



Research Article

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Synthesis and preliminary pharmacological evaluation of mefenamic acid and indomethacin derivatives as anti-inflammatory agents with less GIT side effect

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ABSTRACT

This work aims to design, synthesis and preliminary pharmacological evaluation of mefenamic acid and indomethacin derivatives as potential anti-inflammatory agents with less GIT side effect. The demand for NSAIDs is in rise in the medical treatment but its clinical usefulness is limited owing to gastrointestinal side effects. These derivatives were prepared by conjugated with, 2-Amino-5- Methylpyridine and 2-Amino-5 - Methyl-1, 3-thiazole respectively using N, N-dicyclohexylcarbodiimide (DCC) as coupling agent. The structures of synthesized compounds were confirmed by IR and ¹H NMR spectra .The preliminary pharmacological evaluation as anti-inflammatory activity test and ulcerogenic index screening were performed, in rat using egg-white induced edema model of inflammation. The results showed that most the synthesized derivative performed good anti-inflammatory activity or least maintain the activity of the parent compounds. Compound 5 (2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-2,3-dihydro-1H-indol-3-yl]-N-(5-methyl-pyridin-2-yl)-acetamide) showed maximal anti-inflammatory activity 51.5%, whereas compound 6 (2-[1-(4-Chlorobenzoyl)-5-methyl-2,3-dihydro-1H-indol-3-yl]-N-(5-methyl-thiazol-2-yl)-acetamide compound) 49.78% . Compound 2 (2-(2,3 dichloro phenyl amino)-N-(5-methyl-pyridine2-yl)- Benzamide) and compound (6) showed less ulcer index compared to celecoxib which is, high selective COX-2 inhibitor, safe, and standard of gastric irritation that used in this work .

Keywords: Anti-inflammatory, gastric Ulcer, indomethacin, mefenamic acid.

INTRODUCTION

Inflammation is a term that refer to a complex series of tissue changes that result in pain and fever [1] Traditional Nonsteroidal antiinflammatory drugs (NSAIDs) are used to treat the inflammation as a standard therapy[2]. The prolonged usage of traditional NSAIDs drugs limits their therapeutic use since they cause serious upper gastrointestinal side effect [3] . Traditional NSAIDs act by non selective inhibition of cyclooxygenase (COX) enzymes, which stimulate formation of prostaglandins (PGs) from arachidonic acid [4] . The cyclooxygenase (COX) enzymes have two isoforms. The first isoform COX-1 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 [5]. The selective COX-2 inhibitors (Celecoxib, Rofecoxib) have high activity with less GI toxicity , than traditional NSAIDs but drugs with higher selectivity for COX-2 tend to induce cardiovascular disease [6]. The gastric side effects related to the use of NSAIDs are generally attributed to local and/or systemic mechanisms, the direct or local mechanism involves local irritation produced by NSAIDs and

the systemic mechanism involves the inhibition of synthesis of cytoprotective prostaglandins[7]. Structural modification of available traditional NSAIDs, may improve their specificity for COX-2 enzyme selectivity[8]. The development of new NSAIDs to mask the acidic carboxylic group of NSAIDs has been considered as a promising means of reducing the GI toxicity[9]. The conversion of free carboxylic acid into amide by using heterocyclic coupling ring leads to new derivatives with selective effect towards COX-2 [10]. In the present research work, various derivatives of mefenamic acid and indomethacin were successfully synthesized and screened for their anti-inflammatory and ulcerogenic activities.

EXPERIMENTAL SECTION

1. chemistry :-

1.1 Chemicals:

Mefenamic acid crystalline powder and Diclofenac Sodium crystalline powder kind gift from Modern Yemeni Pharma. Co. Indomethacin crystalline powder was purchased from Ram Pharmaceutical Co. Jordan. 2-amino-5-methylpyridine, 2-Amino-5-methyl-1,3,4-thiazole and N,N-Dicyclohexylcarbodiimide (DCC) were purchased from Apollo scientific chemicals U.K. Dimethyl sulfoxide (DMSO) 99.5% kind gift from laboratory of quality control. Aden. Anhydrous sodium sulphate and dioxane were purchased from Sigma Aldrich Chem. Co. Germany. Zinc dust, propylene glycol, ethanol Sodium carbonate, petroleum ether 60-80% dichloromethane 99.5% and ethyl acetate were purchased from Scharlau Chemicals S.A. European Union. all others chemicals are analytical grades.

1.2 Equipment :

IR spectra were recorded using FT-IR. PerkinElmer spectrometers (USA). and were performed in the Center of Research and Pharmaceutical Studies (CRPS) University of Science and Technology, Sana'a Yemen. (¹H-NMR) spectra were carried out on, JEOL 500 MHz spectrometer (USA), using tetramethylsilane as the internal reference and were performed in the National Research Center (NRC), Cairo, Egypt. Melting points were determined by using a calibrated Stuart SMP11 (U.K.) melting apparatus. The progression of reaction was checked with TLC Kieselgel GF254 (type 60) to make sure the completion of reaction. Rotary evaporator (R-210 V-700 V-850, Buchi, Switzerland). The identification of compounds was done using iodine vapor and mobile phase system: Diethyl ether: Acetic acid: Benzene: Methanol (60: 18:2:20) [11]

1.3 Chemistry Synthesis:

Synthesis of 2-(2, 3-dimethylphenylamino) benzoic anhydride compound 1 (mefenamic anhydride)

Mefenamic acid (5.0 g, 20.77 mmole) was dissolved in dichloromethane (150 ml), and dicyclohexylcarbodiimide (DCCI) (2.12 gm, 10.35 mmole) was added. The reaction mixture was continuously stirred at room temperature for 3.5 hours. A white precipitate of dicyclohexylurea (DCU) was formed, and then removed by filtration. The filtrate was evaporated under vacuum to yield compound 1 (75% yields) [12]

Synthesis of 2-(2,3 dichloro phenyl amino)-N-(5-methyl-pyridine-2-yl)- Benzamide compound 2

Compound (1) (2.5 g, 7.3 mmol), 2-amino-5-methylpyridine (1.3005 g, 7.3 mmol), zinc dust (0.0075 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) were placed in 100 ml round bottom flask, equipped with reflux condenser, and boiling stones were added. The reaction mixture was refluxed for about 2.45 hr. with continuous stirring. The solvent was evaporated under vacuum; the residue was dissolved in ethyl acetate, then washed with NaHCO₃ (10%, 3 times), HCl (1N, 3 times) and 3 times with distilled water, and filtered over anhydrous sodium sulphate. The filtrate was evaporated and the recrystallization was carried out by redissolving the residue in ethyl acetate and filtered. and kept in cold place over-night. Then the mixture was filtered and the precipitate was collected to give compound (2) as pale yellow needle crystal (63% yield) [13]. M.p. 220-223°C, R_f = 0.46, IR N-H of secondary amide, (3313), C-H of aromatic, (2927 and 2851) C-H of CH₃, (1650). C=O of secondary amide, (1576, 1436) (Ar.) v. of secondary amide. ¹H-NMR (DMSO): 7.8 ppm (2H, -CH₃ of pyridine), 9.44 ppm (1H, secondary amine), 5.5 ppm (2H, 2-CH= at position 6'), 12.9 ppm (1H, NH of secondary amide between benzene and pyridine), 7.26-5.56 (m, 4H, of benzene) 3.4 ppm (3H, CH₃ of pyridine)

Synthesis of N-(2-benzothiazolyl)-(2,3dimethylphenylamino) Benz amide (compound 3)

Compound 1 (2.5 g, 5.4 mmol), 2-amino-5-methyl-1,3,4-thiazole (0.81 g, 5.4 mmole), zinc dust (catalytic amount, 0.01 g), glacial acetic acid (0.5 ml, 8.75 mmol) and dioxane (20 ml) were placed in a flask, equipped with reflux condenser. The reaction mixture was continued as in the synthesis of compound 2 to yield compound 3. It was prepared as previously described in (2) to liberate compound (3) as white needle crystals (58% yield). M.p. 188-191°C, R_f = 0.52. IR (KBr, cm⁻¹): 3310 (NH, amide), 2972 (CH, ArH), 1650 (C=O, amide), 1527, 1461 (Ar). ¹H-

NMR(DMSO): 7.17- ppm (2H,-CH₃ of thiazole) ,4.0 ppm(1H,secondary amine), 7.26-5.56 ppm(2H-CH₂ of benzene) 8,0 ppm (1H,NH of secondary amid between benzene and thiazole),7.26-5.56 (m,4H, of benzene) .

Synthesis of Indomethacine anhydride Compound (4)

Indomethacine (10 g, 55.5 mmol) dissolved in 150 ml methylene chloride; dicyclohexyl- carbodimide (5.72 g, 27.7 mmol) was added. The reaction mixture continuously stirred at room temperature for 3.5 hrs. A white precipitate of dicyclohexylurea will be form and will remove by filtration. The solvent will evaporate under vacuum, and an oily product will form to yield the desired anhydride (80% yields) [14]

2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-2,3dihydro-1H-indol-3-yl]-N-(5-methyl-pyridin-2-yl)-acetamide compound 5

Compound (4) (2.5g, 4.414 mmol) , 2-amino 5- methyl pyridine (0.786 g, 4.414 mmol), zinc dust (0.004g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) were placed in 100 ml round bottom flask were prepared as previously described in (2) to liberate afforded compound(5) as white needle crystal. 43% yield, Mp.240-242°C, Rf= 0.61 , IR (KBr, cm-1) 3334 (NH, amide), 2932, 2852 (CH, Ar-H), 1686 (C=O, amid) 1595, 1477 (Ar). 1H-NMR (DMSO-d₆) -pmm: 3.85 (s,3H,-CH₃ of O CH₃),12.22(s, 1H, CONH.),1.7 (3H,CH₃ of pyridine), 6.91-7.04 (m, 7H, H of Aromatic ring), 2.28-2.23(s, 2H-of CH₂), 7.52-7.65-7.67 (m ,3H, H of aromatic pyridine) .

2-[1-(4-Chlorobenzoyl)-5-methyl-2,3dihydro-1H-indol-3-yl]-N-(5-methyl-thiazol-2-yl)-acetamide compound 6

Compound (4) (2.5g, 4.414 mmol) , 2-amino 5-methyl thiazole. (0.786 g, 4.414 mmol), zinc dust (0.004g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) were placed in 100 ml round bottom flask were prepared as previously described in (2) to liberate afforded compound(6) as white crystal. 53% yield, Mp.230-232 °C, Rf= 0.32 , IR (KBr, cm-1) 3334 (NH, amide), 2932 (CH, Ar-H), 1680 (C=O, amide) 1587, 1479 (Ar). 1H-NMR (DMSO.) _ pmm: 3.85 (s,3H,-CH₃ of OCH₃),12.22(s, 1H, CONH),2.28-2.32 (s, 2H of CH₂), 1.91 (m, 3H -CH₃ of aromatic thiophene), 7.03-7.04 (s, 3H ,Hof aromatic thiophen), 6.91-7.70 (m ,7H, H of aromatic ring), 3.34 (d, 3H, CH₃ ofcycloprrl) .

2. PHARMACOLOGY

2.1 Animals:

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model . The adult male albino rats weighing 205 ± 10gm, supplied by the animal house of the pharmacy college, university of Sana'a under standardized conditions .For 5 days animals were provide with water and food .Animal were brought 3 hour before performing the experiments . They were divided into 6groups(for anti-inflammatory activity test) and 7 groups (for ulcerogenic index test) each group of 5 rats . All the experiments were performed by following the approval of research protocols by the Research Animals Ethics Committee, the doses of standard and prepared compounds have to be calculated in equimolecular dose of Indomethacin to rat weight 400mg =0.051*50 mg [15].

2.2 Experimental Design:

2.2.1 Anti-inflammatory Activity Test:

Ovalbumin Paw Edema Method:

Rats are divided into eight groups (n = 5) starved overnight with water ad libitum prior to the day of experiment. The control group were injected intraperitoneally with 0.2ml of vehicle only(DMSO) .Other six animal groups were injected intraperitoneally with tested agents(2,3,5 and 6) with (2.18, 3.72, 3.75,and 3.74 mg. / 0.4 kg.) respectively, and the other 8th animal group were injected intraperitoneally with standard Diclofenac sodium in dose (2.16 mg /0.4 kg.) Then ,one hour after dosing , acute inflammation will be produced by a subcutaneous injection of 0.05ml of undiluted fresh egg-white into the planter side of the left hand paw of the rats. The animals were anaesthetized with diethyl ether , 2.5 hours after challenge the paw is cute, its weight is measured and compared with right one. The weight difference value between two paws was obtained by subtracting right paw from left paw weight and the average weight (mean) is calculated and evaluated statistically. The percentage of inhibition of edema comparative with the treated compounds were calculated for control, Diclofenac, and tested compounds 2,3,5 and 6 respectively[16]

Calculations (Paw Edema and % edema inhibition)

- Paw edema weight was calculated by using the following formula:-

$$W.D. = W_R - W_L$$

Where: WD= weight difference of edema between right and left hand paw

WR= weight of edema of right hand paw

WL= weight of edema of left hand paw

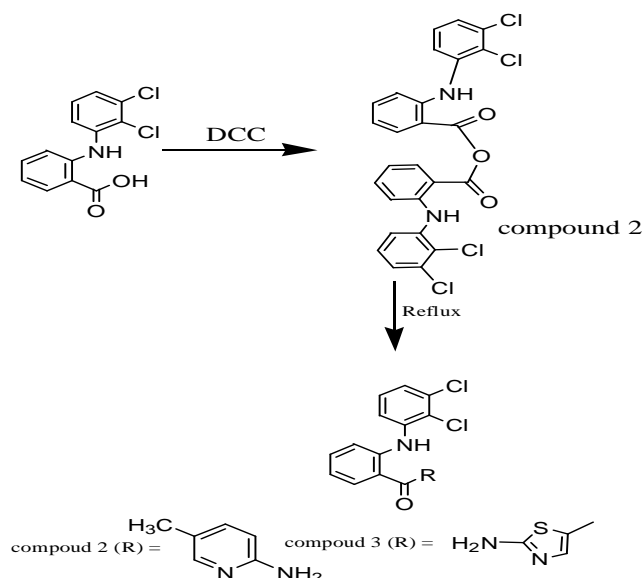
Ulcerogenic index:-

The adult albino rats were divided into 7 groups (n=5). Animals were made to fast 20 hours before drugs administration. The synthesized agents and references (indomethacin and Celecoxib) were given orally in a dose of (2.09, 2.39, 2.73, 2.23, 2.55, 2.48 mg per ml respectively) dissolved in propylene glycol for six groups, while one group received vehicle (propylene glycol) only. Animals were fast for 2 hours, allowed to feed 2 hours, then were fast for another 20 hr and was given another two doses in second and third days. In the fourth day, animals were anaesthetized with diethyl ether, sacrificed, the stomach was removed, opened along with the greater curvature and rinsed with saline 0.9%. The number of mucosal damage (red spot) were counted using magnifying lens and anatomical microscope, then their severity (ulcerogenicity severity) was graded by mean from 0 (no lesion) to 4 (exceptional severe lesion).

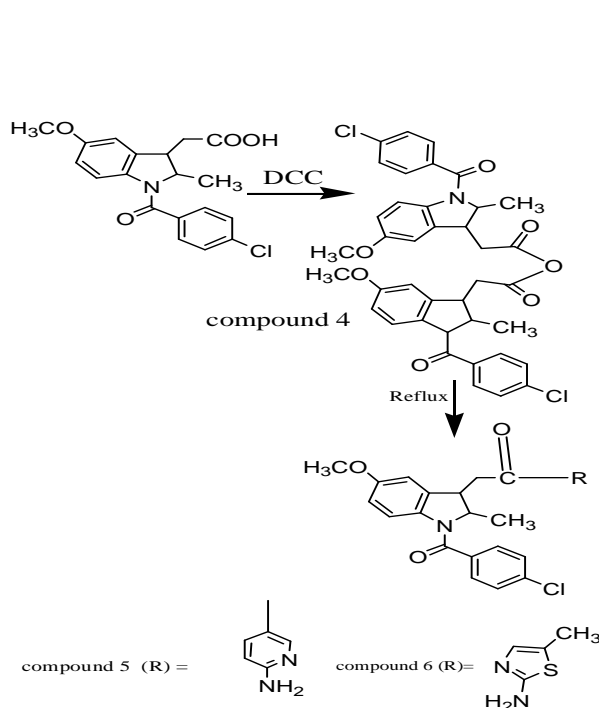
For each stomach specimen, the mucosal damage was assessed and the number of ulcers was noted and the severity was recorded with the following scores: 0.0 = no ulcer, -1.0 = superficial ulcers, -2.0 = severe ulcers, -4.0 = perforation. [17]

2.2.3 Statistical Method :

Students t-test will be used to make comparisons with respect to baseline, while comparisons between different groups at specified time will be done using analysis of variance (ANOVA). P values less than 0.05 were considered significant. This was done by using SPSS version 17.0 (SPSS Inc. Chicago, IL, USA) [18].



scheme(1)



scheme (2)

RESULTS AND DISCUSSION

3.1. Chemistry

The chemistry underlying the scheme showed conversion of carboxylic acid group of mefenamic acid to amide group by conjugating the selected moiety of heterocyclic compound which produce new potential non-steroidal anti-inflammatory agents. Mefenamic acid and indomethacin react with dicyclohexylcarbodiimide to give corresponding

anhydrides compound [1,4] respectively in methylene dichloride as solvent. The Coupling of the anhydride 1 and 4 with amino group of heterocyclic compounds presence of Zn⁺² dust as catalyst to accelerate the compounds formation . This reaction is an example of nucleophilic reaction in which the nucleophile (–NH₂) is added to carbonyl carbon of anhydride in slightly acidic media (by adding glacial acetic acid) and presence of zinc as catalyst. The designed compounds have been synthesized successfully as shown in Scheme (1) and their structures were confirmed, using elemental microanalysis (CHN), infrared spectroscopy (IR spectra) and their purity was confirmed by their physical data (melting points and R_f values).

The conversion of carboxylic acid group of mefenamic acid and indomethacin into amide group by conjugating the selected moiety of heterocyclic compound may produce (2-amino-5-methylpyridine, 2- Amino-5-methyl -1,3,-thiazole) new non-steroidal anti-inflammatory agents with expected selectivity toward COX-2 inhibition and hence less gastric irritation. The anti-inflammatory activity was determined by the induced paw edema method in the rats. According to the method, the particular values for % paw edema inhibition displayed in figure(1) were 40.9%, 46.64%, 51.5% , 49.78% and 45.57 % in compounds 2, 3, 5, 6 and diclofenac respectively . Compared with diclofenac as reference agent and its inhibition percent was 45.74 % . all tested compounds showed better anti-inflammatory activity .

The most potent anti-inflammatory compound were compounds (5 and 6), followed by compounds 3, 2. The effect might be due to the attributing of conjugate heterocyclic rings 2-amino-5-methyl-pyridine and 2-amino-5-methyl-1,3, -thiazole to the parents agent. These heterocyclic rings might incorporated into the side pocket of COX-2 enzyme ,so, achieving a good anti-inflammatory activity toward COX-2 inhibition with less GIT side effect [19] .

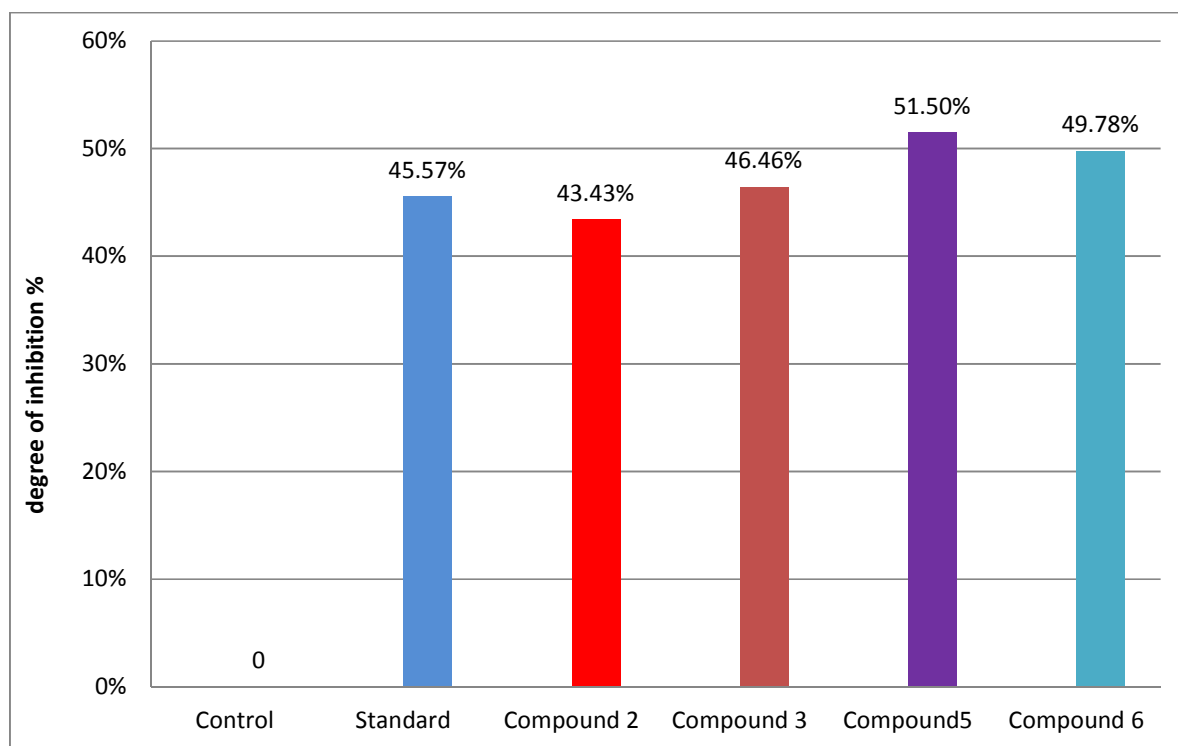


Figure 1: Show %inhibition of edema for diclofenac Na(as standard), compounds 2,3,5 and 6

The NSAIDs such as indomethacin showed effective anti-inflammatory activity with profound ulcerogenic side effects. while highly selective COX-2 inhibitors such as celecoxib has least GI side indicated mild toxicity on gastric mucosa. This property of celecoxib was attributed to COX2 selectivity [20]

The ulcerogenic potential of tested compounds 2,3,5 and 6 were evaluated through acute ulcerogenicity study in which the number of mucosal damage (red spots) were counted using anatomy microscopy (40X) and their

ulcerogenicity was scored by mean from 0 (no lesion) to 4 (exceptional sever lesion) then the ulcer index was calculated. The obtained data in table (2) displayed in figure (2) showed varying degree of ulcerogenic capabilities in which compound (3) showed the least ulcerogenic in compared to Celecoxib (selective COX-2)inhibitor) followed by compound 2, the most compound having a high percent of ulcer index is compound(5 ,6). The ulcer index for tested compounds (2,3,5 and 6), Indomethacin, and Celecoxib as reference are as (5.9 , 5.2 , 11,11.6 ,17.6 and 5.8) respectively .

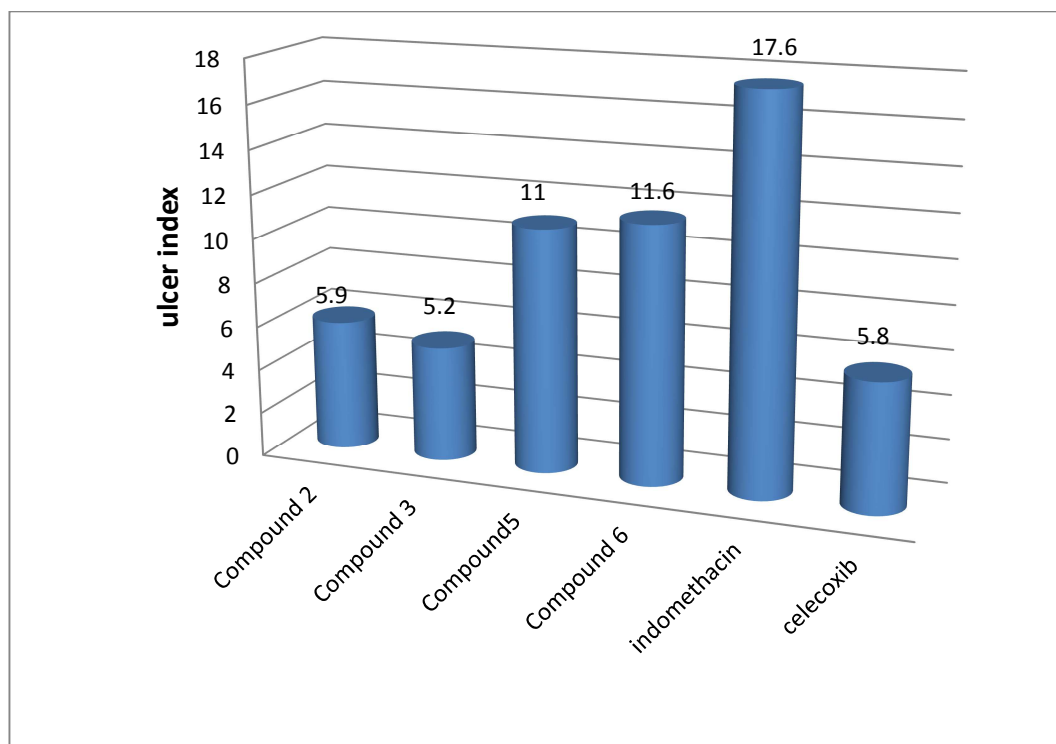


Figure 1: Show ulcer index for indomethacin, celecoxib, compounds 2,3,5 and 6

CONCLUSION

The derivatives of mefenamic acid and indomethacin were successfully synthesized and screened for their anti-inflammatory and ulcerogenic activities . It found that the results obtained support our hypothesis in which , the conversion of free carboxylic group of NSAID to amide group by selective heterocyclic rings lead to increase COX2 selectivity. The preliminary pharmacological evaluation has been found that compounds (5 and 6), were showed the most potent anti-inflammatory activity followed by compounds (3 , 2) .

Compound (3) showed the least GIT side effects (ulcerogenic), followed by compound 2 compared with Celecoxib as high selective COX-2 inhibitor.

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