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Design, Synthesis and Preliminary Pharmacological Evaluation of Mutual Prodrug of Non-Steroidal Anti-Inflammatory Drugs Coupling With Natural Anti-Oxidants *Via* Glycine

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Abstract:

Non-steroidal anti-inflammatory drugs (NSAIDs); naproxen and indomethacin have been conjugated with different antioxidants (thymol, menthol & guaiacol) having antiulcerogenic activity via glycine amino acid as spacer with the objective of obtaining NSAIDs-glycine- antioxidant prodrugs as gastrosparing NSAIDs devoid of ulcerogenic side effects and synergistically with anti-inflammatory action of glycine. Four mutual prodrugs (I-IV) were synthesized using glycine as spacer and their structures were confirmed and characterized using elemental microanalysis (CHNO), IR, and some physiochemical properties.

Invivo acute anti-inflammatory activity of the compounds (I & II) (naproxen derivatives) and the compounds (III & IV) (indomethacin derivatives) was evaluated in rat using an egg-white induced edema model of inflammation in a dose equivalent to 2.5 mg/Kg of naproxen, and 2 mg/Kg of indomethacin respectively.

All tested compounds produced significant reduction of paw edema with respect to the effect of propylene glycol 50% v/v (control group). Moreover, the activity of compound III was significantly higher than that of indomethacin (at 2 mg/Kg), while compound IV expressed a comparable effect to that of indomethacin in the (120–300) minute time of the experiment, while compounds I&II was showed a comparable effect to that of naproxen at (180-300) minute time interval of the experiment. The result of this study indicates that these mutual prodrugs of naproxen & indomethacin maintained or may enhanced their anti-inflammatory activity.

Keywords: NSAIDs naproxen; indomethacin; glycine; anti-oxidant; mutual prodrug; anti-inflammatory; paw edema.

الخلاصة:

الادوية المضادة للالتهاب غير الستيرودية (نابروكسين واندوميثاسين) تم أدماجها مع مجموعة مختلفة من المواد المضادة للاكسدة (الثيمول والمنثول وكواياكول) الحاوية على نشاط مضاد للمقرحات باستخدام الحامض الاميني (الكلايسين) كذراع بهدف الحصول على مقدمات الأدوية المتبادلة لأدوية غير ستيرويدية مضادة للألتهاب مع أنواع مختلفة من مضادات التأكسد بواسطة الحامض الاميني كأدوية غير ستيرويدية مضادة للألتهاب منقذة للمعدة تخلو من المقرح كأثار جانبية مع زيادة فعاليتها مع الكلايسين المتميز بخواصه المضادة للالتهابات.

أربعة من مقدمات الأدوية المتبادلة تم تخليقها وتشخيص تركيبها وخصائصها باستخدام التحليل الدقيق للعناصر (CHNO)، تحليل طيف الأشعة تحت الحمراء (IR) وبعض الخواص الفيزيوكيميائية.

لقد تم تقييم الفعالية الحادة المضادة للالتهابات للمركبين (1 ,2) (مشتقات النابروكسين) والمركبين (3 ,4) (مشتقات الاندوميثاسين) باستخدام طريقة أستحداث وذمة تحت الجلد باستخدام زلال البيض بجرعة مكافئة للنابروكسين (2,5ملغم/كغ) و بجرعة مكافئة للاندوميثاسين (2 ملغم/كغ)على التوالي .
كل المركبات المختبرة أدت إلى انخفاض مؤثر للوذمة مقارنة مع تاثير البروبلين كلايكول 50 % حجم/حجم (مجموعة ضابطة). علاوة على ذلك مركب3 اظهر فعالية مضادة للالتهاب أعلى مقارنة بالاندوميثاسين بجرعة (2 ملغم/كغ) ولكن مركب4 اظهر فعالية مضادة للالتهاب مقاربة للاندوميثاسين للفترة (120-300) دقيقة من التجربة, بينما المركبين (2,1) اظهرت فعالية مضادة للالتهاب مقاربة للنابروكسين للفترة (180-300) دقيقة من التجربة. نتيجة هذه الدراسة تعطي انطباع إلى إن هذه المقدمات من الادوية المتابدلة للنابروكسين والاندوميثاسين قد حافظت على فعاليتها المضادة للالتهابات مع احتمالية زيادتها.

Introduction:

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are among the most commonly prescribed classes of drugs throughout the world. The overall worldwide production of about 50,000 tons a year reflects the importance of this substance even today [1].

NSAIDs are used extensively to alleviate inflammation, pain, rheumatoid arthritis, and osteoarthritis. Long-term regimens of NSAIDs have been greatly shortened due to their gastrointestinal side effects [2].

They are prone to produce certain prevalent side effects such as gastrointestinal irritation though these are more likely with high doses and prolonged use [3].

Owing to their wide spread consumption, a large population taking NSAIDs is reported to eventually develop gastric ulcers and related complications, leading to a condition popularly known as NSAID gastropathy, which is characterized by sub epithelial hemorrhages, erosions and ulcers. Around 50% patients are reported to have gastric erosions and 10-30% suffers from gastric ulcer. [4].

However, recent human epidemiological studies suggest an inverse relationship between intake of NSAIDs and the risk of colorectal cancer [5], and the severity or incidence of Alzheimer's disease [6]. Therefore, efforts are directed to develop NSAIDs with minimal side effects.

The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclooxygenases (COXs) involved in the biosynthesis of prostaglandin H2 (PGH2).[7] It is now well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently.[8]. COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT[9]. COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells.[10]. The suppression of COX-1 and COX-2 is the primary mechanism through which NSAIDs induce ulceration. NSAIDs may be related to suppression of COX-2, this also has implications for gastric mucosal integrity. Suppression of COX-2 leads to leukocyte-endothelial adhesion within the microcirculation, which contributes to ulcer formation. Also, COX-2-derived Prostaglandins (PGs) are essential for healing of mucosal injury, and this process is therefore impaired when COX-2 is inhibited. [10]. There for, design and development of safer agents still remain.

The mutual prodrug is an efficient approach for drug optimization, the term 'mutual prodrug' refers to two or more therapeutic compounds bonded via a covalent chemical linkage. Regardless of being similar to prodrug it differs in having inactive group replacement by active

group, which are coupled directly or indirectly by a cleavable spacer. [11].

A major limitation of the approach is the requirement of specific functional groups for linkage. When two drugs are administered simultaneously they may not be absorbed or transported to the target site of action but, the mutual prodrug has improved absorption rate and can be easily transported to the target site of action.

It has to be stable at the gastrointestinal level, but then it has to be hydrolyzed to provide two (or more) different drugs[11] .

The designated mutual prodrug is oriented into two directions:

1- Coupling of NSAID with glycine does not resulting in the temporarily masking the acidic group of NSAIDs for reducing the GI toxicity, furthermore, glycine as a promoiety was used, because glycine shows broad-spectrum anti-inflammatory, cytoprotective, and immune-modulatory properties and would be expected to synergistic with anti-inflammatory activity of NSAIDs as well as, the colon specific drug delivery of non-steroidal anti-inflammatory drugs involves targeting the drug to the colon, thereby lowering the required dose, reducing the systemic side effects, and thus resulting in a more effective therapy system. [12-13].

   The neutralization of the carboxylate of NSAIDs can generate COX-2-selective inhibitors. The amidation of NSAIDs abolishes COX-1 inhibitory activity while, maintaining COX-2 inhibitory activity. Because many NSAIDs contain a carboxylic acid group, this would represent a general strategy for the conversion of nonselective NSAIDs into selective COX-2 inhibitors.[14-15] Structure activity relationship analysis reveals that structurally diverse functionalities can serve as part of the amide linkage in indomethacin, resulting in highly selective COX-2 inhibitors. [16].

2- During recent years, it has been well established that generation of reactive oxygen species (ROS) plays a significant role in the formation of gastric mucosal lesions associated with NSAIDs therapy[17].

   Based on these observations, it has been suggested that co administration of antioxidants and NSAIDs in pharmaceutical dosage forms may possibly decrease the risk of NSAIDs induced GI ulcerogenicity. [18, 19].

There are potential advantages in giving such agents with complementary pharmacological activities in the form of a single chemical entity.

Such agents are named as mutual prodrugs that are designed with the aim of improving physiochemical properties [20] in comparism to physical mixture of NSAIDs and natural antioxidants ,the reduction in ulcer index is superior due to the polar nature of antioxidant that lead to low bioavailability of antioxidants.

In the view of this background, the present study was conducted to design, synthesis, and preliminary pharmacological study of mutual prodrugs of NSAIDs with different antioxidants to get NSAIDs with lesser ulcerogenic side effects while retaining the anti-inflammatory and analgesic activity.

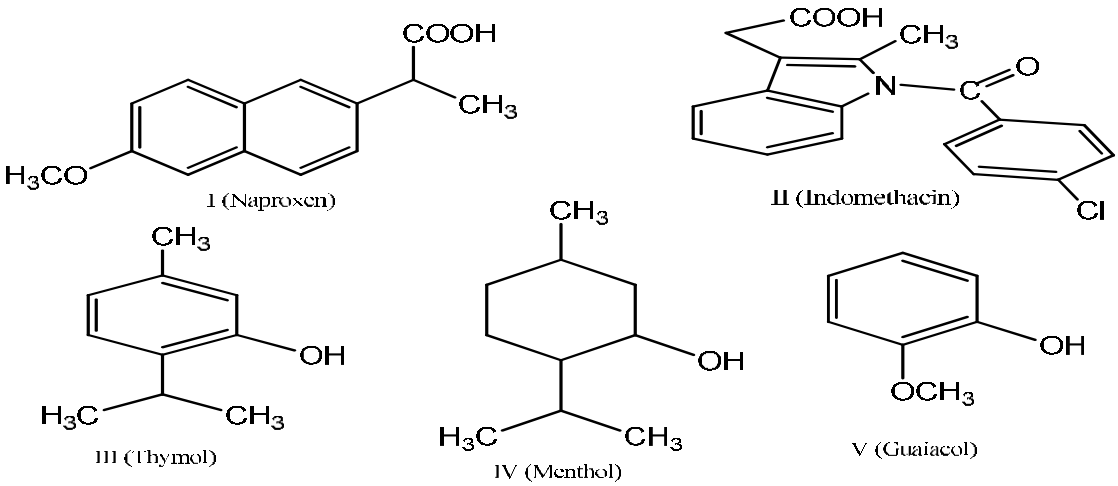


Figure-1: Chemical structures of NSAIDs and antioxidants

Results and Discussion:

Many irritant agents have been used in the paw-edema method like dextran, egg-white and carrageenan solution. The paw edema induced by carrageenan has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins [21]. the intraplantar injection of egg-white into rat hind paw induces a progressive edema. To assess the validity of the method (paw edema) used for the evaluation of newly synthesized antiinflammatory compounds, naproxen, indomethacin were used as a reference compounds of known anti-inflammatory activity profile.

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) also shows the effect of the tested compounds I-IV with respect to control and reference groups (naproxen & indomethacin). All tested compounds effectively limited the increase in paw edema, with statistically significant ($P > 0.05$) reduction in paw edema, as shown in Figure 2.

Table-1: Effect of compounds I,II & naproxen and compounds III, IV & indomethacin & propylene glycol on egg-white induced paw edema in rats.

	Treated groups							
	Time min	**Control (n=6)**	**Naproxen (n=6)**	**Compound (I) (n=6)**	**Compound (II) (n=6)**	**Indomethacin (n=6)**	**Compound (III) (n=6)**	**Compound (IV) n=6**
Paw thick-ness (mm)	0	3.38±0.18	3.20±0.15	3.20±0.18	3.26±0.17	3.34±0.15	3.30±0.18	3.32±0.15
	30	5.49±0.16	5.50±0.20	5.43±0.14	5.47±0.15	5.52±0.15	5.45±0.20	5.57±0.10
	60	6.15±0.20	5.99±0.20	5.94±0.12	6.06±0.13	5.84±0.17	5.65±0.14[b]	6.08±0.14
	120	6.39±0.13	4.99±0.13*a	5.35±0.11*b	5.45±0.13*b	5.39±0.14*a	4.57±0.16[b]	5.46±0.11*a
	180	6.10±0.20	4.54±0.12*a	4.62±0.19*a	4.52±0.14*a	4.96±0.19*a	4.17±0.13[b]	4.82±0.12*a
	240	5.79±0.15	4.30±0.15*a	4.14±0.13*a	4.13±0.18*a	4.44±0.14*a	3.67±0.12[b]	4.26±0.15*a
	300	5.50±0.20	4.07±0.16*a	3.93±0.15*a	3.88±0.20*a	4.09±0.18*a	3.40±0.16[b]	4.01±0.14*a

Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of tested compounds and propylene glycol. Time (30) is the time of injection of egg-white (induction of paw edema). * Significantly different compared to control ($p<0.05$). Non-identical superscripts (a and b) among different groups are considered significantly different ($p<0.05$).

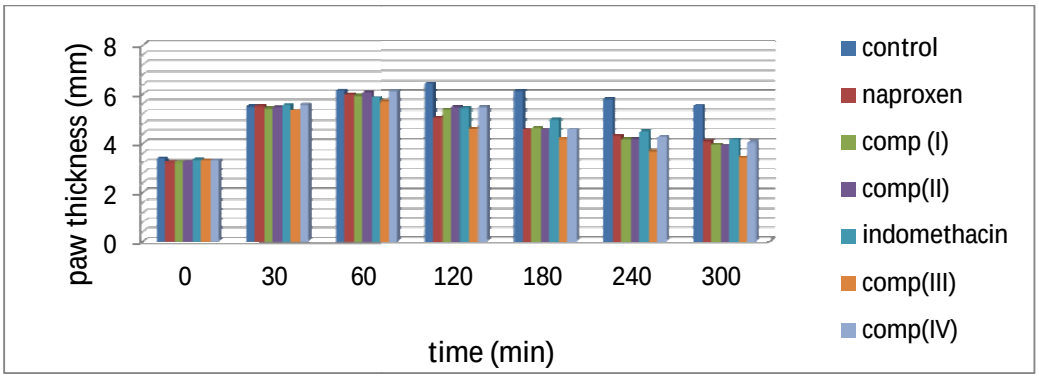


Figure-2: Effect of propylene glycol (control), compounds(I),(II)& naproxen, indomethacin, compound (III) & (IV) on egg-white induced paw edema in rats. Results are expressed as mean ± SEM (n=6 for each group). Time (30) is the time of egg-white injection.

Multi-way comparison between reference drugs and tested compounds revealed the following:

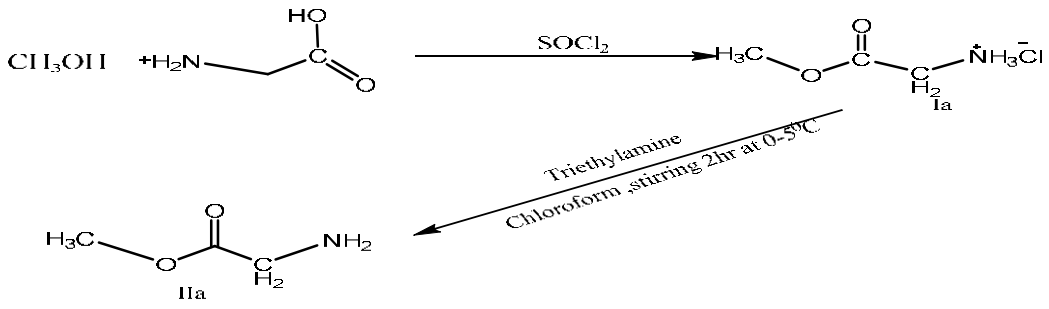
1- All tested compounds were effectively limited the increase in paw edema.
2- The effect of naproxen derivatives (compounds I & II) showed a comparable effect to that of naproxen at the time 180-300 minutes of the experiment, as shown in table (1) & figure (2).
3- The effect of indomethacin derivative (compounds III) started 1 hour after injection of drug, while that for remaining compounds started 2 hours after injection of them, & continued till the end of the experiment, this indicate rapid onset of action of (compound III), as shown in table (1) & figure (2).
4- The effect of (compound III) was significantly higher than that of references and remaining tested compounds at the time 120-300 minutes of the experiment, as shown in table (1) & figure (2).
5- The effect of indomethacin derivative (compound IV) showed a comparable effect to that of indomethacin at the time 120-300 minutes of the experiment, as shown in table (1) & figure (2).

Experimental Part:

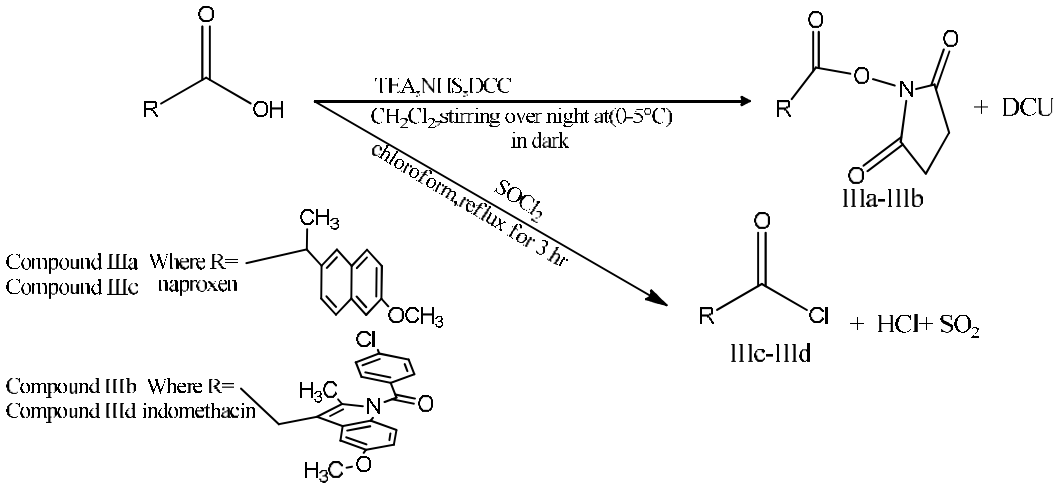
All reagents and anhydrous solvents were of analar type and generally used as received from the commercial suppliers (Merck, Germany, Reidel De-Haen, Germany, Sigma-Aldrich, Germany and BDH, England). Naproxen, indomethacin was supplied by the SDI Company, Iraq. Melting points were determined by capillary method on Bamstead/Electrothermal 9100 an Electric melting point apparatus (England) and ascendig thin layer chromatography (TLC) to check the purity and progress of reactions was run on DC-Kartan SI alumina 0.2 mm plates.

The identification of compounds was done using a U.V. detector and the chromatograms were eluted with THF-ether-cyclohexane (4:4:2). IR spectra were recorded on a FTIR-spectrophotometer Shimadzu as KBr disks.

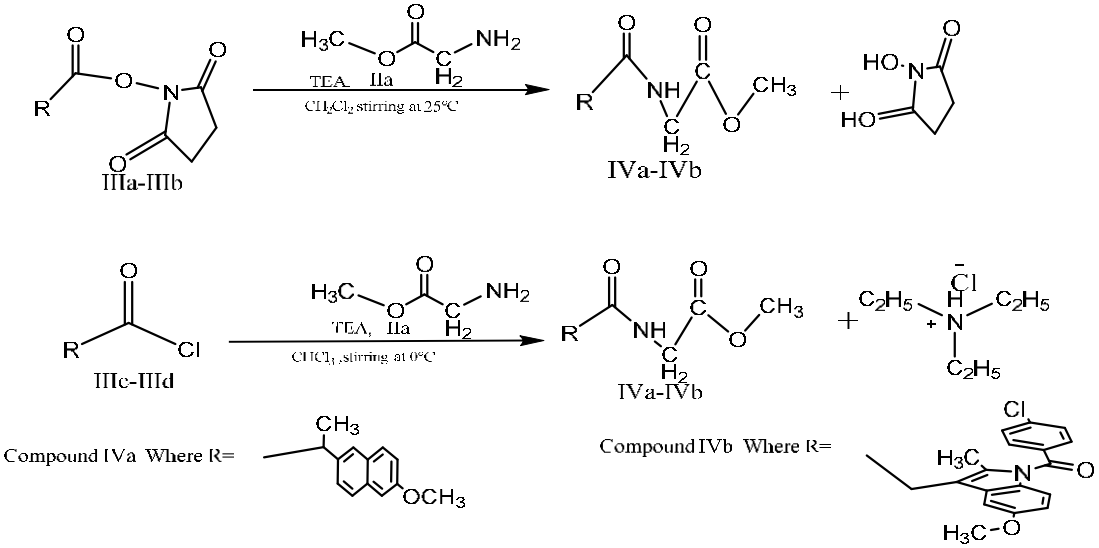
CHNS microanalysis was done using a Euro EA 3000 elemental analyzer (Italy). The general routes outlined in the following schemes were used to synthesize all compounds described here:



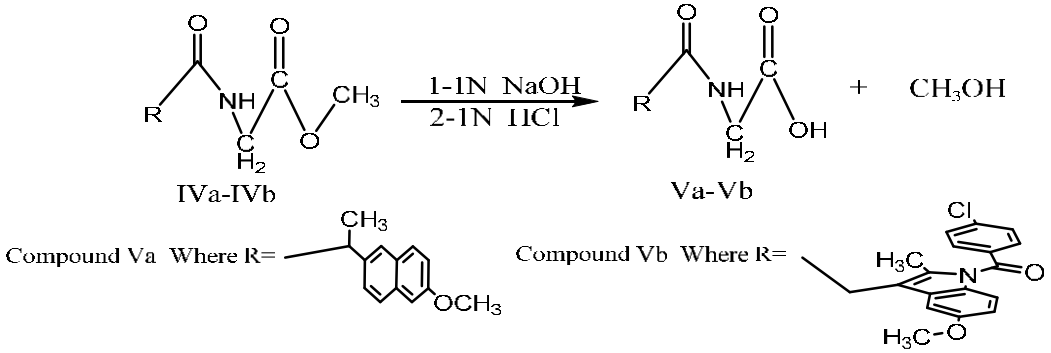
Scheme-1: Synthesis of free glycine methyl ester compound (IIa)



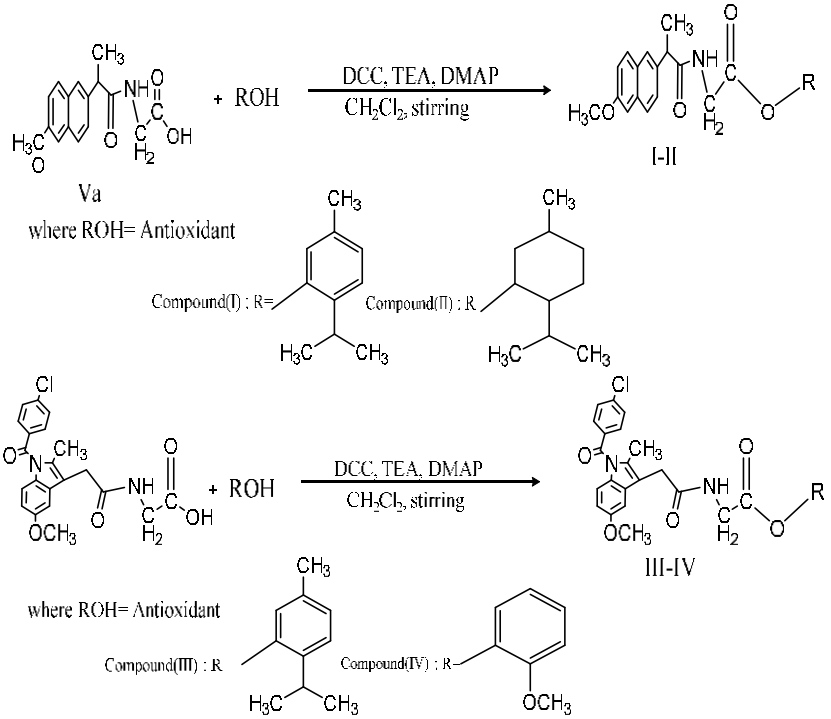
Scheme-2: Synthesis of activated naproxen & indomethacin compounds (IIIa-d)



Scheme-3: Coupling of compounds (IIIa-IIId) & (IIa).



Scheme-4: Saponification of compounds (IVa,IVb)



Scheme5: Coupling of compounds (Va-Vd) & natural antioxidants

Synthesis of glycine methyl ester hydrochlorides (Ia) [21,22]**:**

Thionyl chloride (1.2ml) was slowly added to an absolute methanol (40ml) with cooling to 0°C on ice bath for 15 minute then glycine (0.01mole, 0.7507gm) was added to it.

The mixture was refluxed for 6 h at 65-70 °C with continuous stirring and monitored by evolution of excess HCl gas which is detected by changing the color of pH graduated Litmus paper into Reddish of (1-1.5) pH when was placed on the top of condenser.

The excess of thionyl chloride and solvent was removed under reduced pressure by using rotary evaporator to give glycine methyl ester hydrochloride. The product was recrystalized from methanol by slow addition of diethyl ether (25ml) and cooling at 0°C.

The resulting solid product was collected and dried under vacuum. 2380-3333 Broad, strong band of 1753(C=O) stretching vibration of ester, 1633 & 1573 asymmetrical and symmetrical bending of $+NH_3Cl-$, The percent yield, physical appearance, melting point and TLC are listed in table (2).

Conversion of glycine methyl ester hydrochloride into free glycine methyl ester (IIa) [23,24]**:**

To a suspension of of the glycine methyl ester hydrochloride (10mmole) in chloroform (20ml), triethylamine (2ml, 20mmol) was added over a period of ten minutes at 0°Cwith continuous stirring for 2 hours until completely dissolved and clear solution was obtained.

The clear solution was directly used for the next coupling step

Synthesis of naproxen & indomethacin - N-hydroxy succinamide esters (IIIa-b) [25,26]**:**

Naproxen (5mmol, 1.15g) or indomethacin(5mmol, 1.788gm) was

dissolve in dry dichloromethane (10 ml) and triethylamine (5mmol, 0.5ml) was added under stirring; then N-hydroxy succinamide (NHS) (5mmol, 0.575gm) and N,N-dicyclohexylcarbodiimide (DCC) then (5mmol, 1.031gm) were added.

The reaction was let under stirring overnight at (0-5°C) in the dark. Dicyclohexylurea (DCU) was filtered out and the solution was dropped into diethylether (25 ml), and kept at 0°C , and then filterated and washed with diethyl ether (25ml) and dried under vacuum to produce compound IIIa or IIIb respectively .This compound was directly used for the next coupling step with free glycine methyl ester (IIa).

Synthesis of naproxen and indomethacin acid chloride(IIIc-d) [27,28]:

Naproxen (5mmol, 1.15 g) or indomethacin(5mmol, 1.788gm) was dissolved in dry chloroform (20 ml in a100 ml round-bottomed flask. Thionyl chloride (15mmol, 1.1ml) was added drop wise over a period of 15 minute with cooling on ice bath.

The mixture was refluxed for 3 hr at 65 °C with continuous stirring and monitored by evolution of excess HCl gas which is detected by changing the color of pH graduated Litmus paper into Reddish of 1-1.5pH when was placed on the top of condenser and changing the color of the solution from colorless into deep yellow or green respectively .

The excess of thionyl chloride and solvent was removed under reduced pressure and re-dissolving in dry chloroform (20 ml)and re-evaporated to giving oily yellow residue or greenish oily residue compound IIIc and IIId respectively.

This compound was directly used for the next coupling step with free glycine methyl ester (IIa).

General procedure for synthesis of intermediate compounds (IVa-b): Method A [26,29]:

A mixture of compound IIa (5mmol) and compound III derivative (5mmol) IIIa or IIIb was dissolved in dry dichloromethane (15ml), one drop of triethylamine was added to the mixture and then stirring was continuous for 2 days at temperature 25°C in the dark. The solvent was evaporated under reduced pressure, and the product was re-dissolve in ethyl acetate (10ml)and washed with 5% aqueous solution of sodium bicarbonate (20ml), 5% HC (20ml & distilled water(20ml), & then dried over anhydrous sodium sulfate , filtered& the solvent was evaporated under reduced pressure .the solid product re crystallized from methanol –diethyl ether .

(IVa) 3302 N-H stretching of secondary amide, 3097(C-H)stretching of aromatic, 1762 (C=O) stretching vibration of ester, 1653 (C=O) of secondary amide , 1604,1508,1460 (C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide.

IVb): 3300 N-H stretching of secondary amide, 3050 (C-H) stretching of aromatic, 1760 (C=O) stretching vibration of ester, 1690 (C=O) of secondary amide, 1600, 1580, 1490 (C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide. The percent yield, physical appearance, melting point and TLC results are listed in table (2).

Method B [23, 28]**:**

Compound IIa 5mmol was dissolved in dry CHCl3 (15ml) in a100ml round flask container, then triethylamine (5mmole, 0.5 ml) was added drop wise with stirring for 20 minutes on ice bath and, then compound IIIc or IIId was slowly dropped for 50 minute with continuous

stirring on ice bath, then continuous stirring at room temperature over the night.

The excess of thionyl chloride and solvent was removed under reduced pressure by using rotary evaporator. The resulting solid product was re- dissolved in ethyl acetate (10 ml) and washed with 5 % aqueous solution of sodium bicarbonate (20ml), 5% HCl (20ml) & distilled water (20ml) and then dried over anhydrous sodium sulfate, filtered & the solvent was evaporated under reduced pressure to give the intermediate compounds (IVa-b).

Synthesis of termediate compounds (Va-b):

Intermediate compound (IVa or IVb) (5mmol, 1.5gm or2.14gm) was dissolved in absolute methanol (50ml). The solution was cooled down 18°C, and then sodium hydroxide (1N, 5ml) was added drop wise, with continuous stirring over a period of 30 minutes. Stirring was continued at 18°C for additional five hours.

The reaction mixture was acidified with HCl (1N, 5ml), then excess of cold water was added .The methanol was removed under reduced pressure and the acidic compound was precipitated, and filtered then dried to give compound Va or Vb respectively[29].

The resulting solid product re-dissolve in dichloromethane & dried with anhydrous magnesium sulphate, filtrate & the solvent was dried under vacuum to produce compound (Va or Vb) respectively.

(Va): 3400, 3313, 3180N-H stretching of secondary amide& (OH) stretching vibration, 3043(C-H) stretching of aromatic, 1732 C=O) stretching vibration of acid, 1641 C=O) of secondary amide, 1655, 1544, 1477(C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide.

(Vb): 3300 N-H stretching of secondary amide, 3050(C-H) stretching of aromatic, 1760(C=O) stretching vibration of ester, 1690(C=O) of secondary amide, 1600, 1580, 1490(C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide.

The percent yield, physical appearance, melting point and TLC results are listed in table (2).

Synthesis of final compounds (I-IV) [19]:

Intermediate compound (Va or Vb) (2mmol, 0.57gm, 0.82gm) was dissolved in dry dichloromethane (25ml) in a 100ml round-bottomed flask, then triethylamine (0.1ml) & N ,N-dicyclohexylcarbodiimide (2mmol, 0.412gm) were added with continuous stirring on ice bath. The reaction mixture was stirred at 0°C for 2hours, then, dimethylaminopyridine (DMAP) (20mg) & then antioxidants (2mmol, 0.3gm thymol or 0.33gm menthol or 0.248gm guiacol) were added .The reaction mixture was stirred at room temperature for 24 hour. The precipitated N,N-dicyclohexylurea was removed by filtration. The solvent was removed under reduced pressure to produce the solid product. The resulting solid product was re- dissolved in ethyl acetate (10 ml and washed with 5 % aqueous solution of sodium bicarbonate(2x20ml), 5% HCl(2x20ml) &distilled water(2x20ml) & then dried over anhydrous sodium sulfate , filtered& the solvent was evaporated under reduced pressure to give final compound (I-IV).

1- Compound: **I** (2-isopropyl- 5-methylphenyl 2- (2 methoxynaphthalen-2-yl) propanamido) acetate): 3327, 3267N-H stretching of secondary amide, 3061 (C-H) stretching vibration of aromatic, 1776 (C=O) stretching vibration of ester, 1653 (C=O) of secondary amide, 1627,15541504

(C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide, CHNO microanalysis calculated
C:74.44,H:6.97,N:3.34,O:15.26,
founded:73.976,H:6.89,N:3.30,O:14.01.

2- Compound II (2-isopropyl-5-methylcyclohexyl 2- (2- (6-methoxynaphthalen-2-yl) propanamido) acetate):
3477,3309 N-H stretching of secondary amide, 3059 (C-H) stretching vibration of aromatic, 1751 (C=O) stretching vibration of ester, 1647 (C=O) of secondary amide, 1635, 1608, 1504 (C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide. CHNO microanalysis calculated
C:73.38,H:8.29,N:3.29,O:15.04
founded:73.231,H:8.18,N:3.237,O:15.11.

3- Compound III (2- isopropyl-5-methylphenyl2-(2-(1-(4-chloro-benzoyl)-5- methoxy- 2- methyl-1H-indol-3-yl) acetamido)acetate) :
3327 N-H stretching of secondary amide, 3068 (C-H) stretching of aromatic, 1760 (C=O) stretching vibration of ester, 1687 (C=O) stretching vibration of secondary amide, 1608, 1500, 1481 (C=C) stretching vibration of aromatic overlapping with N-H bending of secondary amide. CHNO microanalysis calculated C:68.06,H: 5.71,N: 5.12, O:14.62 foundedC:68.00,H:5.65,N:5.10,O:14.8.

4- Compound IV 2-methoxyphenyl 2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetamido) acetate:
3483, 3361 asymmetrical and symmetrical N-H stretching of secondary amide,3050 C-H) stretching of aromatic, 1751 (C=O) stretching vibration of ester, 1685 (C=O) stretching vibration of secondary amide, 1597 1477,1456, (C=C) stretching vibration of aromatic overlaping with N-H bending of secondary amide. CHNO microanalysis calculated C: 64.55, H: 4.84, N: 5.38, O: 18.43 founded. C: 64.43, H : 4.67,N:5.29,O:18.33.
The percent yield, physical appearance, melting point and TLC results are listed in table (2).

Table-2: The percent yield, physical appearance, melting point and R_f values of the intermediates and final compounds

Compounds & intermediates	chemical formula	Molecular weight	Description	% yield	Melting point ^oC	R_f* value
Ia	$C_3H_8ClNO_2$	125.5	White crystals	90	175	A=0.76 B=0.61
IIIa	$C_{18}H_{17}NO_5$	327.33	White crystal	69	----------	A=0.9 B=0.78
IIIb	$C_{23}H_{19}ClN_2O_6$	454.86	Yellow powder	72	---------	A=0.88 B=0.70
IIIc	$C_{14}H_{13}ClO_2$	248.70	yellow Oily substance	------	---------	A=0.87 B=0.77
IIId	$C_{19}H_{15}Cl_2NO_3$	376.23	Green Oily substance	------	----------	A=0.78 B=0.65
IVa	$C_{17}H_{19}NO_4$	301.34	White powder	80	110	A=0.83 B=0.64
IVb	$C_{22}H_{21}ClN_2O_5$	428.87	Yellow powder	78	137	A=0.9 B=0.78
Va	$C_{16}H_{17}NO_4$	287.3	White powder	55	141	A=0.88 B=0.70
Vb	$C_{21}H_{19}ClN_2O_5$	414.84	Yellow powder	57	153	A=0.87 B=0.77
I	$C_{26}H_{29}NO_4$	419.51	White crystals	75	142	A=0.73 B=0.60
II	$C_{26}H_{35}NO_4$	425.56	White crystals	78	136	A=0.82 B=0.63
III	$C_{31}H_{31}ClN_2O_5$	547.04	Faint yellow crystal	70	142	A=0.67 B=0.90
IV	$C_{28}H_{25}ClN_2O_6$	520.96	Faint yellow crystal	71	163	A=0.87 B=0.78

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 seven groups (each group consisting of six rats) as follows:

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

Group B: six rats treated with naproxen as reference substance in a dose of 2.5mg/ kg [30] suspended in propylene glycol 50% (v/v).

Group C: six rats treated with indo-methacin as reference substance in a dose of 2mg/kg[31].suspended in propylene glycol 50% (v/v).

Group D-G: six rats/group treated with the tested compounds (I, II, III & IV) in doses that determined below. (Suspended in propylene glycol 50% v/v). as a finely homogenized suspension in 50% v/v propylene glycol in water.

The synthesized compounds (I& II) are derivatives of naproxen which is given in a dose of 2.5mg/kg, so; the doses of synthesized compounds as bellow:

Dose=4.55mg/kg of compound I (i.e. dose of 2.5mg/kg naproxen is equivalent to 4.55mg/kg of compound I).

Dose=4.61mg/kg of compound II (i.e. dose of 2.5mg/kg naproxen is equivalent to 4.16 mg/kg of compound II).

The synthesized compound III & IV are derivatives of indomethacin which is given in a dose of 2mg/kg, so; the doses of synthesized compounds as bellow:

Dose=3.057mg/kg of compound III (i.e. dose of 2mg/kg of indomethacin is equivalent to 3.057mg/kg of compound III).

Dose=2.9mg/kg of compound IV (i.e. dose of 2mg/kg of indomethacin is equivalent to 2.9mg/kg of compound IV).

Anti-Inflammatory Activity:

The anti-inflammatory activity of the tested compounds was studied using the egg-white induced edema model [32].

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 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: Two factors without Replication. Probability (P) value of less than 0.05 was considered significant

Conclusions:

An *in vivo* anti-inflammatory study showed that the conjugation of naproxen & indomethacin with natural antioxidant (thymol, menthol & guaiacol) via glycine amino acid as spacer maintained or increase the anti-inflammatory activity. Compounds **I** and **II** showed a comparable effect to that of naproxen, while compounds **III** & **IV** might show higher effects comparable to that of indomethacin.

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