Design and Synthesis of New Mefenamic Acid Derivatives as Anti-Inflammatory Agents

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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 mefenamic acid derivatives were designed and synthesized as potential NSAIDs. *In vivo* acute anti-inflammatory effect of the synthesized agents (compound 2and 3) was evaluated in the rat using egg-white induced paw edema model of inflammation. Preliminary pharmacological study revealed that compound 2and 3 produced a significant reduction in paw edema with respect to the effect of propylene glycol 50% v/v (control group), moreover compound 2 exhibited comparable anti-inflammatory effect to that of aspirin after 120 and 210 minutes and compound 3 has less anti-inflammatory effect, which encourages the continuation of the search to identify their selectivity toward COX-2 isoenzymes.

Keywords: Nonsteriodal anti-inflammatory drugs (NSAID), mefenamic acid derivatives, cyclooxygenase-1(cox-1), cyclooxygenase-2(cox-2).

Introduction

Inflammation is defined as a complex series of tissue changes that result in pain and fever[1].non steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, inflammatory conditions and fever[2,3] .

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. Although COX-1 and COX-2 have similar structures, there are slight differences that affect the drug binding and lead to different actions [4]. Both enzymes have a long narrow channel into which arachidonic acid enters and be converted into prostaglandins (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].

Selective cox-2 inhibitors are still under development[6, 7], they were proposed that drugs with higher selectivity for cox-2 tend to induce cardiovascular disease [8].

An example of traditional NSAIDs is salicylic acid derivatives, e.g., aspirin, (acetyl salicylic acid) has been in use as a pharmaceutical agent for over 100 years[9], is unique among COX-inhibitors because it covalently modifies the protein of enzymes and irreversibly inhibits them[10].

Another example of TNSAID is N- aryl anthranilic acid derivatives (e.g., mefenamic acid) it has analgesic action 2-3 times more than that of aspirin[11].

A study examining this drug relative to gastrointestinal bleeding indicated a lower incidence of these side effects that exhibited by aspirin [12].

All classic NSAIDs inhibit COX-2 as well as COX-1 to varying degrees, thus they can be considered nonspecific[13,14]. All classical NSAIDs are associated with an increased risk of gastrointestinal (GI) ulcers and serious upper GI complications, including GI hemorrhage, perforation, and obstruction[15,16].

Preferentially Selective COX-2 Inhibitors

For example Meloxicam which is a novel NSAID acting by preferential inhibition of COX-2[17]. It has selectivity towards COX-2

up to 100 fold over COX-1 depending on the test system[18]. Isosteric functional groups to 2-amino-5-methyl thiazole moiety in meloxicam are investigated as a possible bioisosteric analogue[19].

In the view of previous findings, the present study was conducted to design, synthesize and preliminary evaluation of new mefenamic acid derivatives as potential NSAIDs in the area of oxicam derivatives that are class of enolic acid derivatives to give more potent NSAIDs with longer half life, less side effect and may exhibit certain selectivity as cox-2 inhibitors due to the following:

1. Mefenamic acid is well known anti-inflammatory agent.
2. The pocket of cox-1 or cox-2 enzymes to accommodate the corboxamide group in oxicam series are different, sterically specific, and sensitive to the size and isomeric variation on the carboxiamide group. The later may play arole in directing the compound toward one of the isomers of cox[5].
3. The conversion of carboxylic acid group of mefenamic acid to corboxamide group by conjugating the selected moiety of heterocyclic compound as (2-amino pyridine and 2-amino benzothiazole),these conjugates considered as isosteric functional groups of meloxicam which has good selectivity toward cox-2[20].

Experimental

Materials 2-aminobenzothiazole and 2-aminopyrimidine (BDH, England), Acetyl salicylic acid (Judex England), mefenamic acid was supplied from micro company (Indian).

All reagents and solvents were of analar type and used as received from the commercial supplier (Reidal-Dehean Germany), (Sigma-Aldrich Germany) and (BDH England).

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 micro analyzer (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: Methanol: Acetic acid: Diethyl ether: Benzene (2:18:60:20)[21].

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

Mefenamic acid (5.0 g, 20.77 mmole) was dissolved in THF (30 ml), and dicyclohexyl carbodiimide (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)[21, 22].

The percentage yield, physical data and Rf values were given in table (1). IR 3333 NH of secondary amine, 2935 and 2851 C–H st.v. of CH_3 (asymmetric and symmetric), 1815 and 1743 C=O st.v. of anhydride, 1623, 1522 and 1518 C=C st.v. of aromatic ring, 1277 and 1173 C–(C=0)–O–(C=O)–C st.v. of anhydride.

Synthesis of N–(2–pyridyl)–(2,3 dimethylphenylamino) Benz amide (compound2)

Compound 1 (2.5 g, 5.4 mmol), 2-aminopyridine (0.5g, 5.4mmole), Zinc dust (catalytic amount, 0.01 g), glacial acetic acid (0.5 ml, 8.75 mmol) and dioxane (20 ml) are 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 NaHCO3 (10%, 3×10ml), HCL (1N, 3×10 ml) and distilled water (3×10 ml), filtered over 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 ^{o}C) mixture, a white crystalline product was obtained compound (2)[23, 24].

The percentage yield, physical data and Rf values were given in table (1). IR N–H st.v. of secondary amide,3062 C-H st.v. of aromatic, 2935 and 2851 C–H st.v. of CH3, 1675.
C=O st.v. of secondary amide, 1537 and 1446 N–H bending v. of secondary amide. CHN calculated (C20H19N3O): C, 75.69; H, 6.03; N, 13.24.
CHN found: C, 73.12, H, 5.78, N, 12.88.

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

Compound 1 (2.5 g, 5.4 mmol), 2-aminobenzothiazole (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) are placed in a flask, equipped with reflux condenser. The reaction mixture was continued as in the synthesis of compound 2 to yield compound 3.

The percent yield, physical data and Rf values were given in Table (1).
IR 3335 N–H st.v. of secondary amide, 3065 C-H st.v. of aromatic, 2935 and 2851 C–H st.v. of CH3,1696 C=O st.v. of secondary amide.
CHN calculated (C22H19N3OS): C, 70.75; H, 5.13; N, 11.25; S, 8.6.
CHN found: C, 71.24; H, 5.2; N, 11.0; S, 8.3.

Pharmacology

Albino rats of either sex, weighing 200 ± 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 (0.5 ml propylene glycol in water 50% v/v) i.p injection.
B- Six rats received aspirin as a reference substance in a dose of (100mg/kg, i.p.) in propylene glycol[25]. C and D/Six rats received tested compound (2 and 3) respectively in a dose equivalent to 7.5 mg /kg of mefenamic acid as finely homogenized suspension in 50% v/v propylene glycol[22,26] i.p. injection. See Table (2).

The most widely used primary test to screen new anti-inflammatory agents is based on the ability of a compound to reduce local edema induced in the rat paw following injection of an irritant agent[27].

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 paw 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, and 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.

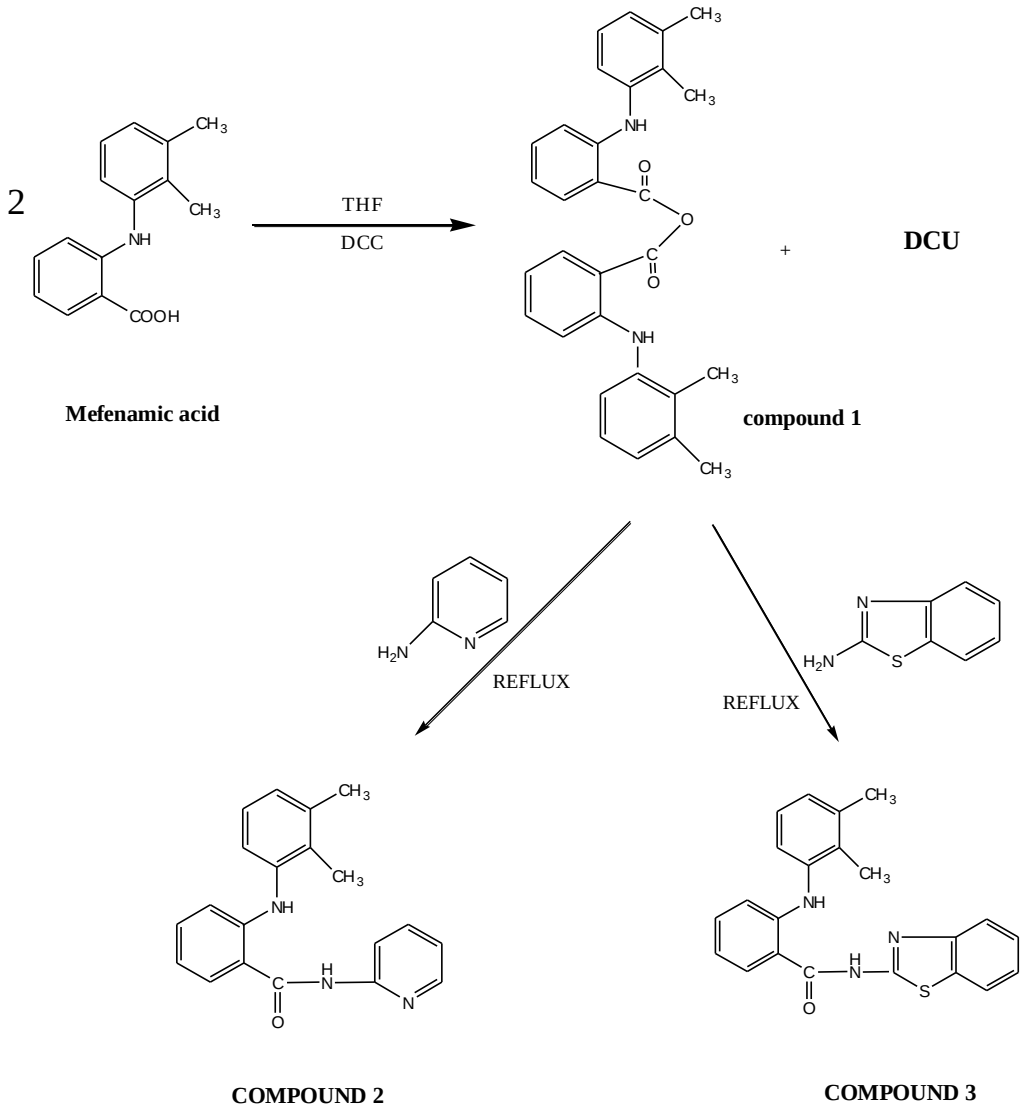
Result and Conclusion

The designed compounds have been synthesized successfully as shown in Scheme (1) 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 to benzamide 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 the designed compounds and it has been found that these compound exhibit anti-inflammatory effects compared to that with aspirin. At time (90) there is a maximum increase in paw edema i.e. maximum edema occur in all control, reference drug and tested drug.

At time (120 min.), there is no difference between reference drug (aspirin) and compound 2 in the reduction of paw edema. At time (210 min) compound (2) show comparable affect to that of aspirin and compound (3) has less anti–inflammatory affect compared with aspirin as shown in Fig.(1).



Scheme (1).

Table (1)
The characterization and physical data of compounds (1, 2 and 3).

Compound	*Empirical formula*	*M.Wt*	*Appearance*	*M.P. observed*	*% yield*	*Rf value*
1	C30H28N2O3	464	White faint powder	143-145*	78	0.74
2	C20H19N30	317.4	White crystals	212-215	32	0.80
3	C22H19N3OS	373.5	White crystals	189-192	36	0.76

Reported M.P. is 141-143[22].

Table (2)
Effect of aspirin, compound (2) and compound (3) on egg-white induced edema in rat.

Time (min.)	Paw thickness (mm)			
	Control	Aspirin	Cpd 2	Cpd 3
0	5.5 ± 0.11 [a]	5.49 ± 0.13 [a]	5.60 ± 0.06 [a]	5.52 ± 0.09 [a]
30	5.9 ± 0.07 [a]	6.23 ± 0.08 [b]	6.13 ± 0.08 [b]	6.12 ± 0.12 [b]
60	6.63 ± 0.09 [a]	6.52 ± 0.07 [b]	6.34 ± 0.13 [b]	6.30 ± 0.19 [b, c]
90	6.85 ± 0.12 [a]	6.41 ± 0.08 [b]	6.1 ± 0.09 [c]	6.28 ± 0.15 [c]
120	6.45 ± 0.05 [a]	5.86 ± 0.05 [b]	5.8 ± 0.17 [b,c]	6.00 ± 0.11 [c]
150	5.93 ± 0.05 [a]	5.45 ± 0.14 [b]	5.41 ± 0.08 [b]	5.63 ± 0.07 [b]
180	5.76 ± 0.09 [a]	5.02 ± 0.07 [b]	4.95 ± 0.06 [b]	5.29 ± 0.11 [c]
210	5.64 ± 0.12 [a]	4.82 ± 0.09 [b]	4.79 ± 0.14 [b]	5.11 ± 0.13 [c]

Data are expressed as mean ± SEM
N=6
Control, aspirin drug, tested compounds were given(30) minutes before the injection of egg-white.
Non-identical superscripts (a, b, c) among groups within the same time interval represent significant difference (P<0.05).

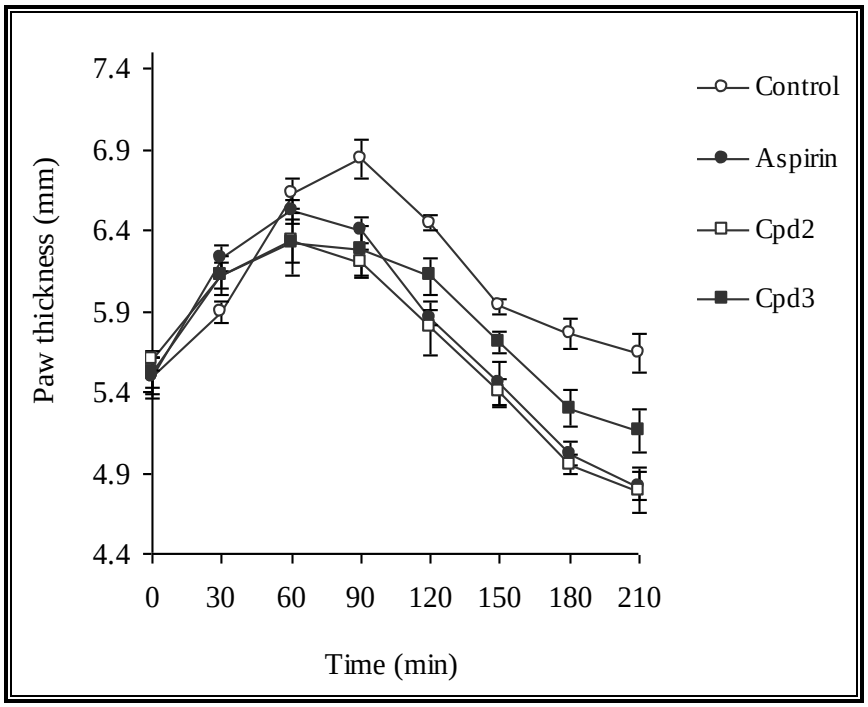


Fig. (1) Effect of vehicle, aspirin, cpd (2) and cpd (3) on egg-white induced paw edema in rats. Results are expressed as mean ± SEM (n =6 / group). Time zero is the time of egg-white injection.
Control, aspirin drug, tested compounds were given (30) minutes before the injection of egg-white.

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الخلاصة

تضمنت الدراسة تصميم وتخليق مركبات جديدة غير ستيرويدية مضادة للالتهابات ذات فعالية متوقعة كمثبطات للانزيم سايكلواوكسجنيز 2(cox-2) للحصول على فعالية أفضل وأعراض جانبية اقل. تم تخليق مركبين اثنين من مشتقات حامض الميفانميك (mefenamic acid) المعروف جيدا كدواء غير ستيرودي مضاد للالتهاب. أجريت دراسة التقييم الدوائي الأولي للفعالية المضادة للالتهابات غير الرثوية للمركبين بطريقة استحداث وذمة تحت جلد يد الجرذ باستخدام زلال البيض (الألبومين). أشارت النتائج الفعالية البيولوجية الأولية إن المركبين قد انتجت انخفاظا مؤثرا للوذمة مقارنة مع البروبلين كلايكول كمجموعة ضابطة علاوة على ذلك ان المركب(2) قد اظهر تاثيرا مضاد للالتهاب مقارب للاسبرين في اوقات 120 و210 دقيقة والمركب (3) تاثيرا اقل مما يشجع على اكمال التقييم الدوائي لمعرفة درجة انتقائها لانزيم السايكلواوكيجنيز.