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Research Article

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Synthesis and anti-inflammatory activity of novel Ketoprofen and Ibuprofen derivatives

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ABSTRACT

The major side effects associated with all currently available NSAIDS are gastrointestinal tract (GIT) hemorrhage and ulceration, due to inhibition of COX-1*, which is responsible for biosynthesis of cyto-protective prostaglandins E_2, while COX–2 is synthesized in response to proinflammatory stimuli such as, cytokines. Structural modification of available traditional NSAIDS, might be improve their specificity for COX–2 enzyme selectivity. These derivatives were prepared from Ibuprofen and Ketoprofen that conjugated with 2-Amino-5 - (Methylthio)—1, 3, 4-thiadiazole, and 2-Amino-5- Methyl-1, 3, 4-thiadiazole respectively using N, N-dicyclohexylcarbodiimide (DCC) as coupling agent. The structures of synthesized compounds were confirmed by IR spectra and ^1H NMR spectra . The preliminary pharmacological evaluation indicate that compounds 3 (2-(4-Isobutyl phenyl) - N-[5-methylthio -2- (1, 3, 4- thiadiazolyl)]–propamide) showed maximal anti-inflammatory activity with less ulcero-genic effect, while compound 2 (N - [5 –Methylthio – 2 - (1, 3, 4 – Thiadiazolyl)]-2- (3 -Benzoylphenyl) Propamide) showed least ulcer indexes these effects may be refer to the presence of certain structural features of heterocyclic ring .*

Keywords: Anti-inflammatory, Ibuprofen, Ketoprofen, Ulcer.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) such as Ibuprofen, Ketoprofen and selective cyclo-oxygenase-2 (COX-2) inhibitors are some of the most commonly prescribed medications worldwide, these drugs are used to treat painful inflammatory conditions such as arthritis, traumatic injuries, pain and fever [1,2].

These agents act via inhibition of the COX enzymes (cox-1 and cox-2 isoforms) which catalyzes the first step of the biosynthesis of prostaglandins[3]. The first isoform COX-l is constitutively expressed particularly in the gastrointestinal tract and the kidneys. It is responsible for the physiological production of prostaglandins. The other isoform COX-2 is induced during inflammation process[4, 5].

The development of selective COX-2 inhibitors leads to discovery of drugs with significantly reduced renal and gastrointestinal side effects[6].

The major side effects associated with all currently available NSAIDS are gastrointestinal (GI) hemorrhage and ulceration[7].Gastrointestinal side effects of NSAIDS resulted mainly from the inhibition of COX-1, which is responsible for biosynthesis of cyto-protective prostaglandins E_2.The PGE2 action includes increase blood flow, increase bicarbonate secretion, stimulate mucus production, and reduce gastric acid secretion this protect the gastrointestinal mucosa, COX–2 is inducible and its synthesis in response to proinflammatory stimuli such as, cytokines and growth factors [8,9].

Traditional NSAIDs such as Indomethacin, Aspirin and Diflunisal are non-selective, COX–1 and COX–2 inhibitors. So that, they have low margin of safety in which one third of patients who were taking long-term nonselective NSAID develop endoscopically proven gastric or duodenal ulcers[10], whereas preferential and high selective COX–2 inhibitor drug namely Meloxicam and Rofecoxib were developed respectively to reduce the GIT side effect [11].

An increasing in the number of studies indicate that the structural modification of available traditional NSAIDS, lead to improve their specificity for COX–2 enzyme selectivity such as Piroxicam, which converted to Meloxicam by chemical modification lead to preferential selective COX–2 inhibitor due to the presence of the enol–carboxamide, heterocyclic ring with methyl substituent at 5position[12].

The objective of this study, to synthesis new anti-inflammatory derivatives of Ibuprofen and Ketoprofen as potential selective COX–2 inhibition with less ulcero-genic effect based on drug development.

EXPERIMENTAL SECTION

2.1. Materials

2.1.1. Chemicals:

Ibuprofen powder was obtained as a gift from ShibaPharma Drug Industry Yemen, Ketoprofen powder was obtained as a gift from Universal Drug Industry Yemen, Indomethacin was obtained as a gift from National Drug Quality Control Laboratory, Sana'a, Yemen. Rofecoxib, 2-Amino-5 - (Methylthio)-1, 3, 4-thiadiazoleand, 2-Amino-5-Methyl-1, 3, 4-thiadiazole, N, N-Dicyclohexylcarbodiimide (DCC), Dimethyl sulfoxide(DMSO) 99.5% were purchased from Sigma Aldrich Chem. Co. Germany. Dichloromethane 99.5% were purchased from Scharlau Chemics S.A. European Union. Zinc dust, and anhydrous sodium sulphate were purchased from Fluka Chemik Switzerland. Sodium carbonate , petroleum ether 60-80%, ether, propylene glycol , ethanol , and dioxane were purchased from Scharlab, Spain. HCl was purchased from Unichem, India. glacial acetic acid was purchased from Himedia, India .

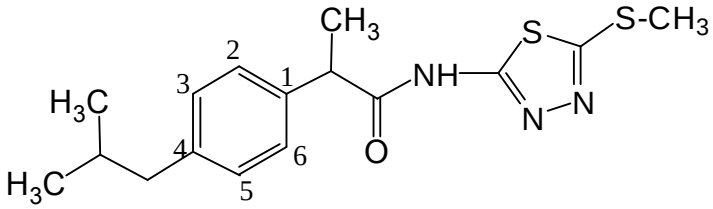
2.1.2. Equipments:

(1H–NMR) spectra were carried out on, mercury 300 MHz spectrometer (Aldenmark), using tetramethylsilane as the internal reference. Melting points(MP) were determined by using a calibrated Thomas–Hoover melting apparatus and uncorrected. IR spectra were recorded using Shimadza FT- (8101 IR) spectrophotometer (Japan).Rotary evaporator(R-210 V-700 V-850, Buchi, Switzerland). Thin layer chromatography (TLC) is performed with precoated silica gel plates (60F-254 with iodine as developing compound.

2.2. Methods

2.2.1. Chemical Synthesis

2.2.1.1. Synthetic Procedures of 2-(4-Isobutyl phenyl) - N-[5-methylthio -2- (1, 3, 4- thiadiazolyl)]–propamide (**Compound 1**)



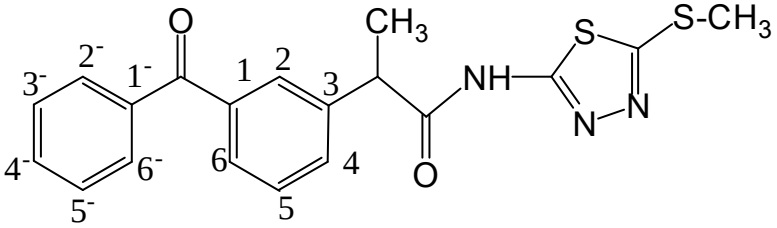
Ibuprofen (3.52 g, 17.11 mmol) was dissolved in dichloromethane (50 ml) and dicyclohexylcarbodiimide (1.76 g, 8.56 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3 hrs, then a white precipitate of dicyclohexylurea was formed and removed by filtration, the solvent was evaporated under vacuum, and semisolid product was obtained to yield Ibuprofen Anhydride know as intermediate compound (A).

Compound (A) (2.5 g, 6.35 mmol), 2-amino-5-(methylthio)-1,3,4- thiadiazole (0.932 g, 6.35 mmol), zinc dust (0.006 g), glacial acetic acid (0.61 ml, 10.67mmol), dioxane (40 ml) were placed in 100 ml rounded bottomed flask, fixed with reflux condenser, boiling stone were added. The reaction mixture was refluxed for about 2.5 hrs with continuously stirring, and the reaction was checked by TLC to make sure that completion of reaction. The solvent was evaporated under vacuum, and residue was dissolved in ethyl acetate, then the reaction mixture was washed, with $NaHCO_3$ (10%) 3 times, HCL (1N) 3 times, and 3 times with distilled water, using (20ml), filtered over anhydrous sodium sulphate. The filtrate was evaporated and the residue was re-dissolved in ethyl acetate and

filtered. The recrystallization was carried out by adding petroleum ether (60-80 C^0) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the crystalswas collected to produce compound (1) in 57.35% yield as white needle crystals[13, 14].

Mp. 190-192. IR, (KBr, Cm^{-1}) 3250 (NH, amide), 1650, 1600, 1550, 1450 (C=C,Ar), 1675 ((-NH-C=O, carbonyl).1H – NMR (DMSO.d6) δ pmm: 7.15(2H, d, at 2&6 positions, Ar),7.25(2H, d, at 3 &5 positions, Ar), 2.55 (s, 3H, 5-S-CH_3), 7.05(s,1H, CONH,H exchangeable with D_2O), 0.84 (6H,CH, iso-but), 1.8 (1H, CH_3iso-but) , 1.45 (3H, CH, Prop.) 3.98 (1H, CH_3,Prop.), 2.2 (s, 3H, $COCH_{3)}$.

2.2.1.2. Synthetic Procedures of N - [5 –Methylthio – 2 - (1, 3, 4 – Thiadiazolyl)]-2- (3 -Benzoylphenyl) Propamide (**Compound 2**).

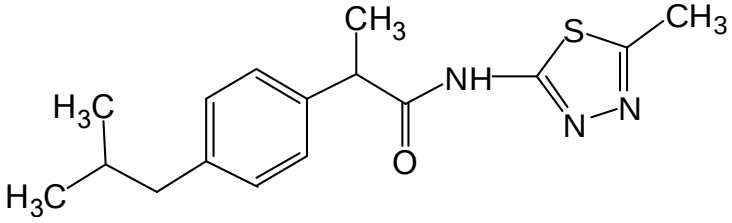


Ketoprofen (4.35 g, 17.11 mmol) was dissolved in dichloromethane (50 ml) and dicyclohexylcarbodiimide (1.765 g, 8.56 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3 hrs, a white precipitate of dicyclohexylurea was formed and removed by filtration, the solvent was evaporated under vacuum, and an semisolid product was formed to yield know as intermediate compound (B).

Compound (B), (2.5 g, 5.10 mmol), 2-amino-5-(methylthio)-1,3,4- thiadiazole (0.75 g, 5.10mmol), zinc dust(0.004 g), glacial acetic acid (0.49 ml, 8.57mmol), dioxane (40 ml) were prepared as described before in compound 1, to generate compound (2) in yield of 52% as white crystals .

Mp. 193-195. IR, (KBr, Cm^{-1}) 3250 (NH, amide), 1650, 1600, 1550, 1450 (C=C,Ar), 1675 (-NH-C=O, carbonyl), 2920 (CH3). 1H – NMR (DMSO.d6) δ pmm: 2.7 (s, 3H,5-S-CH_3), 1.49 (2H, CH3), 4.12 (M, 1H, CH), 7.45-7.82 (1H, s, at 2 position, 1H, m, at 5 position, and 2H, d, at 4 and 6 positions, Ar. 2H, d, at 2^- and 6^- positions,3H, m, at 3^-, 4^-, and 5^- positions **,**Benzoyl),7.o57(s ,1H,
CONH,H exchangeable with D_2O**)**.

2.2.1.3. Synthetic Procedures of 2-(4-Isobutyl phenyl) - N-[5-methyl -2- (1, 3, 4- thiadiazolyl)]–propamide (**compound 3**)



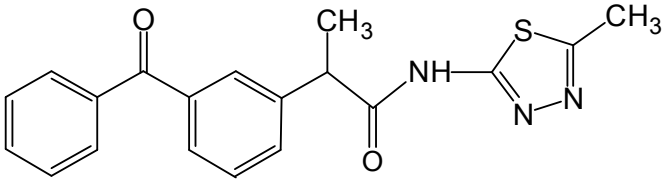
Compound A (5 g, 12.69 mmol), 2-amino -5- methyl -1, 3, 4 thiadiazole (1.460 g, 12.69 mmol), Zinc dust (0.012 g), glacial acetic acid (1.2 ml, 24.040 mmol), and dioxane (50 ml) were added to 100 ml rounded bottomed flask, boiling stones were added and refluxed for about 2 hrs with continuously stirring, the reaction was checked with TLC to make sure the completion of the reaction.

The solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ (10%, 3 times), HCL (1N, 3 times), and three times with distilled water. Ethyl acetate layer was separated and filtered over anhydrous sodium sulphate. The filtrate was evaporated and the residue dissolved in ethyl acetate and filtered. The recrystallization was carried out by adding petroleum ether (60-80C^0) on the filtrate until turbidity occurred then kept in cold place over night. Then the mixture was filtered while it is cold and the crystals was collected to give compound (3) in 55.2% yield as faint White crystals .

Mp. 180-182. IR, (KBr, Cm^{-1}) 3280 (NH, amide), 1675 (-NH-C=O, carbonyl). 1450, 1550, 1600, 1650 (C=C,Ar). 1H – NMR (DMSO.d6) δ pmm: 7.15(2H, d, at 2&6 positions, Ar),7.25(2H, d, at 3&5 positions, Ar), 7.243 (s,1H, CONH, H exchangeable with D_2O), 0.84 (6H,CH, iso-but), 1.8 (1H, CH_3iso-but) , 1.45 (3H, CH, Prop.) 3.98 (1H, CH_3,Prop.), 2.2 (s, 3H, $COCH_{3)}$.

,2.576 (s,3H,-5-CH_3, thiazolyl).

2.2.1.4. Synthetic Procedures of 2-(3-benzoyl phenyl)-N- [5-methyl -2- (1, 3, 4- thiadiazolyl)]–propamide (**compound 4**)



Compound (B), (5 g, 10.20 mmol), 2-amino -5- methyl -1,3,4 thiadiazole (1.173 g, 10.20 mmol), Zinc dust (0.009 g), glacial acetic acid (0.98 ml, 19.692 mmol), and dioxane (50 ml) were prepared as described before in compound 3, to generate compound (4) in yield of 53.1% as white crystals .

Mp. 195-197. IR, (KBr, Cm^{-1}) 3280 (NH, amide), 1650, 1600, 1550, 1450 (C=C,Ar),1675 (-NH-C=O, carbonyl), 2920 (CH3).

1H – NMR (DMSO.d6) δ pmm: 1.49 (2H, CH3), 4.12 (M, 1H, CH),2.45(s,3H,5-CH_3,thiazolyl) 7.45-7.82 (1H, s, at 2 position, 1H, m, at 5 position, and 2H, d, at 4 and 6 positions, Ar. 2H, d, at 2^- and 6^- positions,3H, m, at 3^-, 4^-, and 5^- positions **,**Benzoyl)**,**7.o57(s ,1H,CONH,H exchangeable with D_2O**)**.

2.2.2. Preliminary Pharmacological Study

According to the aim of our study, the anti-inflammatory activity of synthesized compounds was evaluated in comparison with standard potent anti-inflammatory drug (Indomethacin). In addition, the GIT side effects of the tested synthesized agents were evaluated in comparison with selective COX-2 inhibitors (Rofecoxib), and with relatively strong COX-1 inhibitors (Indomethacin).

2.2.2.1. Experimental Design

The animals (guinea pigs) were housed in separated cages. On fasting, the animals were kept in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water. They were divided into six groups each contain 5 animals.

Group A, were served as normal control, they received no drugs; they were injected intraperitoneal (I.P) with vehicle (DMSO).Group B, were injected I.P with Indomethacin in dose 2.55mg/400gbody weight. This dose represented the therapeutic dose in human converted to appropriate dose in guinea pig by Pajet and Barnes method[15]. Groups C-F, were injected I.P with tested compounds [1,2,3, and 4] in doses (2.09 mg,2.895 mg, 2.22 mg, 2.58 mg/400 g body weight I.P) respectively. Tested compounds (compound 1, 1, 3, and 4) are dissolved in DMSO.

2.2.2.2. Anti-inflammatory Activity

Anti-inflammatory activity determined by using Carrageenan paw edema method. Carrageenan 1% is freshly prepared by dissolving in normal saline 0.9%. One hour after dosing, the animals are challenged by a subcutaneous injection of 0.2ml of 1% solution of carrageenan into sub-plantar side of the left hind paw. The animals were anaesthetized with ether, at 2 hours after challenge paw was cut, and its weight was measured in comparison with weight of right one. The weight difference value between two paws was obtained by subtracting right paw from left paw and the average weight (mean) are calculated and evaluated statistically. The percentage of inhibition of edema comparative with the treated compounds were calculated and for control, Indomethacin, and tested compounds 1, 1, 3, and 4 respectively.

Paw edema and % edema inhibition measurement paw edema weight was calculated by; $W_D=W_R-W_L$.

Where: W_D= weight difference of edema between right and left hind paw, W_R=weight of edema of right hind paw, and W_L=weight of edema of left hind paw. % edema inhibition was calculated by the following formula:% edema inhibition = [1- (W_T / W_C)] X 100, where: W_T=weight difference of edema of tested animals, $W_{C=}$weight difference of edema of control animals [16, 17].

2.2.2.3. Determination of the gastric side effects

Determination of the gastric side effects was done by detection of possible ulcero-genic activity for compounds (1,1,3, and 4) that exhibited marked anti-inflammatory activity compared with Indomethacin and Rofecoxib.

Animals were divided into seven groups (n=5),they were fasted 20 hrs before drug administration .The synthesized agents (1,2,3, and 4 compounds), Rofecoxib and indomethacin were given orally in a doses of (2.09, 2.895, 2.22, 2.58, 2.23, and 2.55 mg/400gbody weight)respectively and they dissolved in propylene glycol. The control group received vehicle only (propylene glycol). After that animals were fasted for 2hrs,allowed to feed for 2 hrs, then fasted for another 20 hrs. and given another two doses in the second and third days .In the fourth day, animals were anaesthetized with ether, sacrificed, the stomach removed, opened along with the greater curvature and rinsed with 0.9% saline .The number of mucosal damage (red spots)were counted using magnifying lens and their ulcero-genic severity was graded by mean from 0 (no lesion) to 4 (exceptional sever lesion) [18]. Score assignment; Zero = for normal (no injury), 1= latent small red spot, 2= wide red spot, 3= slight injury, and 4= sever injury.

1- % incidence/10 = (no. of animals showing ulcer divided by total no. of animals in the group *100) / 10.
2-Average number of ulcer = no. of ulcers in the group/ total no. of animals in the group.
3-Average severity = sum (each ulcer * score of severity) / no. of ulcers.
4- Ulcer index = the sum of (1+2+3).

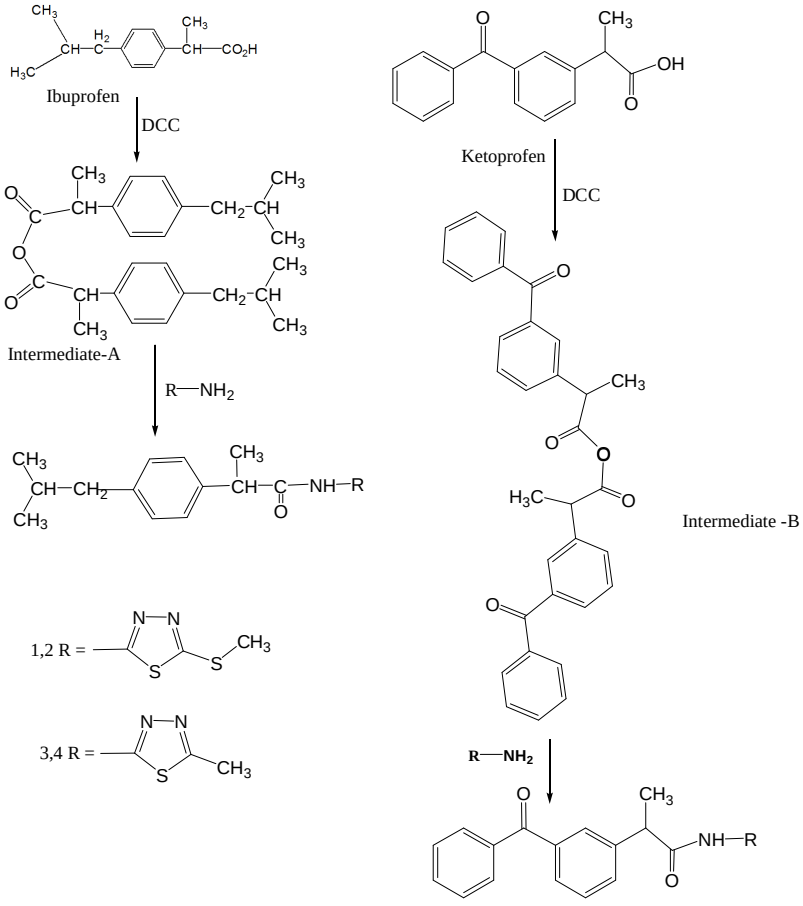
2.2.3. Statistical Analysis

Statistical processing of the result by using the test of analysis of variance (ANOVA test) to show the differences among all groups if it is present, the highly significance is considerable, in which ($p < 0.01$). To conform that the result obtained by ANOVA test using T-test ,in which highly significance if ($p < 0.01$).

RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic pathway to give the target compounds [1,2,3,4]was carried out according to Scheme-1.



In order to prepare the key intermediates(compound A) of Ibuprofen and (compound B) of ketoprofen, the carboxyl group react with DCC as showed in Scheme-1 to liberate very good reactive anhydride intermediate compound (A) and compound (B),these intermediates have a good characteristics like carbonyl carbon with electron deficiency which increased with zinc dust (catalyst). The Coupling of the key intermediates A and B with amino group of

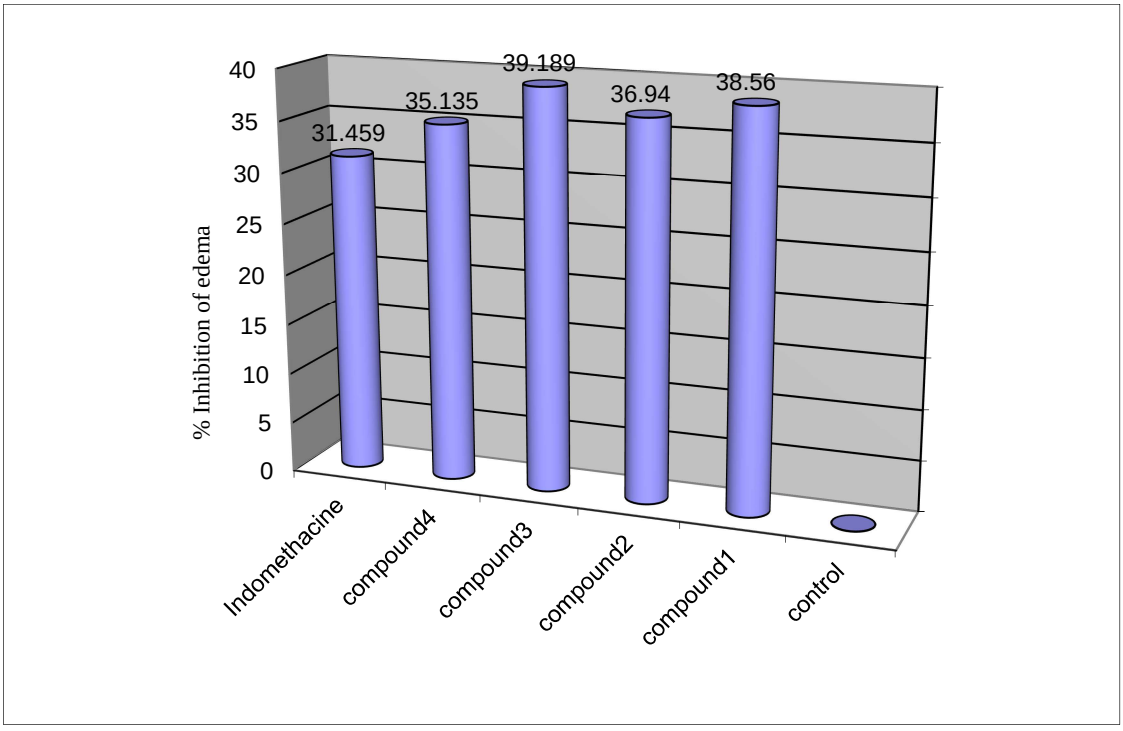
heterocyclic compounds. This procedure is analogous to that reported by Vogel for preparation of amide linkage[19].The acylation of anhydride with amino group of heterocyclic compounds were faster than the using of obnoxious acylchloride. The presence of zinc dust as catalyst to accelerate the reaction. This reaction is an example of nucleophilic reaction in which the nucleophile ($–NH_2$) is added to carbonyl carbon of anhydride in slightly acidic media (by adding glacial acetic acid) and presence of zinc as catalyst.

3.2. Pharmacology

The anti-inflammatory effect of the target compounds (1,2, 3, and4) compared with reference agent were studied on adult male guinea pigs, since these animals are sensitive for induction of inflammation, well responded to anti-inflammatory agents, easily handled, and available[20].Figures 1&2showed comparison among control, references and tested compounds as percent of inhibition edema, and the ulcer index .

The average difference of paw weights(amount of edema developed) as showed in(Fig.1),the control animals was 0.370 g. It's significantly lower in both reference and tested animals than controls; this indicates that such compounds have significant anti-inflammatory activity.

The inhibition percent of edema of tested compounds 1,2, 3, and 4was 38.56%, 36.94%,39.189%, and 35.135% respectively. Compared with indomethacin as reference agent and its inhibition percent was31.459. As shown in fig.1 all tested compounds showed good anti-inflammatory activity. However,compound3 showed maximum anti-inflammatory activity followed by compound1,this effect might due to the attributing of conjugate heterocyclic rings 2-amino-5-methyl-1,3,4-thiadiazole and 2-amino-5-methylthio-1,3,4-thiadiazoleto the parent agent ibuprofen. These heterocyclic rings might incorporated into the side pocket of COX-2 enzyme ,so, achieved a good anti-inflammatory activity toward COX-2 inhibition with less GIT side effect[21].



Figure(1): Graphic display of % Inhibition of edema of control, compound 1, compound 2, compound 3, and compound4,and indomethacin

The ulcero-genic potential of tested compounds1,2, 3, and 4 were evaluated through acute ulcero-genicity study in which the number of mucosal damage (red spots) were counted using magnifying lens and their ulcero-genicity was scored by mean from 0 (no lesion) to 4 (exceptional sever lesion) then the ulcer index was calculated . Indomethacin showed the highest index (15.10) while Rofecoxib showed the least index (3.40). The ulcer indexes of the tested compounds 1, 2, 3, and 4 were 10.53, 5.4, 6.05, and 8.40 respectively as showed in fig.2.

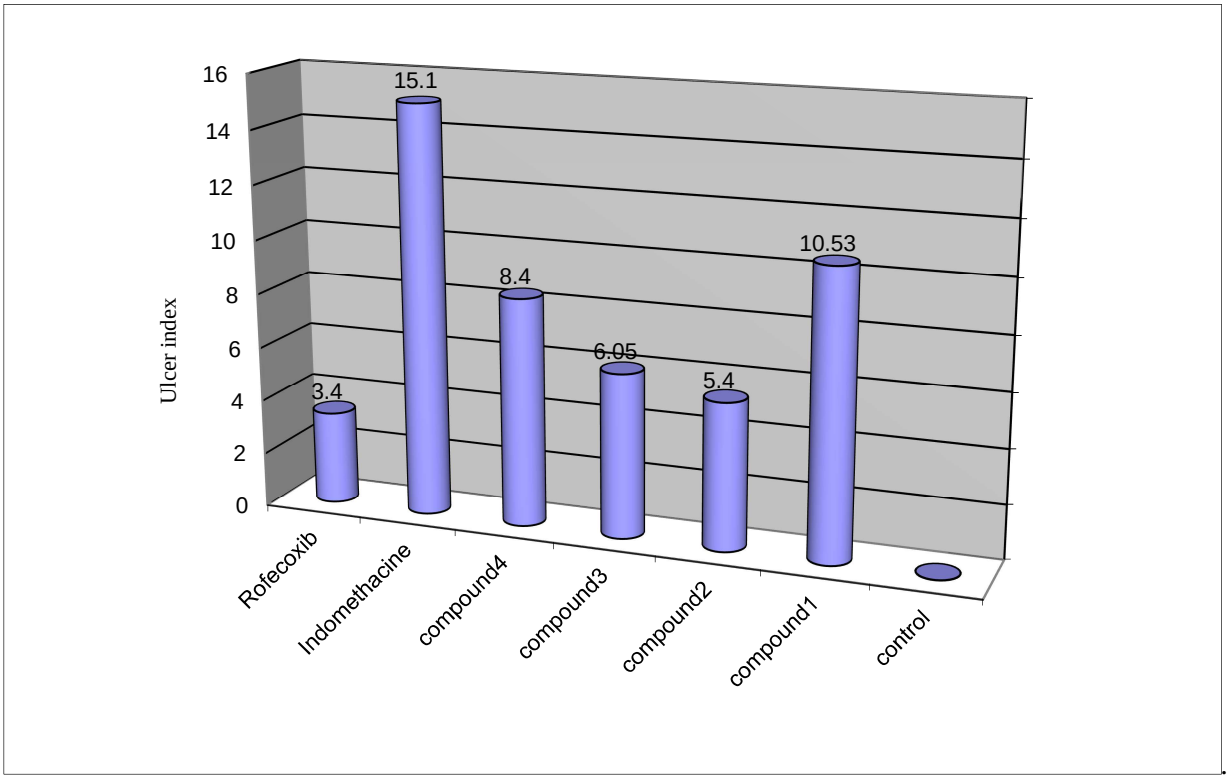


Figure (2): Graphic display of the ulcer index for control, compound 1, compound 2, compound 3, compound 4, rofecoxib, and indomethacin

The ultimate goal of any newly synthesized non-steroidal anti-inflammatory drugs is the achievement of adequate therapeutic effect with least possible side effect. It is well established that most of therapeutically desirable effect of anti-inflammatory drugs is attributed to the inhibition of COX-2 enzyme to the inflammatory prostaglandin synthesis. On the contrary inhibition of COX-1 enzyme may be responsible for undesirable side effect namely ulceration and nephrotoxicity. The NSAIDs such as indomethacin are non-selective, COX-1 and COX-2 inhibitor. They cause GI side effect while highly selectiveCOX-2 inhibitors such as Rofecoxib has a least GI side effect[22]) .In this work the anti-inflammatory activity and GI side effect of novel meloxicam related synthesized compounds were studied, their selectivity was evaluated by comparing their COX-2 dependent anti-inflammatory effectand COX-1 mediated ulcero-genic effect.

Indomethacin showed profound ulcero-genic effect while Rofecoxib showed mild toxic effect on the gastric mucosa this confirmed the gastric ulceration due to the inhibition ofCOX-1 enzyme [23].Compound3 showed maximal therapeutic anti-inflammatory with less ulcero-genic effect, while compound2 showed least ulcer index, these effects may be due to the presence of specific moieties (heterocyclic ring with 5- methyl or 5- methylthio in addition to carboxamide group that involved in synthesized compounds 1,2,3,4).These functional groups may be responsible forCOX-2 inhibition as in Meloxicam [24].

CONCLUSION

Novel non-steroidal anti-inflammatory agents were chemically synthesized and spectroscopically identified. The anti-inflammatory activity and ulcero-genicities of these synthesized agents were preliminary pharmacologically evaluated.Compound3 showed maximal anti-inflammatory activity with less ulcero-genic effect, while compound 2 showed least ulcer index; these effects may be due to the presence of specific heterocyclic ringthat responsible for COX-2 selective inhibition. These heterocyclic rings 5-methyl-1,3,4-thiadiazole and 5-methylthio-1,3,4-thiadiazole,that inserted into side pocket of COX-2 enzyme selectivity.

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