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Research Article

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Synthesis and anti-inflammatory activity of novel aspirin and ibuprofen amide derivatives

Ibrahim Alkabodi[1], Sadik Almekhlafi[1]* and Doa'a A. Ibrahim[2]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy, University of Science and Technology, Sana'a, Yemen*
[2]*Department of Pharmacology, Faculty of Pharmacy, University of Science and Technology, Sana'a, Yemen*

ABSTRACT

This study includes design and synthesis of new non-steroidal anti-inflammatory agents (NSAIDs) to achieve better activity and low gastric side effects. Two series of compounds have been designed and synthesized as potential NSAIDs, these are: aspirin derivatives (compounds 1, 2) and ibuprofen derivatives (compounds 3&4). 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 E2, 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 Aspirin and Ibuprofen that conjugated with 2-Amino-5 – ethyl —1, 3, 4-thiadiazole, and 2-Amino-5- trifluoromethyl -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 1 N-[5- ethyl -1,3,4-thiadiazole]- phenyl acetate showed maximal anti-inflammatory activity with less ulcero-genic effect, while compound 3 (2-(4-Isobutyl phenyl) -N-[5-ethyl-2-(1,3,4- thiadiazolyl)]–propamide) showed least ulcer indexes these effects may be refer to the presence of certain structural features of heterocyclic ring.

Key words: Anti-inflammatory, aspirin, Ibuprofen

INTRODUCTION

The non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most common therapeutic groups of agents used worldwide for the treatment of pain, fever and inflammation [1]. However, the usefulness of these agents is limited due to the higher incidences of the observed gastrointestinal (GI) damage that includes gastric ulceration, perforation and their associated complications [2]. These side effects are a result of two different mechanisms, where the first involves a local action comprising of a direct contact effect due to ion trapping mechanism that resulted from the acidic nature of these drugs and their behavior under the local moderately acidic or neutral condition of the stomach[3].The second mechanism which is considered as a key element in the NSAIDs-induced gastropathy is based on their generalized systemic action which follows their absorption and is related to their intrinsic effect in inhibiting the cyclooxygenases (an enzyme-dependent synthesis of prostaglandins that have gastro-protective properties) responsible for their desired anti-inflammatory activity [4]. Traditional

NSAIDs differ in their relative inhibitory potency against two isoforms of COX: COX-1 and COX-2. The greatest degree of damage is generally caused by NSAIDs that are preferential COX-1 inhibitors and contain a free carboxylic group e.g. aspirin, indomethacin, ibuprofen etc[5]. The objective of this study, to synthesis new anti-inflammatory derivatives of aspirin and Ibuprofen as potential selective COX–2 inhibition with less ulcero-genic effect based on drug development. 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 and ibuprofen are similar that of isosteric functional group of previous Coxibs and its derivatives with selective COX-2 inhibitors.

EXPERIMENTAL SECTION

1.1 Chemicals:

Aspirin crystalline powder, Ibuprofen fine powder and Diclofenac Sodium crystalline powder it were purchased from ModernYemeniPharma.Co.2-Amino-5-(trifluoromethyl) 1,3,4thiadiazole ; 2-Amino-5-ethyl-1,3,4-thiadiazole and N,N Dicyclohexylcarbodimide (DCC)were purchased Apollo scientific chemicals U.K.; all others 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-I.R. PerkinElmer spectrometers (USA). and were performed in the Center of Research and Pharmaceutical Studies(CRPS) University of Science and Technology, Yemen.(^{1}H–NMR) spectra were carried out on, JEOL500 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 Kiesel gel GF_{254} (type 60)to make sure the completion of reaction.

1.3 Chemistry Synthesis:

Aspirin anhydride (1):

Aspirin,(10g,55.5mmol)was dissolved in 150 ml methylenechloride and dicyclohexylcarbodimide (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 (92% yields)[6].

N-[5- ethyl -1,3,4-thiadiazole]- phenyl acetate (1)

Compound (A) (2,5g, 7.3 mmol) , 2-amino-5-ethyl -1,3,4-thiadiazole (0.94g,6.35 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 (IN, 3X) and 3 times with distilled water, and filtered over anhydrous sodium sulphate. The filtrate was evaporated and the recrystallization was carried out by re dissolved 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 (1) as white needle crystal (26% yield) M.p. 122-124°C, R_f = 0.31, IR (KBr, cm^{-1}): 3159 (NH, amide), 3037 (CH, ArH), 1743(C= O, ester), (2928,2850 st.vib. of C-H ,ester) 1690 (C=O, amide), 1600,1576, 1436 (Ar.). ^{1}H–NMR(DMSO.d6) δ pmm : 2.6 (s,3H,-CH_3 of OC=O-CH_3),7.96(s, 1H, CONH,H exchangeable with D_2O), 6.93- 7.19 (m,4H of Ar.H Acetate), 1.19 (t,3H,- CH3 of ethyl-thiazole), 2,90 (q, 2H , -CH2 of ethyl-thiazole).

N-[5-tri fluoro methyl-1,3,4-thiadiazole) phenyl acetate (2)

Compound (A) (2,5g, 7.3 mmol) , 2-amino-5-(trifluormethyl)-1,3,4- thiadiazole (1.23g.6.33 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 (1) to liberate compound(2) as white cotton crystals (38% yield). Mp. 136-138° C, Rf = 0.42, IR (KBr, cm-1): 3308 (NH, amide), 3030 (CH, ArH), 1721 (C= O, ester) 1640 (C=O, amide), 1640, 1520, 1451, (Ar),(2928,2850st.vib. of C-H ,ester). 1H–NMR(DMSO.d6) δ pmm : 2.81 (s,3H,-CH3of OC=O-CH3),8.05(s, 1H, CONH,H exchangeable with D2O), 7.11-7.59 (m,4H of Ar.H Acetate).

Ibuprofen anhydride (b)

Ibuprofen(3.52g,17.11mmol)wasdissolvedin50mlmethylenechlorideanddicyclohexylcarbodimide (DCC) (1.76 g, 8.56 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3hra,than Awhite 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 (B).

2-(4-Isobutyl phenyl) - N-[5-ethyl -2- (1, 3, 4- thiadiazolyl)]–propamide (3)

Compound (B) (2.5g., 6.116 mmol) , 2-Amino-5-ethyl-1,3,4-thiadiazole (0.94g. 6.116 mmol), zinc dust(0.006 g), glacial acetic acid (0.7 ml, 12.241 mmol) and 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 NaHCO3 (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 C0) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the crystals was collected to produce compound (1) in 57.35% yield as white needle crystals[8, 9]. (46 % yield), Mp. 193-195°C. Rf = 0. 29 IR, (KBr, Cm-1) 3271 (NH, amide), 1650, 1600, 1552, 1489 (C=C, Ar), 1699 ((-NH-C=O, carbonyl). 2927 and 2870(-C2H5 aliphatic chain).

1H – NMR (DMSO.d6) δ ppm: 7.06(2H, d, at 2`&6` positions, Ar),7.42(2H, d, at 3`&5` positions, Ar), 7.93 (s,1H, CONH, H exchangeable with D2O), 0.84 (6H,CH, iso-but), 1.8 (1H, CH3iso-but) , 1.45 (3H, CH, Prop.) 3.98 (1H CH3,Prop.), 2.2 (s, 3H, COCH3) 1.19 (t,3H,- CH3 of ethyl thiadiazole), 2,90 (q, 2H , -CH2 of ethyl- thiadiazole).

2-(4-Isobutyl phenyl) - N-[5-trifluoromethyl -2-(1, 3, 4- thiadiazolyl)]–propamid (4)

Compound (B) (2.5 g, 6.35 mmol), 2-amino-5-(trifluormethyl)-1,3,4- thiadiazole (1.23 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 round bottom flask, were prepared as previously described in (2) to generate compound (4) as a White fine crystal (43 % yield).
Mp. 185-187. °C, Rf = 0.12, IR, (KBr, Cm-1) 3326 (NH, amide), 1699 (-NH-C=O, carbonyl). 1450, 1550, 1600, 1626 (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), 7.29 (s,1H, CONH, H exchangeable with D2O), 0.77 (6H,CH, iso-but), 1.73 (1H, CH3iso-but) , 1.375 (3H, CH, Prop.) 3.98 (1H, CH3,Prop.), 2.2 (s, 3H, COCH3).

2. Pharmacology

2.2 Animals:

The adult male albino rats weighing 200 ± 20, supplied by the animal house of the pharmacy college, university of Sana'a 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 6 groups(for anti-inflammatory activity test) and 7 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 400mg =0.051*50 mg [10].

2.2 Experimental Design:

2.2.1 Anti-inflammatory Activity Teste:

Ovalbumin Paw Edema Method:

Animals are divided into six 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 animals groups were treated I.P with tested agents(1,2,3, and 4) with (2.01 , 2.246, 2.151 and 2.422 mg. / 0.4 kg.) respectively, and the other 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 , at2 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 1,2,3 and 4 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

2.2.2 Ulcerogenic Index screening:

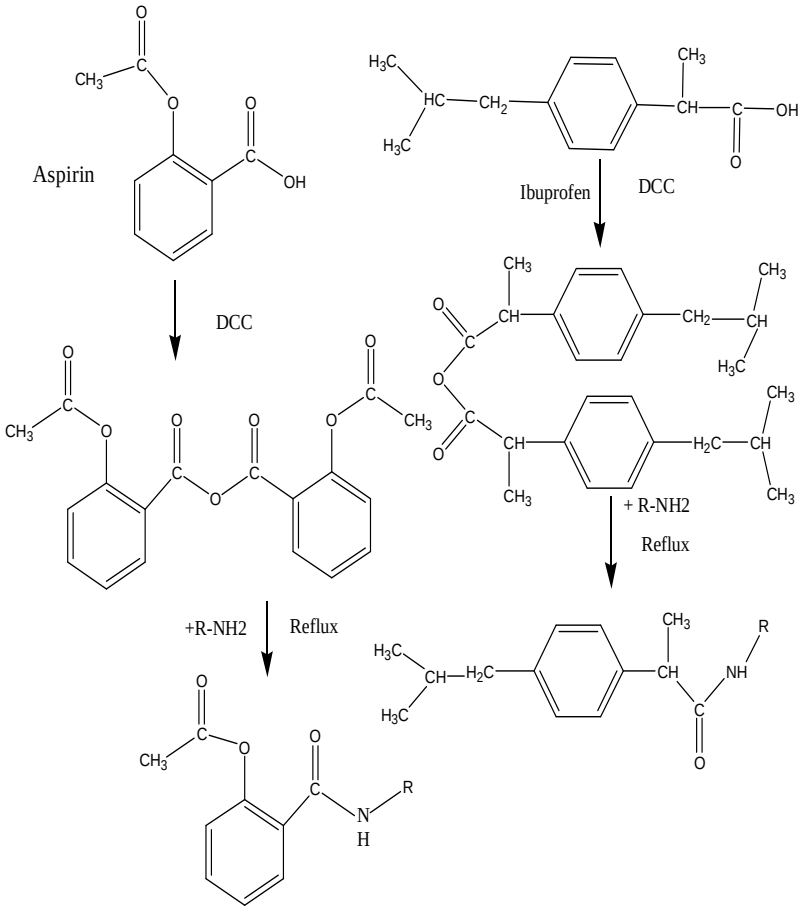
Determination of the gastric side effects was done by detection of possible ulcero-genic activity for compounds(1,1,3, and 4) that exhibited marked anti-inflammatory activity compared with Indomethacin and Celecoxib. Animals were divided into seven groups (n=5),they were fasted 20 hrs before drug administration .The synthesized agents (1,2,3, and 4 compounds), Celecoxib and indomethacin were given orally in a doses of (2.01, 2.246, 2.15, 2.422, 2.59, 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 with0.9% 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) [12]. Score assignment; Zero = for normal (no injury), 1= latent small red spot, 2= wide red spot, 3= slight injury, and 4= sever injury.

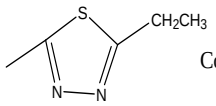
1- % incidence/10 = (no. of animals showing ulcer divided by total no. of animals in the group *100) / 10.
2-Average number of ulcer = no. of ulcers in the group/ total no. of animals in the group.
3-Average severity = sum (each ulcer * score of severity) / no. of ulcers.
4- Ulcer index = the sum of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

2.2.3 Statistical Method:

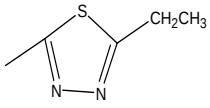
Statistical processing of the result by using the test of analysis of variance (ANOVA test) to show the differences among all groups if it is present, the highly significance is considerable , in which ($p < 0.01$). To conform that the result obtained by ANOVA test using T-test ,in which highly significance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



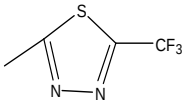
Compound (1)R- =



Compound (3)R- =

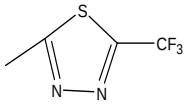


Compound (2)R- =



Scheme (1)

Compound (4) R- =



Scheme (2)

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 Aspirine 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[13].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 (–NH2) 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[14].Figures 1&2showed comparison among control, references and tested compounds as percent of inhibition edema, and the ulcer index .

The inhibition percent of edema of tested compounds 1,2, 3, and 4was 46 %, 40.9 %,45%, and 44% respectively. Compared with diclofenac as reference agent and its inhibition percent was 41. 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-ethyl-1,3,4-thiadiazole and 2-amino-5-methylthio-1,3,4-thiadiazoleto the parent agent aspirin and 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[15].

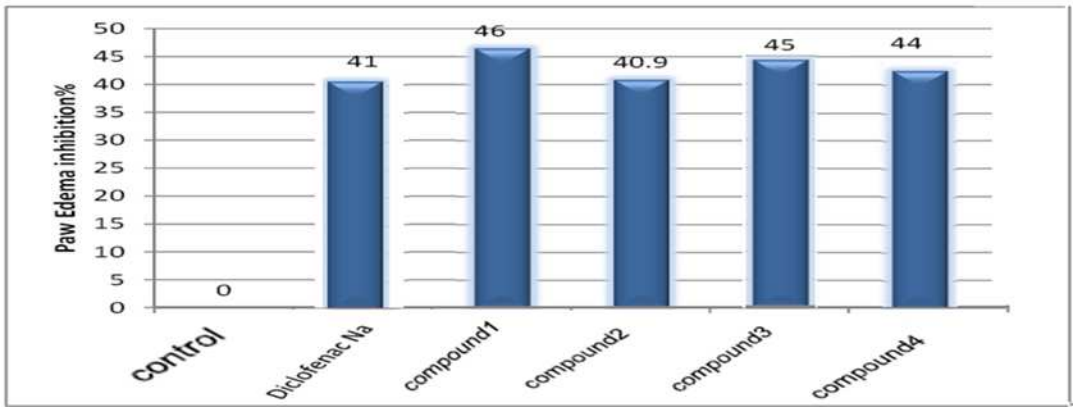


Figure 2: Graphic display of % Inhibition of edema of control, Diclofenac Na, compound 1,2, 3, and 4

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 (17) while celecoxib showed the least index (6). The ulcer indexes of the tested compounds 1, 2, 3, and 4 were 5.6, 10, 5.8, and 8.3 respectively as showed in fig.2

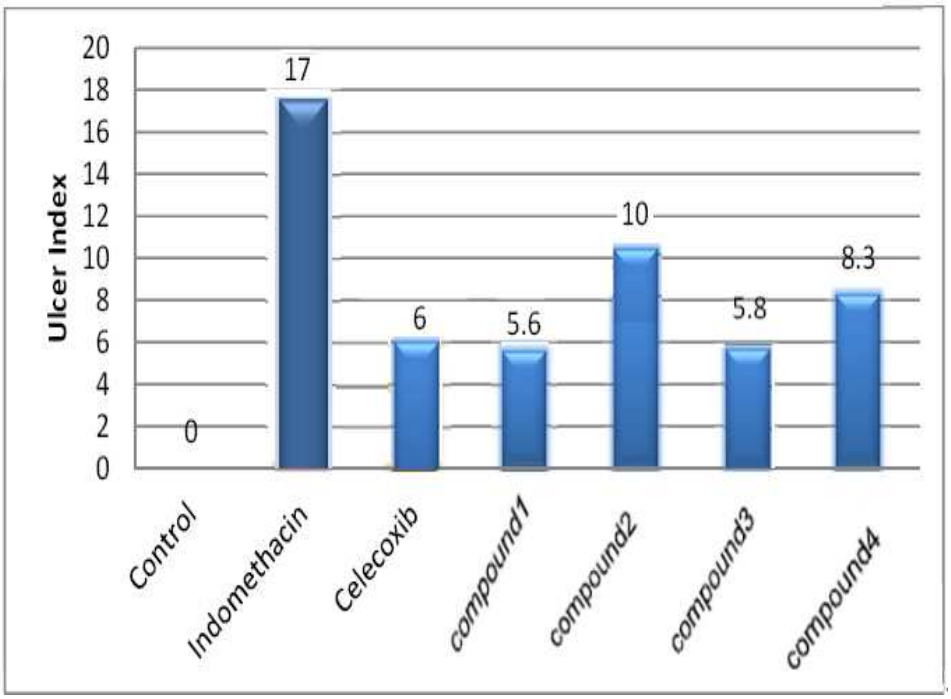


Figure 2: Graphic display of the ulcer index for control, indomethacin, Celecoxib, compounds 1,2,3 and 4

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[16] 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 [17].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 for COX-2 inhibition as in Meloxicam [18].

CONCLUSION

The conversion of carboxylic acid group of aspirin and ibuprofen to corboxamide group by conjugating the selected moiety of heterocyclic 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 1 showed maximal therapeutic anti-inflammatory action with little gastric effects, and compound 3 showed good anti-inflammatory activity with lowest ulcer index.

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