



Synthesis and preliminary evaluation of potential highly selective COX-2 inhibitors of some nonsteroidal anti-inflammatory derivatives

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ABSTRACT

This work include design and synthesis of novel nonsteroidal anti-inflammatory(NSAI) derivatives, with potential selectivity cyclooxygenase 2(COX- 2) inhibitors from well-known NSAIDs ,to increase or at least maintain anti-inflammatory activity, and decrease adverse effects resulting from COX-1 inhibition .Six compounds were synthesized ,(compound 2 and 3) from aspirin with 4-(4-Fluorophenyl)isoxazol-5-amine and 6-chloro,2-aminobenzothiazole, (compound 6 and 7) from diflunisal with 4-(4-Fluorophenyl)isoxazol-5-amine and 3-methyl-4-[3-(trifluoromethyl) phenyl]-5-isoxazolamine, (compound 9 and 10) from Ketoprofen with 2-Amino-5-(trifluoromethyl)1,3,4-thiadiazole and 2-Amino-5-ethyl 1,3,4-thiadiazole. Analysis by IR and ¹H-NMR were performed and consistent with proposed synthesized structures. The preliminary pharmacological evaluation as anti-inflammatory activity test and ulcerogenic index screening were performed. From results of both test, we see most derivative showed good anti-inflammatory activity with range from 46.5%(compound 2) to 36.2% (compound 9) compared to 41% (standard) as %paw edema inhibition, but compound 2 and compound 6 showed ulcer index analogous to celecoxib a selective COX-2 inhibitors as a safe standard of gastric irritation.

Key words: NSAIDs, anti-inflammatory, COX- 2 inhibitors.

INTRODUCTION

Inflammation defined as a complex series of tissue changes that result in pain and fever [1]. Inflammation is the body's effect to inactivate or destroy invading organisms, remove irritation, and set the stage of tissue repair[2]. Inflammation can be divided into three phases; acute, chronic and immune response [3]. There are two cyclooxygenase (COX) enzymes, COX-1 and COX-2. COX-1 is a constitutive enzyme, involved in tissue homeostasis, while COX-2 is induced in inflammatory cells and produces the prostanoids mediators of inflammation. Also COX-3 has recently been described [4]. Although COX-1 and COX-2 have similar structures, there are slight differences that affect the drug binding and lead to different actions .Both enzymes have a long narrow channel into which arachidonic acid enters and be converted into PGs, with COX-2 has an additional side pocket. Selective COX-2 inhibitors have chemical structure with rigid side extension that binds in this side pocket[5]. The present study will conduct to design, synthesize and preliminarily evaluate new Aspirin , diflunisal and Ketoprofen derivatives (figure 1) as potential highly selective COX-2 inhibitor and more safely derivatives of these drugs. The conversion of Carboxyl group of these drugs to carboxamide group and conjugating with specify selected moiety of heterocyclic compounds may impart effect toward selective COX-2 inhibitors with lower side

effects, because these conjugates will make Aspirin, diflunisal and Ketoprofen are similar that of isosteric functional group of previous Coxibs and its derivatives with selective COX-2 inhibitors.

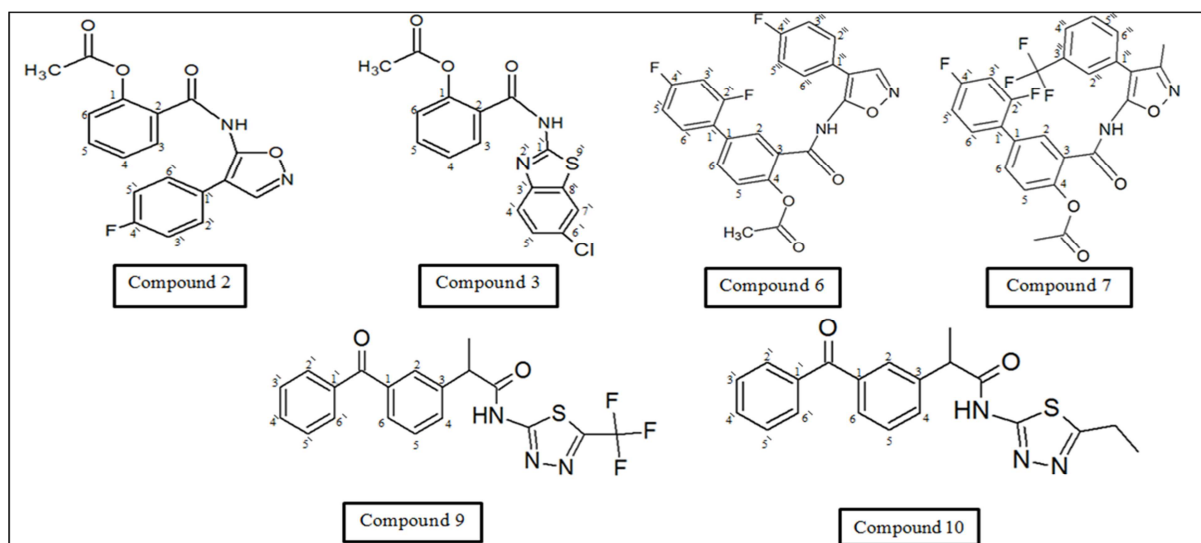


Figure 1: The proposed synthesized derivatives

EXPERIMENTAL SECTION

1.1 Chemicals:

Aspirin crystalline powder, Ketoprofen fine powder and Diclofenac Sodium crystalline powder it were purchased from Modern Yemeni Pharma. Co., Diflunisal crystalline powder it was gift from Ram Pharmaceutical Co. Jordan, 4-(4-Fluorophenyl)isoxazol-5-amine; 2-amino-6-chloro-1,3-benzothiazole; 3-methyl-4-[3-(trifluoromethyl)phenyl]-5-isoxazolamine; 2-Amino-5-(trifluoromethyl)-1,3,4-thiadiazole; 2-Amino-5-ethyl-1,3,4-thiadiazole and N,N-Dicyclohexylcarbodiimide (DCC) were purchased Apollo scientific chemicals U.K.; all other chemicals are analytical grades.

1.2 Equipment:

Melting points were determined by using a calibrated STUART SMP11 (U.K.) melting apparatus. 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, Yemen. ($^1\text{H-NMR}$) 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. The progression of reaction was checked with TLC Kieselgel GF₂₅₄ (type 60) to make sure the completion of reaction.

1.3 Chemistry Synthesis:

Aspirin anhydride (1):

Aspirin, (10 g, 55.5 mmol) was dissolved in 150 ml methylene chloride and dicyclohexylcarbodiimide (DCC) (5.72 g, 27.7 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 oily product was formed to yield the desired anhydride (90% yields) [6].

2-[[4-(4-fluorophenyl)isoxazol-5-yl]carbamoyl]phenyl acetate (2)

Compound (1) (2.5 g, 7.3 mmol), 4-(4-Fluorophenyl)isoxazol-5-amine (1.3005 g, 7.3 mmol), zinc dust (0.0075 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) are placed in 100 ml round bottom flask, equipped with reflux condenser, and boiling stones were added. The reaction mixture was refluxed for about 3 hr. with continuous stirring. The solvent was evaporated under vacuum; the residue was dissolved in ethyl acetate, then washed with NaHCO₃ (10%, 3X), HCl (1N, 3X) 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 [7]. Then the mixture was filtered and the precipitate was collected to give compound (2) as white needle crystal (23% yield) M.p. 220-223°C, $R_f = 0.36$, IR (KBr, cm^{-1}): 3327 (NH, amide), 3037 (CH, ArH), 1771 (C=O, ester), (2928,2850 st.vib. of C-H, ester) 1629 (C=O, amide), 1600,1576, 1436 (Ar). $^1\text{H-NMR}$ (DMSO.d6) δ pmm : 2.81 (s,3H,-CH₃ of OC=O-CH₃),8.05(s, 1H, CONH,H exchangeable with D2O),7.78(s, 1H,CH=N, Isoxazol),7.61(d,2H,2 -CH= at position 3` and 5`),7.61(d,2H,2 -CH= at position 2` and 6`),7.39-7.45 (m,4H, Ar.H Acetate).

2-[(6-chloro-1,3-benzothiazol-2-yl)carbamoyl]phenyl acetate (3)

Compound (1) (2,5g, 7.3 mmol) , 6-chloro,2-aminobenzothiazole (1.35g,7.3 mmol), zinc dust (0.0075 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) are placed in 100 ml round bottom flask, were prepared as previously described in (2) to liberate compound(3) as white cotton crystals (38% yield). Mp. 188-191° C, $R_f = 0.42$, IR (KBr, cm^{-1}): 3376 (NH, amide), 3086 (CH, ArH), 1761 (C=O, ester) 1672 (C=O, amide), 1610, 1527, 1461, (Ar),(2928,2850st.vib. of C-H, ester). $^1\text{H-NMR}$ (DMSO.d6) δ pmm: 2.71(s,3H,-CH₃ of OC=O-CH₃),8.04(s,1H,CONH, H exchangeable with D2O),7.11-7.59 (m,4H of Ar.H Acetate),7.62-7.63(m,2H, at position 4` ,5` of benzothiazole),7.71(s, 1H at position 7` of benzothiazole).

5-(2,4-Difluorophenyl) acetylsalicylic acid (4)

A dry Diflunisal, (10 g, 40 mmol) was placed in 200 ml round conical flask. Acetic anhydride (25 ml, 262 mmol) was added, and 5 drops of concentrated sulfuric acid added drop wise, mixing the contents by rotating the conical flask for 5 minutes, warm in water bath to about 50-60 °C, with stirring for 30 minutes. The reaction mixture was allowed to cold with occasional stirring, and then cold distilled water was added until precipitate was formed, and filtered by using suction pump, washed with cold distilled water several times, and the crude product was collected[8]. Recrystallization was carried out by using ethanol 95%.the precipitate was collected and dried to give compound (4) as a white fine crystals 91 % yield.

5-(2,4-Difluorophenyl)-acetyl salicylic acid anhydride(5)

Compound(4), (8 g, 27.376 mmol) is dissolved in (150 ml) methylene chloride; dicyclohexylcarbodiimide (D.C.C.) (2.824 g, 13.688 mmol) was added. The reaction mixture was continuously stirred at room temperature for about 3 hrs. A white precipitate of dicyclohexylurea was formed and removed by filtration[9]. The solvent was evaporated under vacuum; a solid product will obtained to give(85%yield) as desired anhydride (5).

2',4'-difluoro-3-[[4-(4-fluorophenyl)isoxazol-5-yl] carbamoyl]biphenyl-4-yl acetate (6)

Compound (5) (2,5g, 4.414 mmol) , 4-(4-Fluorophenyl)isoxazol-5-amine (0.786 g,4.414 mmol), zinc dust (0.004 g), 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 needle crystal. 24% yield, Mp. 219-222 °C, $R_f = 0.32$, IR (KBr, cm^{-1}) 3326 (NH, amide), 3036 (CH, Ar-H), 1711 (C=O, ester) (2928,2850 st.vib. of C-H ester) ,1640 (C=O, amide) 1626, 1537, 1436 (Ar). $^1\text{H-NMR}$ (DMSO.d6) δ pmm: 2.12 (s,3H,-CH₃ of OC=O-CH₃), 8.06(s, 1H, CONH,H exchangeable with D2O),7.89 (s, 1H at position 3`of di-F Ar-H), 7.014-7.016 (m, 2H at position 5` and 6` of di-F Ar-H), 7.52(s, 1H at position 2), 6.96-6.99 (m ,2H, at position 5, 6), 7.44 (d, 2H of 2",6"),7.50(d, 2H of 3",5"), 7.66 (s, 1H,CH=N, Isoxazole).

2',4'-difluoro-3-[[4-(3-trifluoromethyl-phenyl)3-methyl-isoxazol-5-yl] carbamoyl]biphenyl-4-yl acetate (7)

Compound (5) (2,5g, 4.414 mmol) , 3-methyl-4-[3-(trifluoromethyl)phenyl]-5-isoxazolamine (1.096 g,4.414 mmol), zinc dust (0.004 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (30 ml) were placed in 100 ml round bottom flask, were placed in 100 ml round bottom flask ,were prepared as previously described in (2) to generate compound (7) as a white long needle crystal (27 % yield). Mp. 217- 220°C, $R_f = 0.37$.IR (KBr, cm^{-1}): 3327 (NH, amide), 1626 (C=O, amide), 3036(CH -ArH), 1713 (C=O, ester), (2930,2853 st.vib. of C-H ester) 1610, 1536, 1460 (Ar). $^1\text{H-NMR}$ (DMSO.d6) δ pmm:2.05 (s,3H,-CH₃ of OC=O-CH₃), 2.47 (s, 3H,-CH₃, Isoxazole), 8.06(s, 1H, CONH,H exchangeable with D2O),8.16 (s, 1H at position 3`of di-F Ar-H), 7.012 -7,014 (m,2H at position 5` and 6` of di-F Ar-H),7.52(s, 1H at position 2), 6.98-6.99 (m , 2H, at position 5, 6), 7.03 (s, 1H,CH at position 2"), 7.12-7.17 (m, 3H at position 4",5" and 6").

Ketoprofen anhydride (8)

Ketoprofen, (10 g, 39.325 mmol) was dissolved in 150 ml methylene chloride and dicyclohexylcarbodiimide (DCC) (4.056 g, 19.663 mmol) was added. The reaction mixture was continuously stirred at room temperature for 2.5 hrs.

A white precipitate of dicyclohexylurea was formed and removed by filtration. The solvent was evaporated under vacuum[6], and an oily product was formed to yield the desired anhydride(8).

***N*-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl] 2-[3-(phenylcarbonyl) phenyl]- propanamide (9)**

Compound (8) (3g., 6.116 mmol) , 2-Amino-5-(trifluoromethyl)-1,3,4-thiadiazole (1.034g. 6.116 mmol), zinc dust (0.006 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (40 ml) are placed in 100 ml round bottom flask, were prepared as previously described in (2) to generate compound (9) as a white crystal powder. (46 % yield), Mp. 155-158°C, $R_f = 0.29$, IR (KBr, cm^{-1}): 3370 (NH, amide), 1925 (C=O ,amide),1699 (C=O, ketone) , 2927 and 2870 ($-\text{C}_2\text{H}_5$ aliphatic chain), 3031(CH -ArH), 1609, 1552, 1489 (Ar). $^1\text{H-NMR}$ (DMSO. d_6) δ pmm: 1.42 (d,3H,-CH₃ of propanamide) , 4.04 (q ,1H,-CH of propanamide),7.91 (s, 1H, CONH, H exchangeable with D2O),7.21 (s, 1H , position 2),7.12 and 7.17 (d, 2H , -CH= and -CH= at position 4 and 6),7.40 (m, 1H,-CH= position 5) 7.71 (d, 2H, 2 -CH=,at 2` and 6` position),7.50-7.52 (m, 3H ,3 -CH= , at 3` , 4` and 5` position).

***N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-[3-(phenylcarbonyl)phenyl] propanamide (10)**

Compound (8) (3g., 6.116 mmol) , 2-Amino-5-ethyl-1,3,4-thiadiazole (0.790g.. 6.116 mmol), zinc dust (0.006 g), glacial acetic acid (0.7 ml, 12.241 mmol) and dioxane (40 ml) are placed in 100 ml round bottom flask, were prepared as previously described in (2) to generate compound (10) as a White fine crystal (43 % yield). Mp. 161-163°C, $R_f = 0.12$, IR (KBr, cm^{-1}): 3162 (NH, amide), 1662 (C=O of amide) , 1692 (C=O of ketone) , 2931 and 2890 ($-\text{C}_2\text{H}_5$ aliphatic chain), 3031(CH -ArH), 1600, 1558, 1460 (Ar). $^1\text{H-NMR}$ (DMSO. d_6) δ pmm: 1.19 (t,3H,-CH₃ of ethyl-isoxazole) , 2,90 (q, 2H , -CH₂ of ethyl-isoxazole), 1.43 (d,3H,-CH₃ of propanamide),4.05 (q ,1H,-CH of propanamide),8.19 (s, 1H, CONH, H exchangeable with D2O),7.37 (s, 1H , position 2),7.30 and 7.33 (d, 2H , -CH= and -CH= at position 4 and 6),7.42 (m, 1H, -CH= position 5) 7.70 (d, 2H, 2-CH=,at 2` and 6` position),7.50 - 7.52 (m, 3H ,3 -CH= , at 3` , 4` and 5` position).

2. Pharmacology

2.1 Animals:

The adult male albino rats weighing 200 ± 20 , supplied by the animal house of the pharmacy college, university of Sanaa were used in this study. Animals were kept under standardized conditions (12 light-12 dark cycle) for 5 days for acclimatization, they were supplied with feed and water. Rats were brought 3 hour before performing the experiment to the laboratory, they were divided into 8 groups(for anti-inflammatory activity test) and 9 groups (for ulcerogenic index test) each group of 5 rat, All the animal experiments were performed by following the approval of study protocols by the Research Animals Ethics Committee, UST(MECA No. 2016/1), the doses of standards and prepared compounds have to be calculated in equimolecular dose of Indomethacin to rat its weight $400\text{mg} = 0.051*50\text{ mg}$ [10].

2.2 Experimental Design:

2.2.1 Anti-inflammatory Activity Teste:

Ovalbumin Paw Edema Method:

Animals are divided into eight groups ($n= 5$) starved overnight with water ad libitum prior to the day of experiment. The control group were treated intraperitoneally with 0.2ml of vehicle only(DMSO),other six animals groups were treated I.P with tested agents(2,3,6,7,9 and 10) with (2.31, 2.35, 3.07, 3.50, 2.75 and 2.48 mg. / 0.4 kg.) respectively, and the other 8th animals group were injected I.P with standard Diclofenac sodium in dose (2.16 mg /0.4 kg.) .Then ,one hour after dosing, the animals are challenged by a subcutaneous injection of 0.1ml of Ovalbumin [11] into the sub-plantar side of the left hind paw. The animals were anaesthetized with Chloroform , at 2 hours after challenge then paw is cute, its weight is measured compared with 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, Diclofenac , and tested compounds 2,3,6,7,9 and 10 respectively.

Calculations (Paw Edema and % edema inhibition)

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

$$W.D. = WR - WL$$

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

WR= weight of edema of right hind paw

WL= weight of edema of left hind paw

- % edema inhibition was calculated by the following formula:-
% edema inhibition = [1- (WT / WC)] X 100

Where: WT = weight difference of edema of tested animals WC= weight difference of edema of control animals[12].

2.2.2 Ulcerogenic Index screening:

Animals were divided into nine groups (n = 5). Animals were fasted 20 hr. before drug administration .The synthesized agents (2,3,6,7 ,9 and 10 compounds), Celecoxib and Indomethacin. were given orally in a dose of (2.31 , 2.35 , 3.07 , 3.50 , 2.75 , 2.48 , 2.59 and 2.55 mg. / ml. respectively) dissolved in propylene glycol 50%v/v for eight groups, while 9th group received vehicle (propylene glycol50%v/v) only, animals were fasted for 2hr ,allowed to feed for 2 hr. then fasted for another 20 hr. and given another two doses in the second and third days .In the fourth day , animals were anaesthetized with chloroform, 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 by Sargent welch microscopic Anatomy (40X) and their severity (ulcerogenicity severity) was graded by mean from 0 (no lesion) to 4 (exceptional sever lesion)[13].

Calculations:

1- % incidence/10=(no. of animals showing ulcer divided by total no. of animals in 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)[7] .

Score assignment: Zero = for normal (no injury), 1= latent small red spot, 2= wide red spot, 3= slight injury, and 4= sever injury.

2.2.3 Statistical Method:

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).

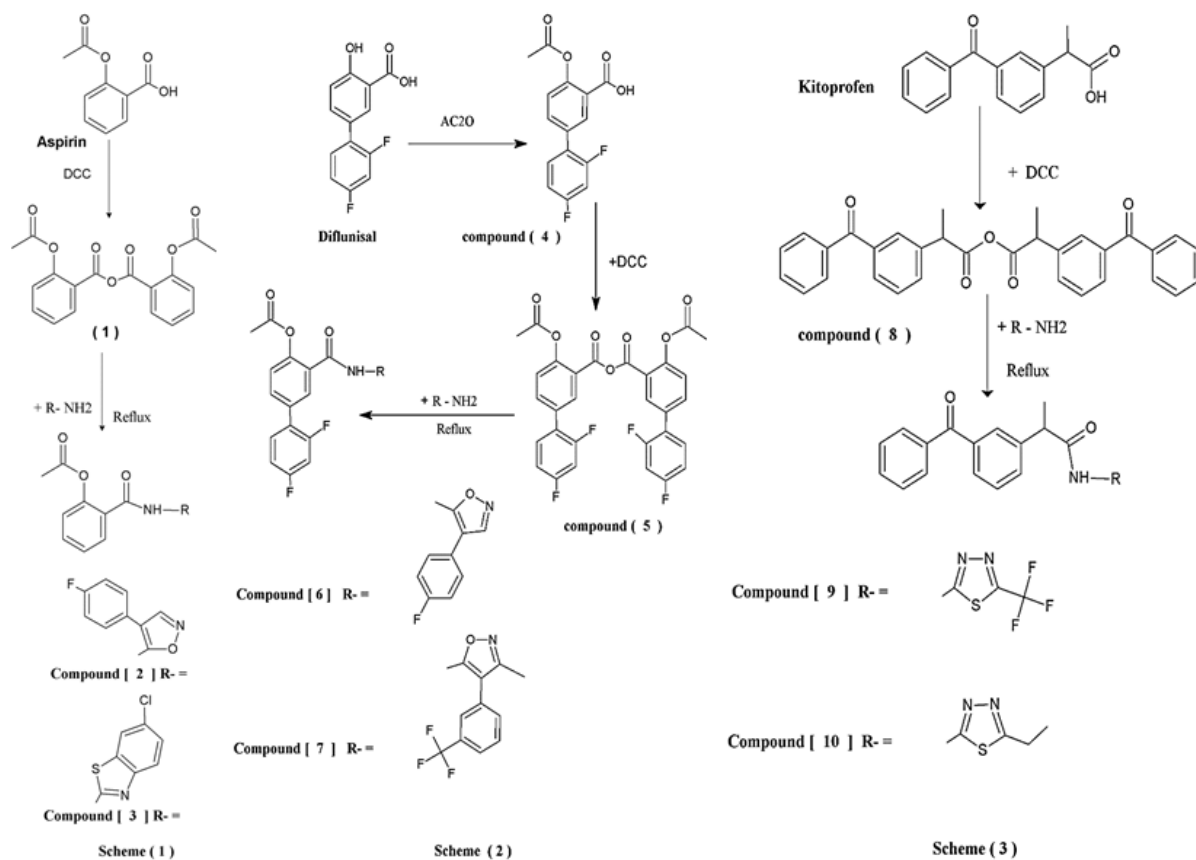
RESULTS AND DISCUSSION

3.1 Chemistry:

The synthetic methods for preparation of the proposed derivatives are outlined in schemes (1 ,2 and 3). Aspirin and Ketoprofen were converted to the corresponding anhydrides (1 , 8) through reaction with dicyclohexylcarbodiimide (DCC) as coupling reagent in methylene dichloride[6,14]. Conversion of the (1 , 8) to their amides (2,3,9 and 10) upon treatment with various amino substituted heterocyclic rings in the presence of Zn⁺² dust as catalyst to accelerate the compounds formation.

Diflunisal derivatives (6 and 7) were prepared according to the method displayed in scheme (2). Acylation of the phenolic group in diflunisal with acetic anhydride as protecting group to prevent the interference of the phenolic group in subsequent reactions to give compound (4). Compound (4) was converted to its corresponding anhydride(5) upon treatment with(DCC) in acidified methylene chloride.

The acylated diflunisal anhydride (5) upon treatment with various selected amino substituted heterocyclic rings, yielded the amide derivatives of acylated diflunisal (6 and 7).IR , H-NMR analysis were consistent with assigned structures as shown in the experimental part, and their purity was confirmed by their physical data (melting points and R_f values).



3.2 Pharmacology:

Table(1) shows the biological evaluation of the test compounds as indicated by % paw edema inhibition. According to the method, the respective values for % paw edema inhibition displayed in figure(2) were 46.5%, 46.2%, 44.3%, 42.6%, 41.%, 40.9% and 36.2 %, compounds 2, 10, 7, 6, diclofenac, 3 and 9 respectively. The most potent anti-inflammatory compound were compounds (2 and 10), followed by compounds 7, 6, diclofenac, 3 and 9.

The ulcerogenic potential of tested compounds 2,3,6,7,9 and 10 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 (3) showed varying degree of ulcerogenic potentialities in which compounds (2) and (6) showed the least ulcerogenic in comparing to Celecoxib (selective COX-2 inhibitor) followed by compounds (3, 7, 9), the most compound have a high percent of ulcer index is compound (10). The ulcer index for tested compounds (2,3,6,7,9 and 10), Indomethacin, and Celecoxib as reference are showed in table (2) as following (5.8, 8.05, 5.6, 8.3, 8.4, 10, 17.4 and 5.9) respectively.

From results of both tests i.e. anti-inflammatory and ulcerogenic activity, we see all synthesized compounds showed good anti-inflammatory activity with the range between 46.5% for 2-[[4-(4-fluorophenyl)isoxazol-5-yl]carbamoyl]phenyl acetate (compound 2) and 36.2% for N-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl] 2-[3-(phenylcarbonyl) phenyl]- propanamide (compound 9) as % paw edema inhibition comparing to standard Diclofenac Na., while 2-[[4-(4-fluorophenyl)isoxazol-5-yl]carbamoyl]phenyl acetate (compound 2) and 2',4'-difluoro-3-[[4-(4-fluorophenyl)isoxazol-5-yl] carbamoyl]biphenyl-4-yl acetate (compound 6) show ulcer index analogous to selective COX-2 inhibitors Celecoxib as a safe standard of gastric irritation, but N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-[3-(phenylcarbonyl) phenyl] propanamide (compound 10) show the highest value of ulcer index (10) comparative to non-selective COX-2 TNSAIDs as a strong irritating drug standard Indomethacin.

The anti-inflammatory effect of the tested compounds compared with reference drug compatible with many comparative studies involve selective COX-2 inhibitors and TNSAIDS in treatment of inflammatory condition, in which COX-2 inhibitors such as Rofecoxib and Celecoxib showed promised equivalent efficacy [15].

The first main and ultimate goal of many newly synthesized nonsteroidal anti-inflammatory drugs is the performance of adequate therapeutic effects with lowest possible undesirable side effect. It is well established that most of therapeutically desirable effect of anti-inflammatory drugs is attributed to inhibition of COX-2 enzyme to the inflammatory prostaglandin synthesis. On the contrary inhibition of COX-1 enzyme may be responsible for undesirable side effects (peptic ulceration and nephrotoxicity). TNSAIDS such as Indomethacin are non-selective, COX-1 and COX-2 inhibitors, they have low margin of safety[12].

2-[[4-(4-fluorophenyl)isoxazol-5-yl]carbamoyl]phenyl acetate (compound 2) showed maximal therapeutic anti-inflammatory action with little gastric effects, and 2',4'-difluoro-3-[[4-(4-fluorophenyl)isoxazol-5-yl]carbamoyl]biphenyl-4-yl acetate (compound 6) showed good anti-inflammatory activity in comparative to standard with lowest ulcer index, the bulky structure of (2 and 6) derivatives which have same heterocyclic nucleus(4-(4-Fluorophenyl)isoxazol-5-amine) may enhance a good liability to occupy the side pocket of COX-2 enzyme with maintenance of anti-inflammatory activity.

Table1: The % paw edema inhibition of Control ,Diclofenac, Compounds 2,3,6,7,9 and 10

| Group | Mean of Difference weights paw of 5 rats | S.D. | % Inhibition | P* | P** |
|-------------|--|----------|--------------|--------|--------|
| Control | 0.438 | 0.101666 | - | - | - |
| Standard | 0.258 | 0.087878 | 41 | < 0.01 | < 0.01 |
| Compound 2 | 0.234 | 0.075980 | 46.5 | < 0.01 | < 0.01 |
| Compound 3 | 0.258 | 0.023773 | 40.9 | < 0.01 | < 0.01 |
| Compound 6 | 0.251 | 0.038503 | 42.6 | < 0.01 | < 0.01 |
| Compound 7 | 0.243 | 0.047726 | 44.3 | < 0.01 | < 0.01 |
| Compound 9 | 0.279 | 0.044802 | 36.2 | < 0.01 | < 0.01 |
| Compound 10 | 0.235 | 0.025832 | 46.2 | < 0.01 | < 0.01 |

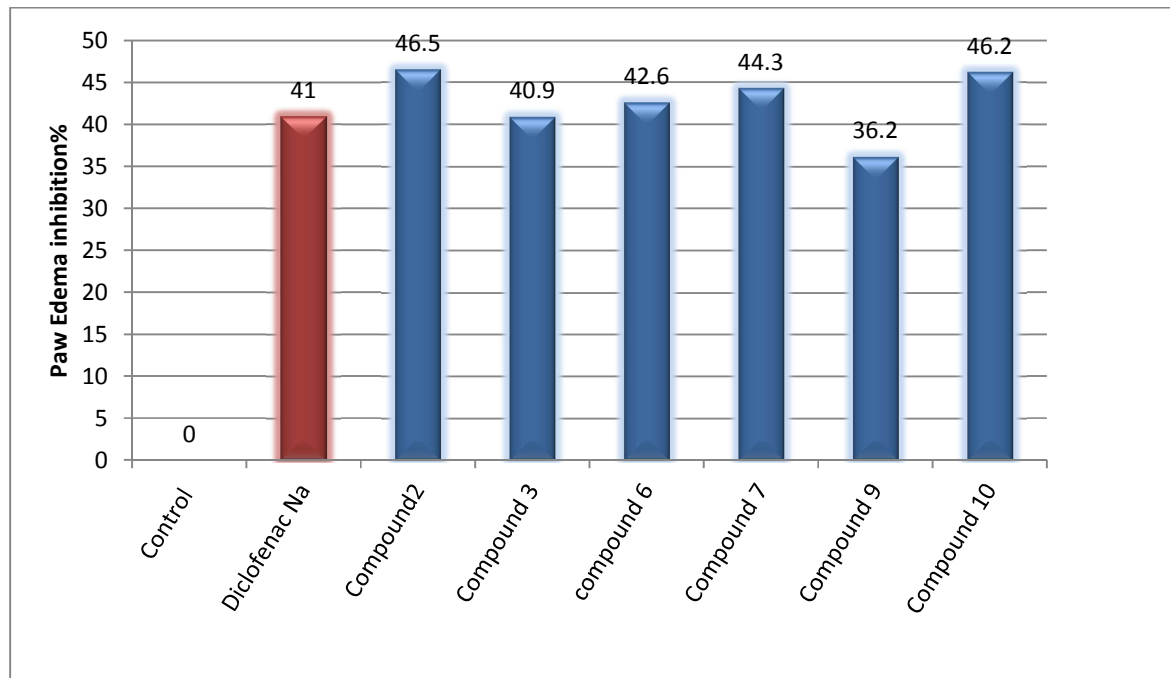


Figure 2: Graphic display of % Inhibition of edema of control, Diclofenac Na, compound 2,3, 6,7,9 and 10

Table 2: Ulcerogenic Index screening of control, Indomethacin, Celecoxib Compounds 2,3,6,7,9 and 10

| Group | Ulcer Index |
|---------------|-------------|
| Controls | 0 |
| Indomethacin | 17.4 |
| Celecoxib | 5.9 |
| compound (2) | 5.8 |
| compound (3) | 8.05 |
| compound (6) | 5.6 |
| compound (7) | 8.3 |
| compound(9) | 8.4 |
| compound (10) | 10 |

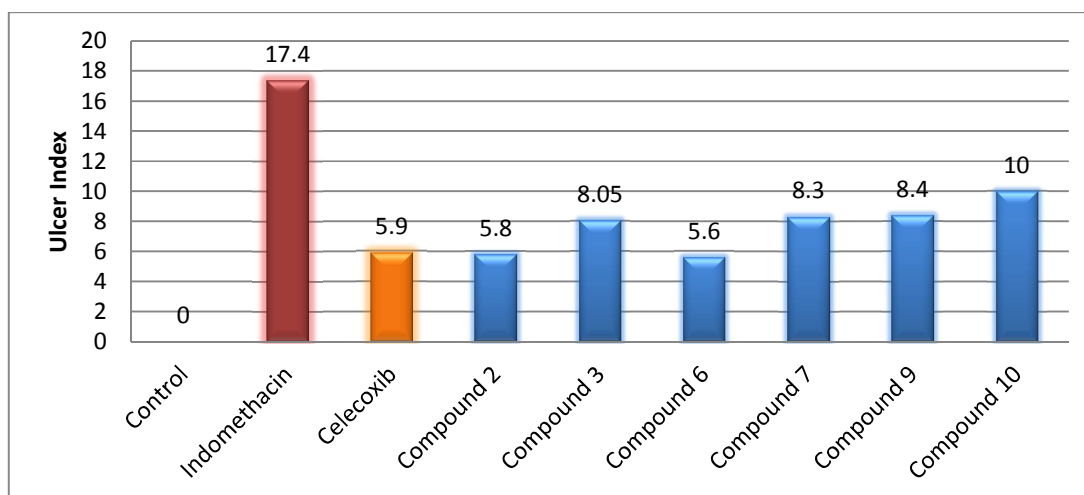


Figure 3: Graphic display of the ulcer index for control, indomethacin, Celecoxib, compounds 2,3,6,7,9 and 10

CONCLUSION

The conversion of carboxylic acid group of aspirin, diflunisal and Ketoprofen to carboxamide group by conjugating the selected moiety of heterocyclic may produce new non-steroidal anti-inflammatory agents with expected selectivity toward COX-2 inhibition and hence less gastric irritation. Preliminary evaluation has been found that compound 2 showed maximal therapeutic anti-inflammatory action with little gastric effects, and compound 6 showed good anti-inflammatory activity with lowest ulcer index, the bulky structure of 2 and 6 derivatives which have same heterocyclic nucleus (4-(4-Fluorophenyl)isoxazol-5-amine) may enhance a good liability to occupy the side pocket of COX-2 enzyme with maintenance of anti-inflammatory activity.

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