Synthesis and Preliminary Pharmacological Evaluation of Esters and Amides Derivatives of Naproxen as Potential Anti-Inflammatory Agents

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Abstract

4-chloro and 4- nitro substituted phenol and aniline incorporated to a carboxylic group of naproxen a well-known non-steroidal anti-inflammatory drug (NSAID) to increase bulkiness were synthesized for evaluation as a potential anti-inflammatory agents with expected COX-2 selectivity. In vivo acute anti-inflammatory activity of these compounds (I-IV) was evaluated in rats using egg-white induced edema model of inflammation in a dose equivalent to (2.5 mg/Kg) of naproxen. All tested compounds produced a significant reduction in paw edema with respect to the effect of propylene glycol 50% v/v (control group). Moreover, compounds I and IV might show higher effect comparable to that of naproxen and to that of compounds II & III which may attribute to the higher effect of nitro group than chloride group. The results of this study indicate that esterification and amidation of naproxen with selected pharmacophoric groups enhance or maintain its anti-inflammatory activity.

Keywords: anti-inflammatory; naproxen derivatives.

تخليق وتقييم دوائي اولي لمشتقات استرية وامايدية للنابروكسين كمضادات التهاب محتملة

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الخلاصة

4– كلورو و4- نايترو معوضات الفينول والانيلين تم ادخالهم كل على حدى على مجموعة الكاربوكسيل للنابروكسين وهو احد ادوية مضادات الالتهابات غير الستيرويدية لمعرفة زيادة فعاليتها وانتقائها لأنزيم كوكس المعروفة لزيادة ضخامتها قد صنعت لتقييمها كعنصر قوي مضاد للالتهاب مع انتقائية متوقعة لأنزيم كوكس-2.

لقد قيمت الفعالية المضادة للالتهاب الحاد للمركبات (1 – 4) داخل جسم الجرذ بطريقة استحداث وذمة بأستخدام زلال البيض وبجرعة تكافئ (2.5 ملغم / كلغم) من النابروكسين . جميع المركبات التي تم فحصها اعطت نتائج ايجابية بالمقارنة مع تأثير البروبلين كلايكول 50% حجم / حجم (كمجموعة قياس). بالأضافة لذلك المركبين 1 و 4 اثرت بقوة على فعالية النابروكسين اعلى من المركبين 2 و3 ويعود ذلك ربما لقوة تأثير مجموعة النايترو مقارنة مع الكلورايد .نتائج هذه الدراسة تشير الى ان استرات وامايدات النابروكسين مع مجموعة علاجية تركيبية منتقات تزيد او تحفظ فعاليته كمضاد للالتهابات.

الكلمات المفتاحية:مضادات التهاب ، نابروكسين

Introduction

Non-steroidal anti-inflammatory drugs represent one of the most widely used classes of drugs, and are used primarily for treatment of osteoarthritis , rheumatoid arthritis and other inflammatory disorders; however, the use of NSAIDs is significantly limited by their ability to induce the formation of erosions and ulcers in the gastrointestinal (GI) tract [(1)]. The mechanism of action principally responsible for most of the NSAIDs seems to act by inhibition of prostaglandin (PG) synthesis causing almost complete blockade of the activity of the precursor enzymes , cyclooxygenase[(2)] .

Cyclooxygenase is a rate limiting enzyme for Prostaglandin synthesis [(3)]. The three isoenzymes of COX (COX-1, COX-2 and COX-3) have been identified [(4, 5)] though COX-3 activity in human has not been confirmed [(6)].COX-1 is constitutively expressed, widely distributed and has "housekeeping" function. It is of particular importance in maintaining gastric mucosal integrity, renal function and homeostasis [(7)]. COX-2 is highly induced in settings of inflammation by cytokines and inflammatory mediators or physiological stress [(8, 9)]. However, COX-2 also is constitutively expressed in certain areas of kidney, brain, reproductive tract [(10)], the vascular system [(11)], in wound healing, lung and bone [(12)].

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However, since the identification of cyclooxygenase-2 (COX-2), the field of inflammation and particularly the search for effective NSAIDs with fewer adverse effects has

greatly intensified. Increasing number of experimental and clinical data support the role of selective COX-2 inhibitor in anti-inflammatory processes and the involvement of COX-1 inhibition in the side effects associated with using NSAIDs [13].

Selective COX-2 inhibitors differ from traditional NSAIDs in two major ways; Coxibs are less likely to result in NSAID-induced gastropathy, and they do not inhibit platelet function [14]. As a result , selective COX-2 inhibitors elicit less clinically significant GI damage and bleeding than conventional NSAIDs [15].

Naproxen (1) is one of the most used NSAIDs for the treatment of arthritic pain. It can induce GI side effects ranging from stomach irritation to ulceration and bleeding. These GI complications are believed to be determined from the mixed effect of irritation caused by blockage of PG biosynthesis in the GI tract and direct action of free carboxylic groups in NSAIDs. [16,17]

Studies have shown that derivitization of the carboxylate moieties in NSAIDs such as indomethacin (2) and meclofenamic acid(3) result in the generation of potent and selective COX-2 inhibitors. [18]

Also amidation of diclofenac (4) with 4 - (methyl sulfonyl) aniline pharmacophore [19]and amidation of ibuprofen (5) with 4 -amino benzene sulphonamide [20] results in the generation of potent COX-2 inhibitors.

In the view of this background, the present study was conducted to synthesis, and preliminary evaluate ester and amide derivatives of naproxen as new non-steroidal anti-inflammatory agent with expected selectivity toward COX-2 enzyme using 4-chloro and 4-nitro substituted phenol and aniline.

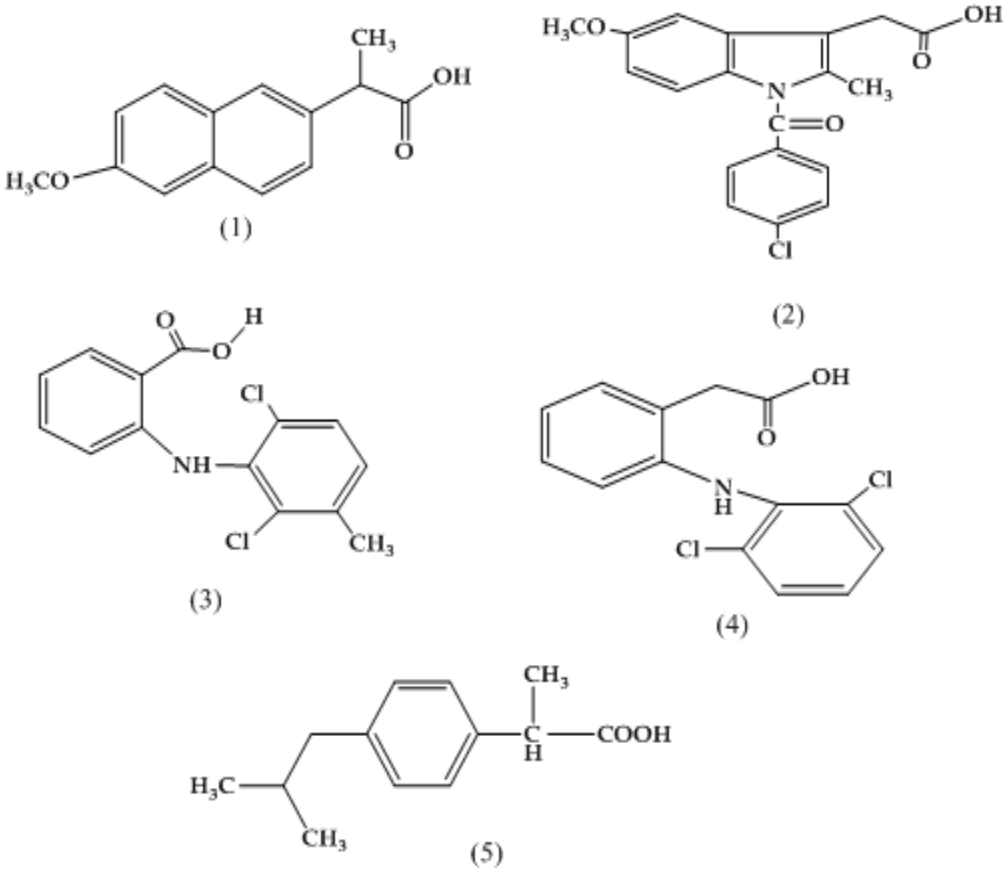
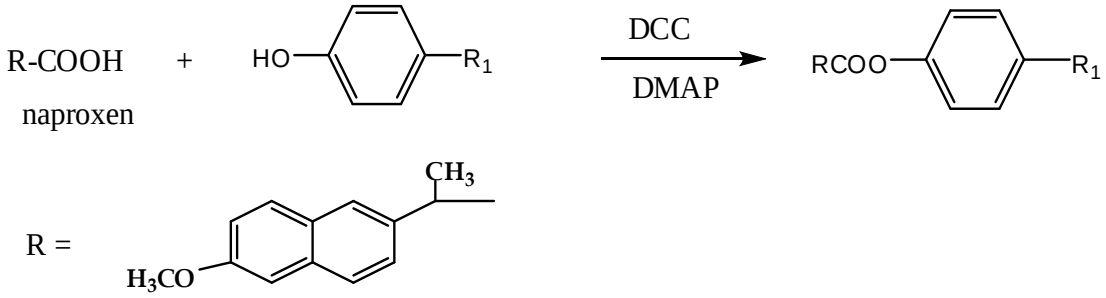


Figure 1: Chemical structure of some NSAIDs

Experimental Section

Chemistry

The synthetic pathways for the designed target Compounds (I-IV) are illustrated in schemes 1 & 2.



compound I $R_1 = NO_2$

compound II $R_1 = Cl$

Scheme1: Synthesis of target compounds (I and II)

Scheme2. Synthesis of target compounds (III and IV).

Pharmacology

Albino rats of either sex weighing (150 ± 10 g) were supplied by the animal house of the College of Pharmacy, University of Baghdad, and were housed in the same location under standardized conditions. Animals were fed commercial chaw and had free access to water ad *libitum*. Animals were divided into six groups as follow:

Group A: six rats served as control; and treated with the vehicle (propylene glycol 50% v/v).

Group B: six rats treated with naproxen in a dose of 2.5mg/ kg [21].suspended in propylene glycol 50%.

Group C-F: six rats/group treated with the tested compounds (I-IV) respectively in doses that determined below. (Suspended in propylene glycol 50%).

Anti-inflammatory activity:

The anti-inflammatory activity of the tested compounds was studied using the egg-white induced hind paw edema model [22]. Acute inflammation was produced by a subcutaneous injection of undiluted egg-white (0.05 mL) into

the plantar side of the left hind paw of the rats; 30 min after i.p. administration of the drugs or their vehicle. The paw thickness was measured by vernea at seven time intervals (0, 30, 60, 120, 180, 240, and 300 min) after drug administration.

The data was expressed as the mean ± SEM (standard error of the mean) and results were analyzed for statistical significance using student t-test (Two Sample Assuming Equal Variances) for comparison between mean values. While comparisons between different groups were made using ANOVA (analysis of variance): Two factors without Replication. Probability (P) value of less than 0.05 was considered significant.

General

All reagents and anhydrous solvents were used as received from the commercial supplier (Merck _Germany, sigma – Aldrich – Germany , BDH _ England and Fluka _USA). Naproxen was supplied from SDI Company, Iraq. Melting points were determined by capillary method on Electric melting point apparatus _ England. Thin layer chromatography (TLC) was run on Silica gel (60) F_{254}, Merck _Germany to check the purity of the products as well as monitoring the progress of reactions.

The identification of compounds was done using U.V. detection and chromatograms were eluted by chloroform: methanol (85:15). FT-IR spectra were recorded by using Shimadzu _Japan spectrophotometer and the determination of spectrum was performed by using KBr disk. CHN microanalysis was done by using Euro EA3000 elemental analyzer –Italy. HPLC analysis was done using KNAUER analyzer – Germany.

General procedure for the esterification of naproxen: synthesis of compounds (I & II).

A reaction mixture containing naproxen (0.84 mmol) in 6mL of anhydrous CH_2Cl_2 was treated with DCC (0.92 mmol), DMAP (0.084 mmol) and 4-chlorophenol (or 4-nitrophenol) (0.92 mmol). After stirring at room temperature for 5 h, the reaction mixture was filtered and the filtrate was concentrated in a vacuum (in vacuo). The residue was diluted with water (30mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic solution was washed with 5% acetic acid (AcOH) (2 × 30 mL), 1 N sodium hydroxide (NaOH) (2 × 30) mL), and water (100 mL) dried magnesium sulfate ($MgSO_4$), and filtered, and the solvent was removed in vacuo to give the final compounds I & II. [23] .

The final products were obtained as solids and the recrystallization was carried out by dissolving the compound in ethyl acetate and addition of petroleum ether (80–100) °C to the filtrate until turbidity occurred and then keeping in a cold place overnight. The mixtures were filtered while cold and the precipitate was collected to give the final compounds I & II.

4-nitrophenyl 2- (6 – methoxynaphthalen – 2 – yl)propanoate (compound I):

White crystal (62% yield); m.p. 208–209 °C; $\mathbf{R_f}$ = 0.64; IR (cm^{-1}): 2,945, 2,843 (C-H) , 1,742 (C=O) of ester, 1,597 and 1,518 (C=C of aromatic), 1,141(C-O); CHNO calculated ($C_{20}H_{17}NO_5$): C, 68.37; H, 4.88; N, 3.99; O, 22.77 found: C, 68.75; H, 4.73; N, 3.81; O, 23.44.

4-chlorophenyl2- (6- methoxynaphthalen-2-yl) propanoate (compound II):

White powder (55% yield); m.p. 185-186 °C; $\mathbf{R_f}$ = 0.73; IR (cm^{-1}): 2,941, 2,852 (C-H), 1,753 (C=O) of ester, 1,602 and 1,523 (C=C of aromatic), 1,146(C-O); CHNO calculated ($C_{20}H_{17}ClO_3$): C, 70.49; H, 5.03; O, 14.08 found: C, 71.82; H, 5.15; O, 14.75.

***Synthesis of Acid Anhydride Derivatives of naproxen* . 2- (6 – Methoxynaphthalene -2-yl) propanoic anhydride**

The anhydrides intermediate was obtained when two mmols of naproxen (0.46 g) was dissolved in tetrahydrofuran (THF) (30 mL), and then one mmol of dicyclohexyl carbodiimide (DCC) (0.206 g) was added. The reaction mixture was continuously stirred at room temperature for 4 hours, whereby a white precipitate of dicyclohexylurea (DCU) was formed, which then removed by filtration. The solvent was evaporated under vacuum to yield anhydride [24] as a white powder (75% yield); m.p. 128–130 °C; R_f = 0.45. IR (cm−1): 1,801 and 1,737 of anhydride (symmetric and asymmetric), 1,606 and 1,471 (aromatic), 1,313, 1,224, 1,159 C-(C=O)-O-(C=O)-C of anhydride.

General procedure for the amidation of naproxen: synthesis of compounds (III & IV).

A mixture of anhydride derivatives of naproxen (2.65 g, 6 mmol), 4-chloro aniline (1.53 g, 12 mmol) or 4-nitro aniline (1.65g, 12 mmol), zinc dust (0.011 g, 0.168 mmol), glacial acetic acid (1.1 mL, 19.2 mmol) and dioxane (35 mL) were placed in a flask equipped with reflux condenser, and boiling stones were added. The reaction mixture was refluxed gently for 90 min, the solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate, washed with sodium bicarbonate ($NaHCO_3$) (10%, 3×),

hydrochloric acid (HCl) (1 N, 3×), and distilled water (3×), and filtered over anhydrous magnesium sulfate ($MgSO_4$). The filtrate was evaporated under vacuum to give the final compounds I-IV. The final products were obtained as solids and the recrystallization was carried out by dissolving the compound in ethyl acetate and addition of petroleum ether (80–100°C) to the filtrate until turbidity occurred and then keeping in a cold place overnight. The mixtures were filtered while cold and the precipitate was collected to give the final compounds (IIII & IV) [24].

2- (6- methoxynaphthalen – 2 – yl) – N - (4 - chlorophenyl)- propanamide (compound III)

White crystal m.p. 218–220 °C; R_f = 0.68; IR (cm^{-1}): 3,298 (N-H) of secondary amide, 1,658 (C=O) of secondary amide, 1,595 and 1,516 (C=C of aromatic); CHNS calculated ($C_{20}H_{18}ClNO_2$): C, 70.69; H, 5.34; N, 4.12; O, 9.42; found: C, 71.23; H, 5.29; N, 3.89; O, 9. 82.

2- (6 – methoxynaphthalen – 2 - yl) – N - (4 - nitrophenyl)propanamide (compound IV)

White crystal m.p. 175-176 °C; R_f = 0.68; IR (cm^{-1}): 3,286 (N-H) of secondary amide, 1,667 (C=O) of secondary amide, 1,597 and 1,523 (C=C of aromatic); CHNS calculated ($C_{20}H_{18}ClNO_2$): C, 68.56; H, 5.18; N, 8.00; O, 18.27; found: C, 67.94; H, 5.27; N, 7.89; O, 18.81.

Results and Discussion

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 [25]. When egg-white is injected into the paw of rats, a substantial induction of COX-2 is observed at 2 hours coinciding with enhanced prostaglandins (PGs) and local edema [26].

Table (1) shows the effect of naproxen (reference) and propylene glycol (control) on egg-white induced paw edema in rats. The differences in paw thickness readings among control and naproxen groups indicates that the method used in this study (paw edema) is a valid method and can effectively be used for the assessment of the anti-inflammatory effect of the newly synthesized compounds as shown in Figure 2.

Table 1: Effect of naproxen (reference) and propylene glycol (control) on egg white induced paw edema in rats.

Time (min)	*Control (n = 6)*	*Naproxen (n = 6)*
0	4.48 ± 0.08	4.43 ± 0.06
30	6.55 ± 0.14	6.48 ± 0.13
60	7.63 ± 0.04	6.80 ± 0.07 *
120	7.05 ± 0.16	6.64 ± 0.03 *
180	6.75 ± 0.11	6.16 ± 0.11 *
240	6.50 ± 0.09	5.86 ± 0.05 *
300	6.12 ± 0.08	5.70 ± 0.07 *

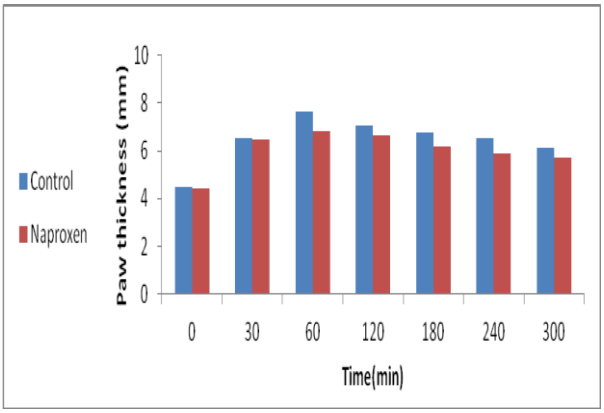


Figure 2. Effect of naproxen (reference), and propylene glycol (control) on egg-white induced paw edema in rats. Time (30) is the time of egg-white injection.

Table (2) shows the effect of the tested compounds (I-IV) with respect to control and reference group (naproxen). All tested compounds effectively limited the increase in paw edema, the effect of all tested compounds started at 60 minutes (significantly different compared to control). However, the effect of all tested compounds continued till the end of the experiments with statistically significant (P > 0.05) reduction in paw edema, as shown in Figure 3.

Table 2: Effect of control, naproxen and compounds (I-IV) on egg-white induced paw edema rats.

Time (min)	Control (n = 6)	Naproxen (n = 6)	Compound I (n =6)	Compound II (n =6)	Compound III (n =6)	Compound IV (n =6)
0	4.48 ± 0.08	4.43 ± 0.06	4.40 ± 0.07	4.45 ± 0.16	4.41 ± 0.11	4.46 ± 0.13
30	6.55 ± 0.14	6.48 ± 0.13	6.43 ± 0.05	6.46 ± 0.11	6.56 ± 0.07	6.50 ± 0.11
60	7.63 ± 0.04	6.80 ± 0.07 *	6.70 ± 0.11*	6.86 ± 0.03 *	6.88± 0..13*	6.71 ± 0.07 *
120	7.05 ± 0.16	6.64 ± 0.03 * a	6.26 ± 0.13 * b	6.56 ± 0.03 * a	6.70 ± 0.13 * a	6.30 ± 0.12 * b
180	6.75 ± 0.11	6.16 ± 0.11 * a	5.49 ± 0.09 * b	6.10 ± 0.15 * a	6.26 ± 0.11 * a	5.77 ± 0.11 * c
240	6.50 ± 0.09	5.86 ± 0.05 * a	5.23 ± 0.04 *b	5.80 ± 0.05 * a	5.98 ± 0.05 * a	5.38 ± 0.05 * b
300	6.12 ± 0.08	5.70 ± 0.07 * a	4.95 ± 0.07 *b	5.40 ± 0.07 * c	5.67 ± 0.07 * a	5.09 ± 0.07 * b

Non-identical superscripts (a & b) among different tested compounds are considered significantly different ($p < 0.05$); * significantly different compared to naproxen ($p < 0.05$).

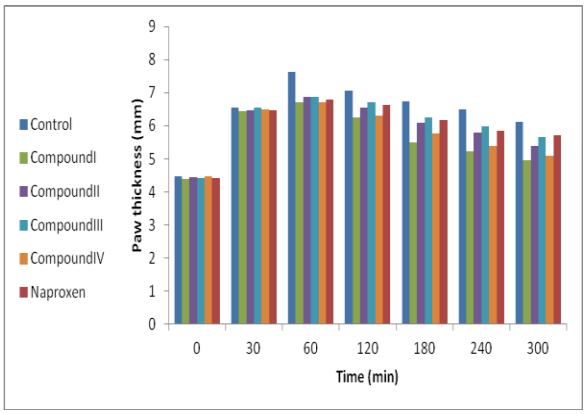


Figure 3: Effect of naproxen, propylene glycol, compounds I, II, III and IV on egg-white induced paw edema in rats. Results are expressed as mean ± SEM (n = 6 for each group). Time (30) is the time of egg-white injection.

The comparison between the tested compounds and naproxen shows that at time 0–60 minutes there are no differences with naproxen between groups; however at the interval time 120–300 minute, compounds I and IV show significantly higher effects than naproxen while compound II expressed a comparable effect to that of naproxen at the interval time 60–240 minute, and compound III showed a comparable effect to that of naproxen for all the experimental times.

Conclusions

An *in vivo* anti-inflammatory study showed that esterification and amidation of naproxen maintained or increased its anti-inflammatory activity. These structural pre-requisites for COX-2 selectivity can mainly attributed to the occupancy of the side pocket (val.509) in COX-2 enzyme.

Compounds II and III showed a comparable effect to that of naproxen, while compounds I and IV might show higher effects comparable to that of naproxen and to that of compounds II & III which may attributed to the higher effect of nitro group than chloride group.

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