C=O).

Propyl 2-(4-isobutylphenyl)propanoate Z3. Off-white powder; yield 81%; m.p. 161-163°C. FT-IR (KBr, cm−1): ν 3061 (C-H), 1722(C=O), 1585(C=C). ^{1}H-NMR (DMSO-d6, ppm): δ 0.85 (d, 3H, 2CH3), 1.03 (t, 3H, CH3), 1.38 (d, 3H, CH3), 1.48-1.56 (m, 2H, CH2), 1.71-1.82 (m, 1H, CH), 2.41 (d, 2H, CH2), 3.74 (q, 1H, CH), 3.96 (t, 2H, CH2), 7.10, 7.19 (H-aromatic). ^{13}C-NMR (DMSO-d6, ppm): δ 10.55, 18.87, 21.93 (3CH3), 22.60 (CH2), 30.08 (CH-C=O), 44.65 (CH2-Ph), 65.99 (OCH2), 127.50-140.21 (C-aromatic), 174.43 (C=O).

Propyl 3-(4,5-diphenyloxazol-2-yl)propanoate Z4. Brown powder; yield 75%; m.p. 184-186 °C. FT-IR (KBr, cm^{-1}): ν 2924 (C-H), 1725 (C=O), 1645 (C=N), 1575 (C=C). ^{1}H-NMR (DMSO-*d6*, ppm): δ 0.85 (t, 3H, CH_3), 1.75-1.83 (m, 2H, CH_2), 2.87 (t, 2H, CH_2), 3.11 (t, 2H, CH_2), 4.01 (t, 2H, CH_2), 7.23-7.77 (H-aromatic). ^{13}C-NMR (DMSO-*d6*, ppm): δ 10.67 (CH_3), 21.99 (CH_2), 23.37 (CH_2-C=O), 30.68 (C-CH_2), 66.04 (O-CH_2), 126.77-162.62 (C-aromatic), 172.18 (C=O).

propyl 3-phenoxybenzoate Z5. Yellow powder; yield 76%; m.p. 172-174 °C. FT-IR (KBr, cm^{-1}): ν, 3054 (C-H), 1728 (C=O), 1619 (C=C). ^{1}H-NMR (DMSO-*d6*, ppm): δ 0.92 (t, 3H, CH_3), 1.64-1.72 (m, 2H, CH_2), 3.85 (t, 2H, CH_2), 6.96-8.65 (H-aromatic). ^{13}C-NMR (DMSO-*d6*, ppm): δ 10.25 (CH_3), 20.45 (CH_2), 55.88 (CH_2-O), 114.89-163.54 (C-aromatic), 175.71 (C=O).

Antifungal and antibacterial activity. *Escherichia coli* and *Candida albicans* were selected to investigate the antimicrobial activity of the synthesized compounds using the well-diffusion method. The culture medium (Muller-Hinton medium) was prepared according to the supplier's instructions. After solidifying the medium in the plates, five wells with a diameter of 5 mm were created using a cork drill with leaving suitable space between wells. Next, the bacterial suspension (0.1 mL) was transferred and evenly spread on the nutrient agar surface using a sterile cotton swab. After 30 minutes, 0.1 mL of the standard and study compounds with concentrations of 100 μM and 250 μM were individually added to each well in separate plates. The plates were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 days (fungi), zone of inhibition was measured in mm.

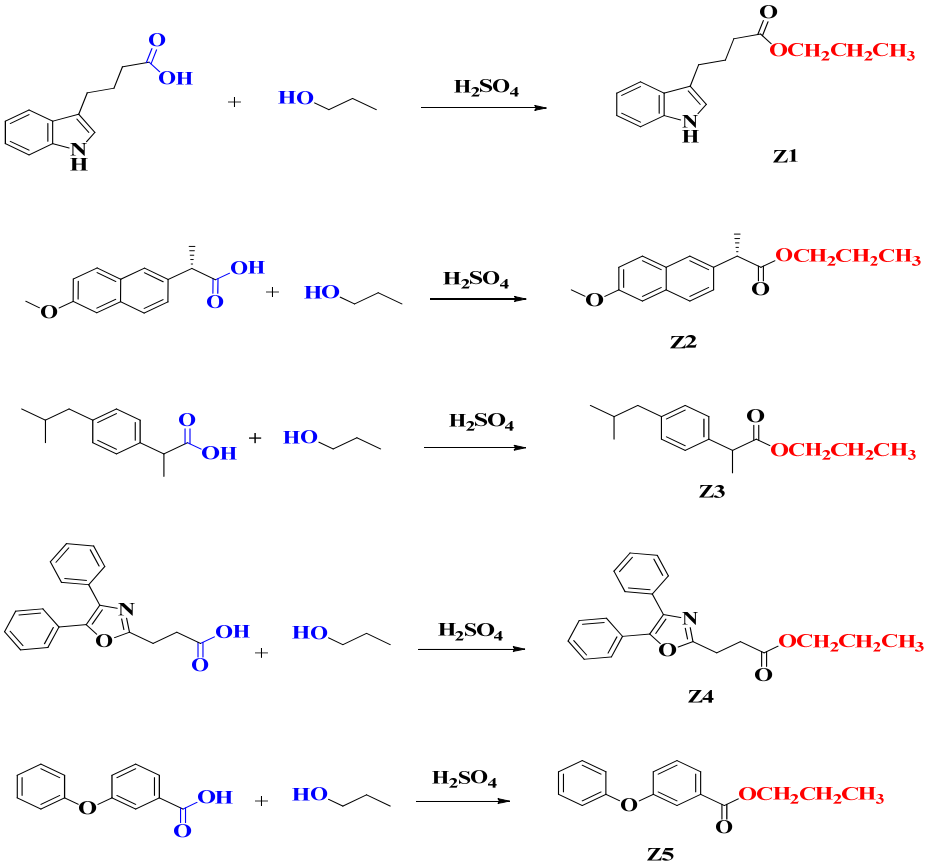
Antioxidant assay. The antioxidant effect of compounds Z1-Z5 was evaluated in vitro using the DPPH assay. Practically, a solution of DPPH (60µM/5ml MeOH) was added individually to different concentrations of compounds Z1-Z5 (25, 50, 100, 200, 300, and 500 µM), and then the homogenized mixture was incubated for 30 min in the dark. After that, the absorbance of the solution was determined at wavelength 515 nm on a UV/Vis spectrophotometer (Amersham Biospectro). The results obtained were compared with an ascorbic acid activity and then used to calculate the reduction percentage of the DPPH according to the following formula:

$$\textbf{\% of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: *Ac* is control reaction absorbance; *As* is testing specimen absorbance.

Results and discussion

According to the Fischer-esterification reaction, the derivatives Z1-Z5 were synthesized via a reaction of carboxylic acid compounds with 1-propanol using concentrated sulfuric acid as a catalyst, as shown in Scheme 1. The reaction's mechanism includes the addition of a proton (H_2SO_4) to the carbonyl group of carboxylic acid to form a more reactive electrophile. Next, the nucleophilic attack of the alcohol gives a tetrahedral intermediate containing two equivalent hydroxyl groups. One of these hydroxyl groups is eliminated after a proton shift (tautomerism) to give water and the ester, as shown in Fig. 2. The structures of all synthesized compounds were spectroscopically characterized by IR, ^{1}H-NMR, and ^{13}C-NMR, in addition to microelements analysis. The spectroscopic data obtained were included in the experimental section.



Scheme 1. The experiment for preparation of compounds Z1-Z5

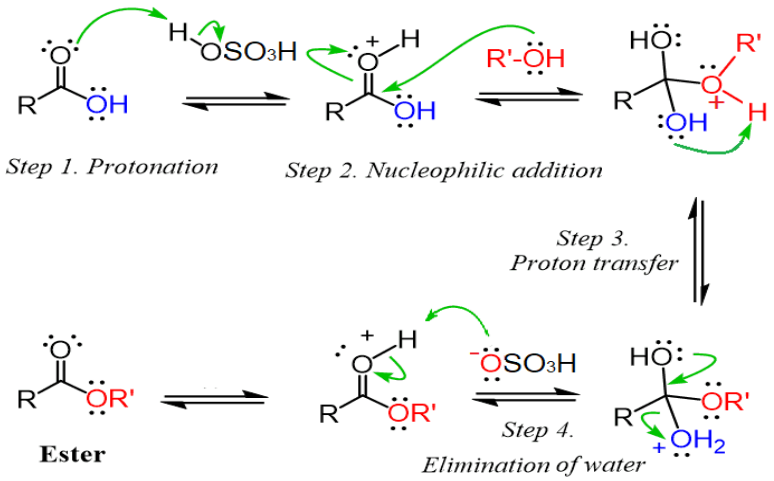


Fig. 2. Mechanism of the Fischer Esterification

According to the Fischer-esterification reaction, the derivatives **Z1-Z5** were synthesized from the reaction of carboxylic acid compounds with alcoholic compounds using concentrated sulfuric acid, as shown in Scheme 1. The structures of all synthesized compounds were spectroscopically characterized by IR, ^{1}H-NMR, and ^{13}C-NMR, as well as microelement analysis.

Antifungal and antibacterial activity. In this test, the biological activity of synthesized compounds was evaluated in vitro against both *E. coli* and *C. albicans* using the agar diffusion method. These microorganisms were chosen because they are a medical challenge due to their resistance to treatment and their ability to cause several diseases. Overall, the test results indicate that some of these compounds exhibited potent inhibition against the growth of both *E. coli* and *C. albicans* as shown in Table 1. At a concentration of 250 µM, the compounds **Z1** and **Z4** exhibited ideal inhibition against *E. coli* and *C. albicans,* compared to Ciprofloxacin and Fluconazole. Other compounds exhibited good inhibitory activity against the chosen types of bacteria and fungi except for the compound **Z3,** which exhibited weak activity toward *E. coli* and wasn't active toward *Candida albicans*.

Table 1. Antimicrobial activity of the compounds Z1-Z5

Antimicrobial activity					
E. coli			***C. albican***		
No.	Size, mm		No.	Size, mm	
	100 µM	250 µM		100 µM	250 µM
Z1	17	25	**Z1**	13	20
Z2	16	24	**Z2**	15	22
Z3	15	18	**Z3**	No A.	No A.
Z4	14	20	**Z4**	20	25
Z5	13	22	**Z5**	12	18
Ciprofloxacin	22 mm	26 mm	**Ciprofloxacin**	12 mm	18 mm
Fluconazole	10 mm	15 mm	**Fluconazole**	20 mm	25 mm

Antioxidant assay. The antioxidant activity of targeted compounds 5a-d was evaluated using a DPPH assay. The test mechanism uses H-donor molecules to reduce the free-radical DPPH and thus form the DPPH-H. The ascorbic acid is used as a reference for comparison. Generally, the tested compounds showed potent activity towards free radical DPPH compared to ascorbic acid, which was used as a reference. The results in Table 2 are obtained at a concentration of 500 µM, where the compounds **Z1, Z4,** and **Z5** showed inhibition percentages of 91.22, 84.38, and 86.51%, respectively. In comparison with standard ascorbic acid, it can be proven that compounds 5a, 5c,

and 5d have potent activity levels. The free radical capture activity may be due to the structural properties of the target compounds.

Table 2. Results of DPPH assay of derivatives Z1-Z5 at wavelength 515 nm and concentration 500 μM

Compounds	Absorbance of Sample	% Inhibition
Z1	0.045	91.22±2.52
Z2	0.113	60.36±3.93
Z3	0.148	47.75±2.24
Z4	0.048	84.38±3.46
Z5	0.064	86.51±3.07
Ascorbic-acid	0.041	93.6±2.82

Conclusions

In summary, the new classes of ester compounds were synthesized with encouraged yields via the Fischer-esterification reaction. These compounds were evaluated *in vitro* as antimicrobial and antioxidant agents. The results obtained through the DPPH test revealed that the compound propyl 4-(1H-indol-3-yl)butanoate exhibited ideal activity against free radicals compared to ascorbic acid, while the derivatives propyl 3-(4,5-diphenyloxazol-2-yl)propanoate and propyl 3-phenoxybenzoate exhibited good activity against free radicals. In addition, the antimicrobial activity evaluating tests proved that some of the tested compounds showed potent inhibition toward the growth of the bacteria and fungi examined compared to standard drugs.

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