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Research Article

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DESIGN, SYNTHESIS, AND HYDROLYSIS STUDY OF MUTUAL PRODRUGS OF NSAIDS WITH DIFFERENT ANTIOXIDANTS VIA GLYCOLIC ACID SPACER

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ABSTRACT

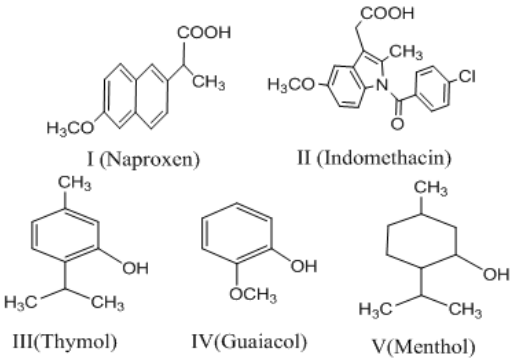
Non-steroidal anti-inflammatory drugs (NSAIDs); naproxen and indomethacin have been conjugated with different antioxidants (thymol, guaiacol, and menthol) having antiulcerogenic activity with the objective of obtaining NSAIDs- antioxidant prodrugs as gastrosparing NSAIDs devoid of ulcerogenic side effects. Six mutual prodrugs (1a-c and 2a-c) were synthesized using glycolic acid spacer and their structures were confirmed and characterized using elemental microanalysis (CHNO), IR, and some physiochemical properties. *In-vitro* chemical and enzymatic hydrolysis studies for naproxen derivatives (1a-c) revealed that these compounds were chemically stable in pH 1.2 and pH 7.4, with $t_{1/2}$ range from 6.22- 20.98 hr; while in 80% diluted plasma were found to be susceptible to enzymatic hydrolysis with more than 40% hydrolysis occur after 15 min the results indicate higher chemical stability of ester prodrugs in non enzymatic simulated gastro-intestinal fluid and rapid conversion to the parent drugs in 80% human plasma.

Keywords: NSAIDs, mutual prodrug, antioxidant, ulcerogenicity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world, owing to their analgesic, anti-inflammatory and antipyretic properties.[1,2] However, the use of "traditional" NSAIDs results in serious upper gastrointestinal (GI) adverse events e.g. (**figure 1**) Naproxen (**I**) and Indomethacin (**II**).[3] 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).[4] It is now well known that COX exists in two isoforms, namely COX-I and COXII, which are regulated differently.[5] COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT.[6] COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells.[7] Since most of the NSAIDs used clinically inhibit both isoforms, long term use of these agents results in gastric ulcer and there is enough evidence that inhibition of COX-I rather than that of COX-II underlies gastric ulcer formation.[8] But initial enthusiasm for selective COX-II inhibitors as safer NSAIDs has faded due to emergence of serious cardiovascular side effects on long term use and need for design and development of safer agents still remain.[9,10] It has been well known that local generation of various reactive oxygen species (ROS) plays a significant role in the formation of gastric ulceration associated with NSAID therapy.[11] These observations indicate that antioxidants may be used to prevent NSAIDs induced gastric ulcers. During the past few decades, a large number of naturally occurring compounds have been identified as antioxidants e.g. (**figure 1**) thymol (**III**), guaiacol (**IV**) and menthol (**V**), which are viewed as promising therapeutic agents for treating free radical mediated diseases including NSAID induced peptic ulcers.[12] Large number of herbs and spices are recognized as source of natural antioxidants and studies have confirmed their efficacy for the treatment of gastrointestinal ulcers.[13] Based on these observations, it has been suggested that coadminstration of antioxidants and NSAIDS in formulated dosage form may possibly decrease the risk of NSAIDs induced gastrointestinal side effects.[14] However, there are potential advantages in giving such coadministered drugs having complementary pharmacological activities in the form of a single chemical entity. Such agents are named as mutual prodrugs which are designed with improved physicochemical properties.[15,16] In the view of this background, the present study was conducted to design, synthesis, and preliminary kinetics 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



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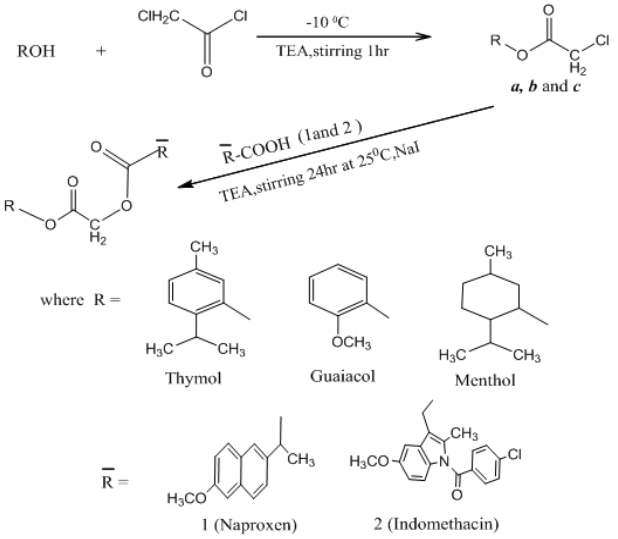
CHEMISTRY

The synthetic pathways for the designed target compounds (**1a-c** and **2a-c**) are illustrated in (scheme **1**).

Scheme1. Synthesis of target compounds (1a–c and 2a-c) & their intermediates.



Intermediate ***a, b*** and ***c*** if R= Thymol, Guaiacol and Menthol respectively.
Compound 1a if R = Thymol, R^- = Naproxen
Compound 2a if R = Thymol, R^- = Indomethacin
Compound 1b if R = Guaiacol, R^- = Naproxen
Compound 2b if R = Guaiacol, R^- = Indomethacin
Compound 1c if R = Menthol, R^- = Naproxen
Compound 2c if R = Menthol, R^- = Indomethacin

Hydrolysis study of naproxen analogues (1a, 1b, 1c)

Hydrolysis at pH 1.2 and pH 7.4 (chemical stability): The hydrolysis of the naproxen prodrugs was studied in aqueous HCl and phosphate buffer solution of pH 1.2 and pH 7.4 at 37°C. the total buffer concentration was 0.1 M and the ionic strength (μ) of 1.0 was maintained for each buffer by addition of calculated amount of sodium chloride. The rate of hydrolysis was followed spectrophotometrically by recording the decreases in the absorbance of naproxen prodrugs accompanying the hydrolysis. The reactions were initiated by adding 1 ml of stock solutions (1mg /ml) of the derivatives in ethanol to preheated buffer solution to give final concentration of derivatives 0.02mg /ml. The solutions were kept in a water bath at 37°C and samples (3ml) were withdrawn at appropriate time interval (15, 30, 60, 120, 240 min) and the absorbances were recorded. The observed first rate constants were determined from the slopes of the linear plots of log concentration versus time.

Hydrolyses in plasma (enzymatic stability): The hydrolysis rates of naproxen derivatives were studies in 80% human plasma diluted with isotonic phosphate buffer (pH 7.4) at 37°C. The reactions were initiated by adding 0.5 ml of stock solutions (1mg /ml) of the naproxen derivatives in ethanol to preheated diluted plasma to give final concentration of derivatives 0.02mg /ml. Samples (3ml) were withdrawn at appropriate time interval (15, 30, 60, 120, 240 min),after each incubation time sample was centrifuged and the supernatant was analyzed by UV spectrophotometer.

RESULTS AND DISCUSSION

All the compounds were evaluated for chemical and enzymatic hydrolysis.

Chemical and Enzymatic hydrolysis evaluation

Under experimental conditions used the hydrolysis of the naproxen analogues in the aqueous HCl and phosphate buffer solution of pH 1.2 and pH 7.4 at 37°C followed first order kinetics, since plots of log concentration vs. time resulted in straight lines, from their slopes, the observed rate constants of hydrolysis were calculated. Figures **2**, **3** and **4** are representative graphs for compounds **1a**, **1b** and **1c** respectively; while table **1** shows the pH values, the corresponding *Kobs* and half-life of the hydrolysis of naproxen analogues. The half-life was calculated using equation (**1**), which derives from the first order kinetic law.[17]

$$t_{1/2} = \frac{0.693}{Kobs} \quad \text{---------- equation (1)}$$

Table 1. The rate constant of hydrolysis of compounds 1a, 1b and 1c at pH 1.2 and pH 7.4 at 37°C, and μ=1.

compound	pH	*K*obs(min^{-1})	*t*½(min)
1a	1.2	1.0248×10^{-3}	676.22
	7.4	7.9223×10^{-4}	874.74
1b	1.2	9.304×10^{-4}	744.8
	7.4	1.856×10^{-3}	373.38
1c	1.2	6.862×10^{-4}	1010
	7.4	5.504×10^{-4}	1259

Figure 1. The hydrolysis of compound *1a* in 0.1M HCl and phosphate buffer of pH 1.2 and 7.4 respectively at 37°C (μ=1), (R^2 =0.998 and 0.997 respectively)

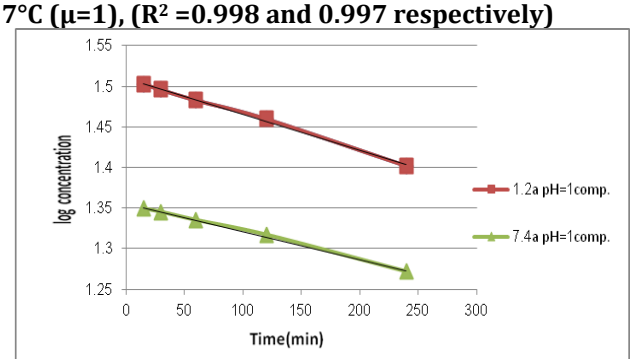


Figure 2. The hydrolysis of compound 1b in 0.1M HCl and phosphate buffer of pH 1.2 and 7.4 respectively at 37°C (μ=1), (R2 =0.998 and 0.997 respectively)

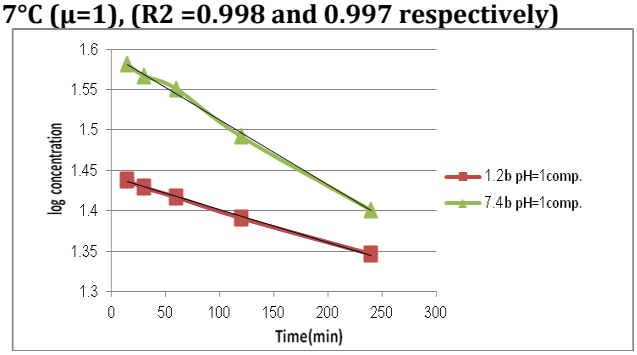
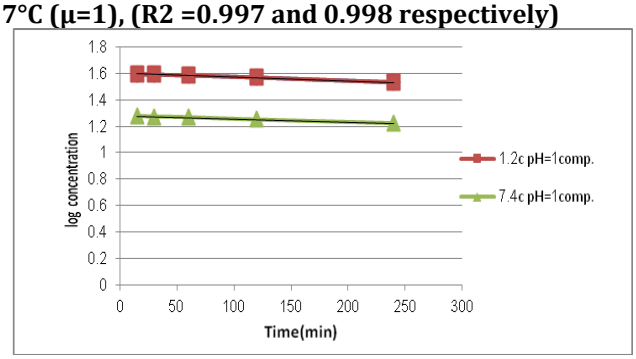


Figure 3. The hydrolysis of compound 1c in 0.1M HCL and phosphate buffer of pH 1.2 and 7.4 respectively at 37°C (μ=1), (R2 =0.997 and 0.998 respectively)



One of the crucial requirements for a prodrugs to be used, they should show a good stability in aqueous solutions and in gastrointestinal fluid, and it should be readily hydrolyzed following gastrointestinal absorption to release the parent drug.[18] Incubation of compounds (**1a**, **1b** and **1c**) in plasma shows significant hydrolysis of the ester bonds, as shown in table **2**.

Table 2. Percent hydrolysis of compounds 1a, 1b and 1c in 80% plasma

Compound	Hydrolysis (%) in plasma				
	15min	30min	60min	20min	240min
1a	44.3	59.21	70.94	80.52	87.73
1b	39.86	54.76	64.97	75.34	85.23
1c	48.42	62.54	73.86	82.38	91.38

CONCLUSION

Preliminary kinetic study for compound ***1a, 1b*** and ***1c*** revealed that these compounds were chemically stable at pH 1.2 and pH 7.4 with half-life range from 6.22-20.98 hr, while undergo significant enzymatic hydrolysis in 80% diluted plasma with about 70% hydrolysis in first hour.

EXPERIMENTAL

Chemistry

All reagents and anhydrous solvents were of analytical grade and were used as received from the commercial supplier (Merck-Germany, Riedal-Dehaen-Germany, BDH-England and Fluka-Switzerland). NSAIDs (naproxen and indomethacin) were purchased from SDI Company, Iraq. Thin layer chromatography (TLC) was run on Kieslgel GF254 (60), Merck (Germany), to check the purity of the products as well as monitoring the progress of reactions. FT-IR spectra were recorded at College of Science, Al-Mustanseriya University by using Shimadzu-Japan spectrophotometer and the determination of the spectra were performed by using KBr discs. CHNO microanalysis has been done in AL Al-bayt University, AL-Mafraq, Jordan; by using CHNO analyzer Euro-vector EA3000A (Italy).

Synthesis of antioxidant-chloroacetyl derivative (a-c): A mixture of an appropriate antioxidant (0.01 mole) and TEA (0.01 mole) in dichloromethane (25ml) was cooled in an ice salt mixture to 10 °C.To this reaction mixture, chloroacetylchlorid (0.01 mole) in chloroform (25ml) was added drop wise with constant stirring over a period of 1 h, maintaining the temperature constant. The reaction mixture was stirred over night at room temperature , washed with 5% HCl (3×50 ml), 5% NaOH (3×50 ml) and finally with brine solution (2×25ml). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain the corresponding antioxidant-chloroacetyl derivative. This general procedure was used with different antioxidants (thymol, guaiacol and menthol) to prepare corresponding chloroacetyl derivative (**a**, **b** and **c**). These derivatives were recrstyllized from petroleum ether and ethyl acetate. The percent yield, physical appearance, melting point and TLC are listed in table **3**.

Synthesis of NSAIDs-antioxidant mutual prodrugs: A mixture of appropriate antioxidant- chloroacetyl derivatives (0.01mole), NSAID [naproxen, indomethacin] (0.01mole), TEA (0.01mole) and sodium iodide (0.01mole) in DMF (25 ml) was stirred over night at room temperature. The reaction mixture was poured into finely crushing ice with stirring and extracted with chloroform (4×25ml). The combined organic layer was washed with 2% sodium thiosulphate (3×50ml), 5% HCl (3×50 ml), 5% NaOH (3×50 ml) and finally with brine solution (2×25ml). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain the NSAIDs-antioxidant mutual prodrugs (**1a**, **1b**, **1c** and **2a**, **2b**, **2c**).

The final products were obtained as solids (*) and recrstyllized from petroleum ether and ethyl acetate. The yield percent, physical appearance, melting point and TLC are listed in table **3**. *Indomethacin-thymol was obtain as semi-solid

Table 1. The percent yield, physical appearance, melting point and R_f of the intermediate & final products.

Compounds and intermediates	Empirical formula	Molecular weight	Description	% yield	Melting point °C	R_f value
a	$C_{12}H_{15}ClO_2$	226.7	Semi-solid	86.2	----------	A=0.85 B=0.65
b	$C_9H_9ClO_3$	200.6	White crystals	80.5	59-61	A=0.88 B=0.80
c	$C_{12}H_{21}ClO_8$	389	White crystals	80.1	30-32	A=0.93 B=0.82
1a	$C_{26}H_{28}O_5$	420.5	White crystals	70.3	61-63	A=0.83 B=0.86
2a	$C_{31}H_{30}ClNO_6$	548	Semi-solid	53	----------	A=0.92 B=0.89
1b	$C_{23}H_{22}O_6$	394.42	yellow powder	68.9	58-60	A=0.76 B=0.88
2b	$C_{28}H_{24}ClNO_7$	521.95	Yellow powder	50.6	79-81	A=0.92 B=0.8
1c	$C_{26}H_{34}O_5$	426.55	White powder	40	90-92	A=0.87 B=0.82
2c	$C_{31}H_{36}ClNO_6$	554.07	Gray powder	30.5	104-106	A=0.9 B=0.78

- ***2-isopropyl-5-methylphenyl 2-chloroacetate (a)***
  IR (KBr): 2964 (C-H), 1776 (C=O), 1504 and 1456 (C=C), 1151(C-O), 815(C-Cl) cm^{-1}.
- ***2-methoxyphanyl 2-chloroacetate (b)***
  IR (KBr): 2995, 2949 (C-H), 1770(C=O), 1602, 1502 and 1454(C=C), 1147(C-O), 756(C-Cl) cm^{-1}.
- ***2-isoprpyl-5-methylcyclohexyl 2-chloroacetate (c)***
  IR (KBr): 2955, 2868 (C-H), 1735 (C=O), 1182(C-O), 790(C-Cl) cm^{-1}.
- ***2-(2-isopropyl-5-methylphenoxy)-2-oxoethyl 2-(6-methoxynaphthalen-2-yl) propanoate (1a)***
  IR (KBr): 2935, 2874 (C-H), 1770 and 1745(C=O) ester, 1604, 1506 and 1458 (C=C), 1166(C-O) cm-1.CHNO Calculated: C, 74.26; H, 6.71; O19.02. Found: C, 73.976; H, 6.804; O, 19.22.
- **2-(2-isopropyl-5-methylphenoxy)-2-oxoethyl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetate (2a)**
  IR (KBr): 2931, 2872 (C-H), 1772, 1749 (C=O) ester, 1682 (C=O) amid (indomethacin), 1597 and 1477 (C=C), 1139(C-O) cm-1. CHNO calculated: C, 67.94; H, 5.52; N, 2.56; O, 17.52. Found: C.68.1; H, 5.601; N, 2.617; O, 17.61.
- **2-(2-methoxyphenoxy)-2-oxoethyl 2-(6-methoxy naphthalen-2-yl) propanoate (1b)**
  IR (KBr): 2943, 2841 (C-H), 1786, 1747 (C=O) ester, 1606, 1500 and 1460 (C=C), 1141(C-O) cm-1.CHNO Calculated: C, 70.04; H, 5.62; O, 24.34. Found: C, 69.791; H, 5.739; O, 24.47.
- **2- (2-methoxyphenoxy)- 2-oxoethyl 2-(1-(4-chloro benzoyl)- 5-methoxy- 2-methyl- 1*H*-indol-3-yl)acetate**

(2b)
IR (KBr): 2941, 2839 (C-H), 1780, 1751 (C=O) ester, 1680 (C=O) amid (indomethacin), 1600, 1492 and 1469(C=C), 1141(C-O) cm-1.CHNO Calculated: C, 64.43; H, 4.63; N, 2.68; O, 21.46. Found: C.64.561; H, 4.731; N, 2.781; O, 21.61.

- **2-(2-isopropyl-5-methylcyclo hexyloxy)-2-oxoethyl 2-(6-methoxynaphthalen-2-yl) propanoate (1c)**
IR (KBr): 2953, 2862 (C-H), 1747 (C=O) ester, 1610, 1496 and 1462(C=C), 1157(C-O) cm^{-1}. CHNO Calculated: C, 73.21; H, 8.03; O18.65. Found: C, 73.337; H, 7.93; O, 18.733.
- **2-isopropyl-5-methylcyclohexyl 2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetoxy)acetate (2c)**
IR (KBr): 2928, 2868 (C-H), 1745 (C=O) ester, 1683 (C=O) amid (indomethacin), 1597, 1475 and 1460 (C=C), 1147(C-O) cm-1.CHNO Calculated: C, 67.2; H, 6.55; N, 2.53; O, 17.33. Found: C.67.5; H, 6.71; N, 2.67; O, 16.51.

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