Design and Synthesis of New Non-Steroidal Anti-inflammatory Agents with Expected Selectivity toward Cyclooxygenase-2 Inhibition

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Abstract

This study includes design and synthesis of new non-steroidal anti-inflammatory agents (NSAIDs) with expected cyclooxygenase-2 (COX-2) selective inhibition to achieve better activity and low gastric side effects. Two series of compounds have been designed and synthesized as potential NSAIDs,these are: Salicylamide derivatives (compounds 3,4,5) and Diflunisal derivatives (compounds 10&11). *In vivo* acute anti-inflammatory effect of one of the synthesized agents (compound 3) was evaluated in the rat using egg-white induced paw edema model of inflammation. Preliminary pharmacological study revealed that compound 3 exhibited less anti-inflammatory effect compared to that of aspirin after 120 and 210 minutes, which encourage the continuation of the search to demonstrate or identify the preliminary pharmacological activity for the synthesized compounds and to identify their selectivity toward COX-2 isoenzymes.

Keywords:nonsteriodal anti-inflammatory drugs(NSAID),aspirin derivatives ,diflunisal, Cyclooxygenase -1(cox-1),cyclooxygenase-2(cox-2)

الخلاصة

تتضمن الدراسة تصميم وتخليق مركبات جديدة غير ستيرويدية مضادة للالتهابات ذات فعالية متوقعة كمثبطات للانزيم سايكلواوكسجنيز 2 (cox-2) للحصول على فعالية افضل واعراض جانبيية اقل. تم تخليق مجموعتين من المركبات وهي:مشتقات السلسيل أمايد (المركبات 5,4,3)ومشتقات الديفلونيسال (المركبان 10و11).أجريت دراسة التقييم الدوائي الأولي للفعالية المضادة للالتهابات غير الرثوية لواحد من المركبات المخلقة الجديدة (المركب 3) بطريقة استحداث وذمة تحت جلد يد الجرذ باستخدام زلال البيض (الألبومين)..أشارت النتائج الفعالية البيولوجية الأولية إن المركب 3 قد أظهر تأثيراً مضاداً للالتهابات اقل من الأسبرين بعد 120 و 210دقيقة، مما يشجع على إكمال البحث لمعرفة التقييم الدوائي الأولي لبقية المركبات المخلقة وكذلك معرفة درجة انتقائها المثبط لإنزيم السايكلوأوكسجنيز.

Introduction

Inflammation defined as a complex series of tissue changes that result in pain and fever [(1)]; or it is a normal, protective response to tissue injury caused by physical trauma, noxious, chemical, or microbiologic agents. 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 prostanoid 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 [(5)]. 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 .NSAIDs have three major pharmacological desirable actions, all of which result mainly from the inhibition of COX-2 in inflammatory cells and the resultant decrease in prostanoid synthesis; these are: anti-inflammatory action,antipyretic effect and analgesic effect. We have two types of NSAID these are traditional NSAID and selective NSAID[(5)] .Traditional NSAIDs (TNSAIDs) are mainly carboxylic acid containing compounds that are either aromatics or aliphatics. They include different chemical classes with different physical properties [(6)].They are frequently prescribed for muscloskeletal complications and are often taken without prescription for minor aches and pains. There are new many different NSAIDs on the market and non of these is ideal in controlling or modifying the sign and symptoms of inflammation [(7)] .

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For example salicylic acid derivatives e.g. aspirin, (acetyl salicylic acid) has been in use as a pharmaceutical agent for over 100 years [8]. Aspirin is unique among COX-inhibitors because it covalently modifies the protein of enzymes and irreversibly inhibits them [9]. Aspirin transfers its acetyl group to serine-530 (Ser530), which prevents proper binding of arachidonic acid in the COX active site .No other residues on either COX-1 or Cox-2 are acetylated [10,11,12].Aspirin suffers from the side effects associated with local gastrointestinal irritation; therefore a number of attempts or modifications have been carried out to overcome this problem such as salt formation, e.g. calcium acetyl salicylate ,buffered aspirin (magnesium or aluminum salts) and such as nuclear modification such as diflunisal [13].Diflunisal ,5-(2',4'-difluorophenyl) salicylic acid, is not converted to salicylic acid *in vivo*. It is largely devoid of antipyretic effect, perhaps because of poor penetration into the CNS. It is 3-4 times more potent than aspirin [6].The higher potency of diflunisal may be resulted from the existence of two vanderwaals binding sites (aromatic rings) and the ortho fluoro atom which promotes non-coplanarity between the two aromatic rings which is required for effective binding to the COX enzyme. In addition, the presence of para-fluoro atom will retard metabolism, by preventing oxidation of the para-position, hence increase duration of action [13].COX-2 selective inhibitors, or Coxibs, were developed in an attempt to inhibit prostacyclin synthesis by COX-2 isoenzyme induced at the site of inflammation without affecting the action of the constitutively active COX-1 isoenzyme found in the gastrointestinal tract, kidney, and platelets [3].

Preferentially Selective COX-2 Inhibitors

for example : Meloxicam which is a novel NSAID acting by preferential inhibition of COX-2 [14]. It has a selectivity towards COX-2 up to 100 fold over COX-1 depending on the test system [15].. An isosteric functional groups to 2-amino-5-methyl thiazole moiety in meloxicam are investigated as a possible bioisosteric analogues [16].

Highly Specific COX-2 Inhibitors

Since the loss of selectivity is a potential problem with large dosage of NSAIDs that, preferentially inhibit COX-2; thus new agents that are more specific towards COX-2 even at large dose were synthesized, these agents include:celecoxib,roficoxib,valdicoxib,etoricoxib, and imricoxb.

Experimentals

A.chemistry

Materials: *2-aminobenzothiazole,2-aminopyridine&2-aminopyrimidine (BDH,England), Acetyl salicylic acid(Judex England),DCCI, Diflunisal(Jordon),*Ethanol 95%and 99%,all solvents were of analar type and used without further purification

General procedure

melting points(uncorrected) were determined by capillary method on Thomas hoover apparatus (England)and IR spectra were recorded on model 500 scientific IR spectrophotometry ,Buck company(USA) in pharmacy collage ,Baghdad university.Ascending thin layer chromatography (TLC) was run on DC-Kartan SI Alumina 0.2 mm to check the purity and progress of reaction. The CHN analysis was done using an Exeter CE-440 elemental microanalyzer (Germany). The analysis was carried out at micro analytical center, Faculty of Science-Cairo University.The identification of compounds was done using iodine vapor and the chromatograms were eluted by two solvent systems:

A:THF : Diethyl ether : Cyclohexane (40:40:20) [17]

B:Methanol : Acetic acid : Diethyl ether : Benzene (1:18:60:20)[18]

Method of preparation of aspirin anhydride

Aspirin (5.0gm, 27.77mmole) was dissolved in methylene chloride (75ml), and dicyclohexyl carbodiimide (DCCI) (2.86gm, 23.84mmole) was added. The reaction mixture was continuously stirred at room temperature for 3.5 hours. A white precipitate of dicyclohexylurea (DCU) was formed, then removed by filtration. The filtrate was evaporated under vacuum and an oily product was formed to yield compound (1) [18].

Synthesis of N-(2-pyridyl)-acetyl salicylamide

Compound 1: (4.0gm, 11.69mmole), 2-aminopyridine (1.1gm, 11.69mmole), zinc dust (catalytic amount, 0.01gm), glacial acetic acid (1.12ml, 19.64mmole) and dioxane (32ml) were placed in a flask, equipped with reflux condenser. The reaction mixture was refluxed gently for 90 minutes, the solvent was evaporated under vacuum, the residue was dissolved in the minimum volume of ethyl acetate, washed with $NaHCO_3$ (10%, 3X), HCl (1N, 3X), and distilled water (3X), dried using anhydrous magnesium sulfate. The filtrate was evaporated under vacuum to give a crude product 2. The recrystilization was carried out using ethyl acetate-petroleum ether (60-80^oC)

mixture, a white crystalline product was obtained compound(2) [19, 20].

Compound (2) :melting point (115-117),yield (41.14 white powder),IR in KBR disk :3390 N-H stretching vibration of secondary amide,1768 c=0 stretching vibration of acetate ester ,1604-1577&1532 c=o stretching vibration of aromatic and N-H bending (amide II)is also included in this region.

Synthesis of N-(2-pyridyl)-salicylamide

Compound 2 (0.5gm, 1.953mmole) was dissolved in a minimum volume of absolute ethanol 99%: tetra hydro furan(THF) (3:1) mixture. The solution was cooled to 18°C, and then sodium hydroxide solution (2N, 1.162ml, 2.325mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Stirring was continued at 18°C for additional three hours. The reaction mixture was neutralelized with equivalent quantity of HCl, excess of cold water was added to precipitate the phenolic compound, which was then filtered and dried to give compound (3)[20].The same method of synthesis has been done for the other salicylamide derivatives but with two different heterocycles (2-amino benzothaiazole) and (2-amino pyrimidine) to yield the final compounds (4)&(5).

Compound (3):melting point (218-220),yield(51% white crystals),Rf value (0.75A&0.79B) ;IR in KBr disk :3000-3500 broad O-H stretching vibration of phenol,3238 N-H stretching vibration of secondary amide , 1675 C=O stretching vibration of secondary amide (amide I),
1611-1604, 1541-1537 C=C stretching vibration of aromatic and N-H bending vibration of secondary amide,1234C-O stretching vibration of phenol (figure 1) .
CHN analysis found C 66.71, H 4.63,N 12.74.
CHN calculated C 67.28,H 4.67,N 13.08

Compound (4):melting point 290 decomposed ,yield (47% white crystals).

Compound (5):melting point (191-193),yield(56%white crystals).
IR in KBr disk for both compounds :3000-3500 broad band stretching vibration of phenol , 3330 &3301 N-H stretching vibration of secondary amide ,1678&1623 c=o stretching vibration of secondary amide (amide I),1245&1224 c-o stretching vibration of phenol.

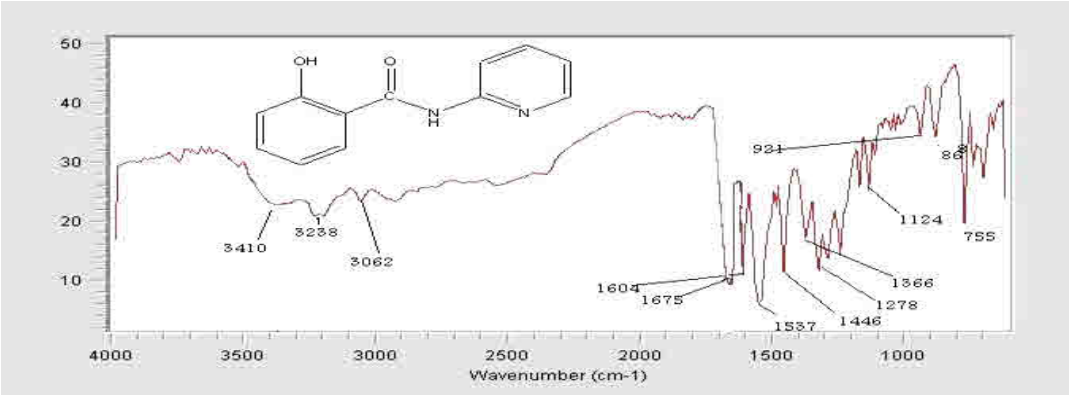


Figure 1 : IR spectrum of compound(3) in KBr disk.

Synthesis of 5-(2',4'-difuorophenyl)-acetylsalicylic acid, Compound (6)

In a 250ml boiling flask, equipped with reflux condenser, diflunisal (5.0gm, 20mmol) and acetic acid anhydride (15ml, 159mmole) were placed and 3 drops of concentrated sulfuric acid were added drop wise. The reaction mixture was refluxed gently for 1 hour, then allowed to cool with occasional stirring. Ice-water was then added until a precipitate was formed, which was filtered using pump, washed with cold distilled water several times, the crude product was collected. The recrystalization was carried out using ethanol 95% to give compound(6) [19] .

Compound (6): melting point (172-174),yield (89% white crystals) IR values in KBr disk 3000-2676 O-H stretching vibration (H-bonded) of carboxylic acid ,1768 c=o stretching vibration of acetate ester ,1700 stretching vibration of carboxylic acid ,1319 O-H bending vibration of carboxylic acid ,1274 c-o stretching vibration of carboxylic acid.

Synthesis of 5-(2',4'-difluorophenyl)-acetyl salicylic acid

Anhydride compound(7): compound 6 (5.0gm, 17.1mmole) was dissolved in THF (30ml), then DCCI (1.75gm, 8.55mmole) was added. The reaction mixture was continuously stirred at room temperature for 4 hours. A

white precipitate of DCU was formed which was then removed by filtration. The solvent was evaporated under vacuum and a solid product of compound (7) was obtained[18].

Compound (7): melting point (149-150),yield (80% white powder) IR value in KBr disk :1815&1743 c=o stretching vibration of anhydride (asymmetric &symmetric bands),1277&1173 c-(c=o)-o(c=o)-c stretching vibration of anhydride.

Synthesis of 5-(2,4-difluorophenyl)-N-(2-pyridyl) acetyl salicylamide compound(8)

Compound 5 (2.5gm, 4.4mmole), 2-aminopyridine (0.418gm, 4.4mmole), zinc dust (0.004gm), glacial acetic acid (0.425ml) and dioxane (25ml) were placed in round-bottom flask, equipped with reflux condenser. The reaction was carried out as in the synthsis of compound (2). The recrystilization was carried out using ethyl acetate-petroleum ether (60-80^{o}C) mixture, a white crystalline product was obtained((compound 8) [19, 20].

Compound (8): melting point (180-181),yield (42.2 white powder) .

The same method of preparation has been done with 2-amino benzothiazole to yield compound (9)

compound (9):melting point (161-164),yield(39.5% white crystals) .

IR values in KBr disk for compounds (8&9): 3335&3333 N-H stretching vibration of secondary amide ,1774&1753 c=o stretching vibration of acetate ester ,1696&1686 c=o stretching vibration of secondary amide (amide I).

Synthesis of 5-(2,4-difluorophenyl)-N-(2-pyridyl) salicylamide, Compound (10)

To a cooled solution(18 ^{o}C) of compound (3) (0.4gm, 1.085mmole) in absolute ethanol 99%: THF (3:1) mixture, sodium hydroxide (2N, 0.651ml, 1.302mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Then the reaction mixture was worked up as described previously in preparation of compound (3).The same method of preparation has been done for diflunisal anhydride with (2-amino benzothiazole) to yield the final product compound (11).

Compound (10):melting point (230-232),yield (48,%of white powder),RF value (0.53 A&0.69B),IR in KBR disk: 3376-3227 O-H stretching vibration of phenol, 3304 N-H stretching vibration of secondary amide,1660 C=O stretching vibration of secondary amide (amide I), 1598 and 1530 C=C stretching vibration of aromatic and N-H bending of secondary amide (amide II), 1262 C-O stretching vibration of phenol (figure 2).

CHN analysis found :C 64.93,H 3.80, N 8.33

CHN calculated : C 66.26,H 3.68, N 8.59.

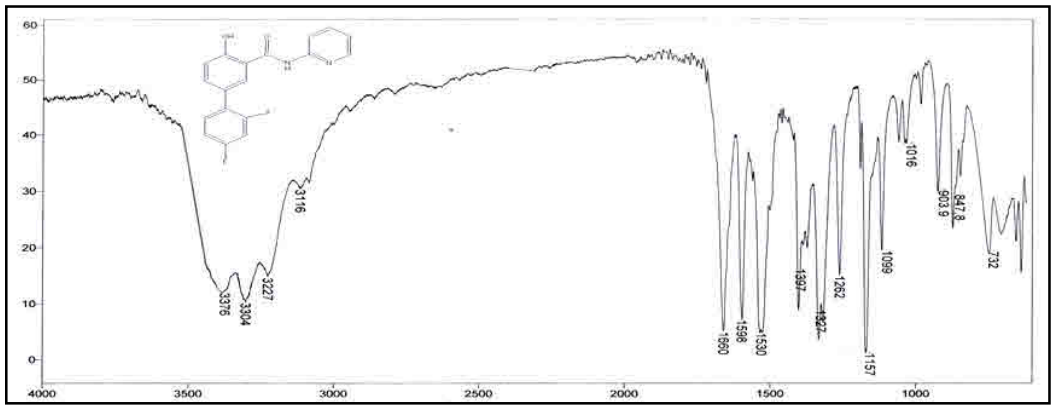


Figure 2 : IR spectrum of compound 10 in KBr disk.

Compound (11): melting point (251-253),yield 42% faint yellow crystals).IR value in KBr disk :3450-3100 O-H stretching vibration of phenol and N-H stretching vibration of amide is buried within this band ,1677 C=O stretching vibration of secondary amide (amide I),1604-1534 C=C stretching vibration of aromatic and bending vibration of secondary amide (amide II).

B.Pharmacology

Acute anti-inflammatory activity of one of the chemically synthesized compounds, compound(3) was evaluated *in vivo* using egg-white to induce paw edema in rats. The decrease in paw thickness is the basis of screening the newly synthesized compound for its anti-inflammatory activity.Eighteen albino rats of either sex, weighing 300 ± 10 gm supplied by the animal house of the College of

Pharmacy, University of Baghdad were used in this study. Animals were kept under standardized conditions (12 light-12 dark cycle) for 7 days for acclimatization; and were fed commercial chaw and had provided with water. Rats were brought 1 hour before performing the experiment to the laboratory, and were allocated into 3 groups (each of 6 rats) as follows:

A/ Six rats served as control; they received drug vehicle (0.5ml propylene glycol in water 50% v/v).

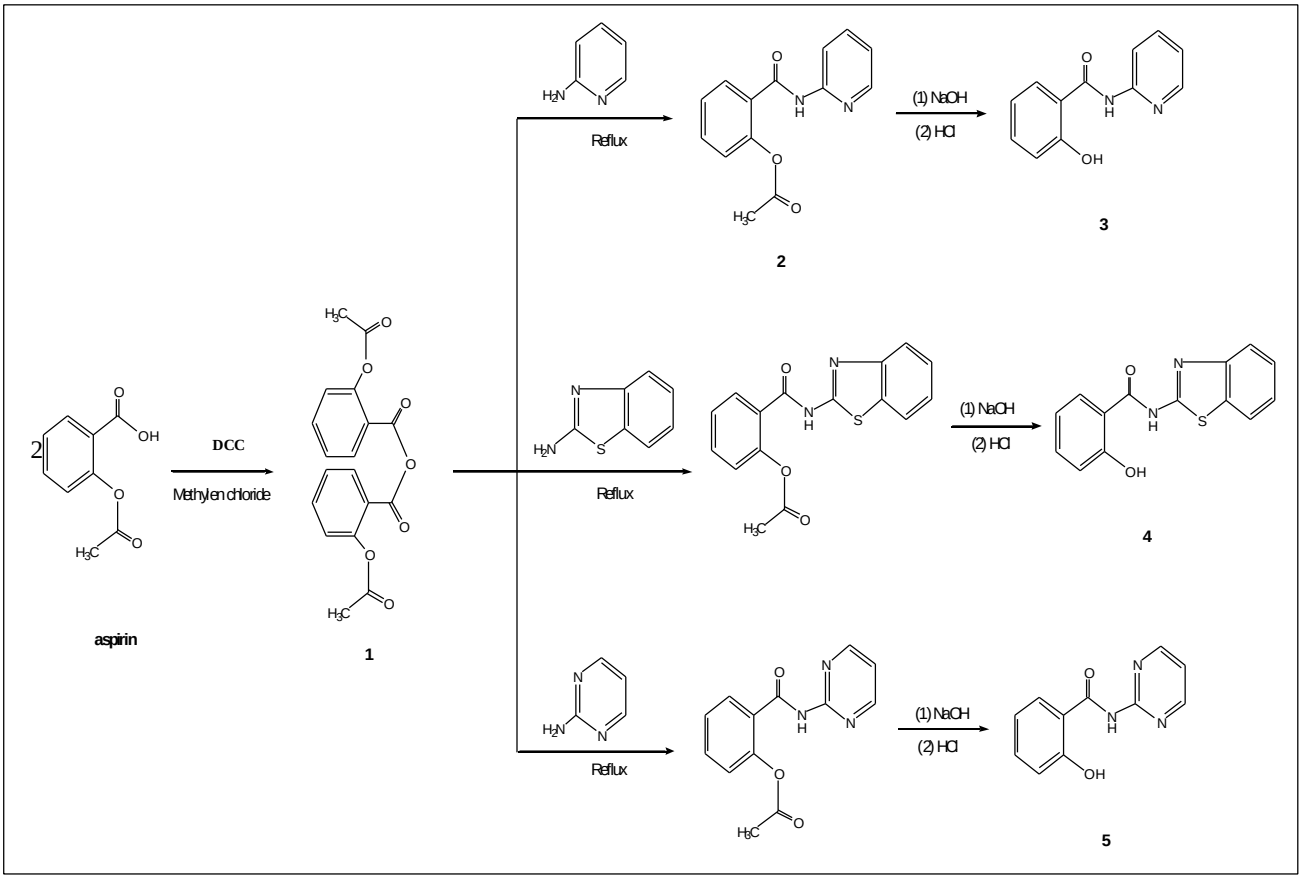
B/ Six rats received aspirin as a reference substance in a dose of (100mg/kg, i.p.) in propylene glycol [21].C/ Six rats received compound 3 suspended in propylene glycol (118.8mg/kg, i.p.) [22]. The anti-inflammatory activity of the tested compound was studied using egg-white induced edema model. Acute inflammation was produced by a subcutaneous injection of 0.05ml of undiluted fresh egg-white into the planter side of the left hind of the rats; 30 minutes after intraperitoneal injection of the drug or the control. The paw thickness was measured by vernea at eight time intervals (0, 30, 60, 90, 120, 150, 180 and 210 minutes) after the drug administration.

Statistical Analysis

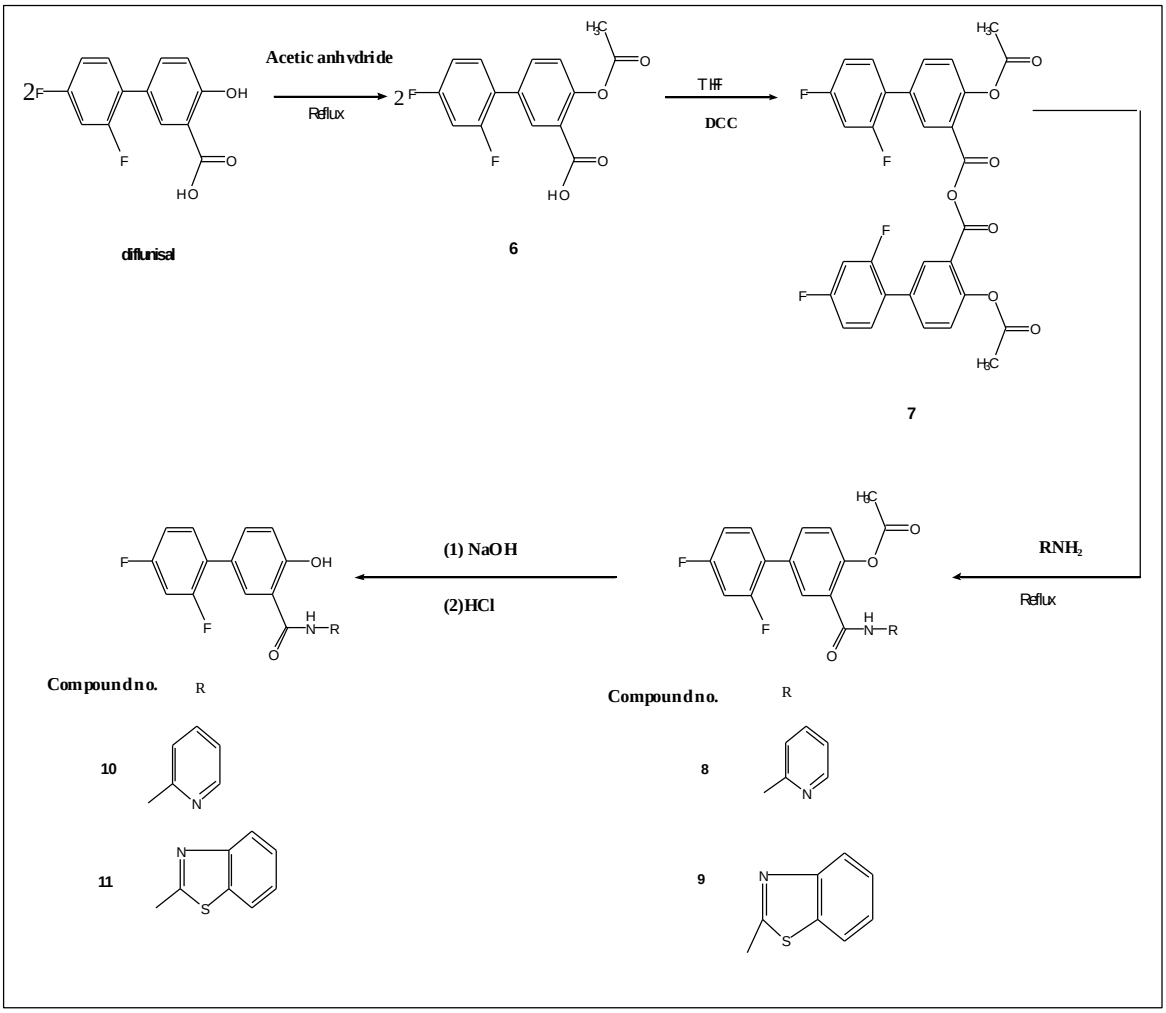
Students *t*-test was used to make comparisons with respect to baseline, while comparisons between different groups at specified time was done using analysis of variance (ANOVA). P values less than 0.05 were considered significant.

Result and conclusion

The designed compounds have been synthesized successfully as shown in scheme (1) & (2) 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 aspirin and diflunisal to corboxamide group by conjugating the selected moiety of heterocyclic compound may produce new non-steroidal anti-inflammatory agents with expected selectivity toward COX-2 inhibition and hence less gastric irritation. Preliminary pharmacological evaluation has been done for one of the designed compounds (compound 3) and it has been found that this compound exhibit slightly less potent anti-inflammatory effect than aspirin as shown in (figure 3 and table 1).



Scheme (1): Synthesis of compounds (3,4 ,5).



Scheme (2): Synthesis of compounds (10 and 11).

Figure 3: Effect of vehicle (propylene glycol), aspirin and compound 3 on paw edema in rat after 120 and 210 minutes of egg-white injection. Results are expressed as mean ± SEM (n=6/group). Time zero is the time of egg-white injection. Non-identical superscripts (a,b) among different groups represent significant difference (P<0.05).

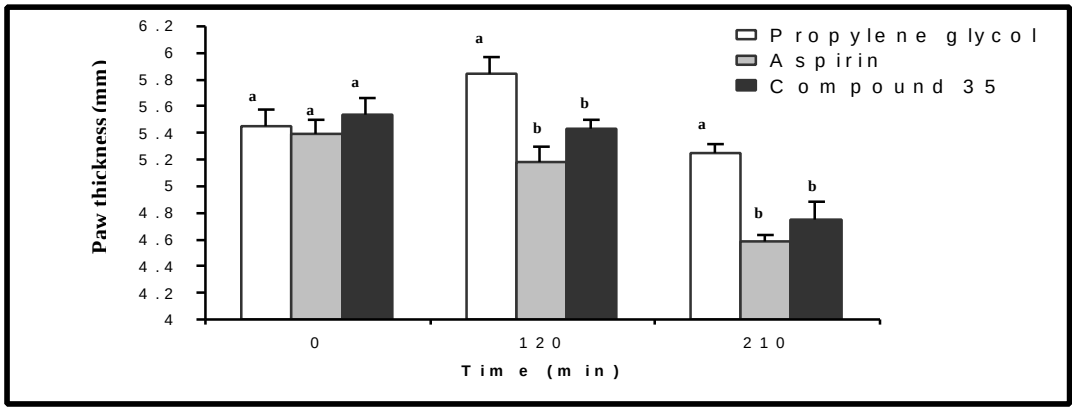


Table 1:Effect of control (propylene glycol), reference drug (aspirin) and tested compound (compound 3) on egg-white induced paw edema in rats.

Time (min)	Paw thickness (mm)		
	Control (n=6)	Aspirin (n=6)	Compound 3 (n=6)
0	5.45 ± 0.12 [a]	5.39 ± 0.11 [a]	5.54 ± 0.12 [a]
30	6.15 ± 0.15	5.85 ± 0.13	5.79 ± 0.09 *
60	6.41 ± 0.07 *	5.64 ± 0.14 *	5.85 ± 0.11 *
90	6.56 ± 0.11 *	5.48 ± 0.12 *	5.68 ± 0.09 *
120	5.85 ± 0.12 * [a]	5.18 ± 0.12 * [b]	5.43 ± 0.07 * [b]
150	5.41 ± 0.09 *	4.95 ± 0.15 *	5.21 ± 0.11 *
180	5.30 ± 0.12 *	4.70 ± 0.11 *	4.98 ± 0.09 *
210	5.25 ± 0.07 * [a]	4.58 ± 0.06 * [b]	4.75 ± 0.13 * [b]

- Data are expressed as mean ± SEM.
- n= number of animals.
- Control, reference drug and tested compound were given 30 minutes before the injection of egg-white.
- Time (0) is the time of injection of egg-white (induction of paw edema).
- *P<0.05 with respect to time (0).
- Non-identical superscripts (a, b) among different groups represent significant difference (P<0.05).

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