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Research Article

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DESIGN AND SYNTHESIS OF POSSIBLE MUTUAL PRODRUGS BY COUPLING OF NSAIDS WITH SULFA DRUGS BY USING GLYCOLIC ACID AS SPACER

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ABSTRACT

This study includes synthesis of mutual prodrugs of NSAIDs and different sulfa drugs using glycolic acid spacer (-OCH2COO-) to reduce the ulcerogenic side effects of NSAIDs, by esterfication the free carboxyl group of the NSAIDs that responsible for the local irritation. Two NSAIDs, ibuprofen and naproxen each one of them were linked to two different sulfa drugs sulfathiazole and sulfadiazine through glycolic acid spacer (-OCH2COO-) as possible mutual prodrugs to reduce the ulcerogenic side effects of NSAIDs by esterfication of the free carboxyl group of the NSAIDs that responsible for the local irritation. The structures of these compounds were confirmed and characterized using elemental microanalysis (CHNO), infrared spectroscopy (IR) and some physicochemical properties (melting point m.p. and thin layer chromatochraphy TLC). *In-vitro* preliminary kinetic study was done for two target compounds at different pH (1.2 and 7.4) to identify the expected hydrolyses of these mutual prodrugs in the gastrointestinal tract. *In-vitro* preliminary kinetic study for target compounds (I) and (III) at pH (1.2 and 7.4) was revealed that these compounds were chemically stable in simulated gastrointestinal fluid, while 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 prodrugs in non enzymatic simulated gastro-intestinal fluid and rapid conversion to the parent drugs in 80% human plasma.

Keywords: mutual pro-drugs; NSAIDs; ibuprofen; naproxen; sulfa drugs.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in the world.[1] (NSAIDs) are commonly used for the treatment of chronic inflammatory diseases such as arthritis. Prolonged administration of these drugs exhibits several undesired side effects, most important are gastrointestinal irritation and ulceration.[2,3]

The patients who use NSAIDs on chronic basis have about three times greater relative risk for serious adverse GI events compared to the population of non-user.[4,5] There is therefore a need for anti-inflammatory and analgesic drugs that will provide symptomtic relief without causing GI injury.[6]

All the pharmacologically desirable actions of NSAIDs, result from the suppression of prostanoid synthesis in inflammatory cells through inhibition of cycloxygenase -2 (cox-2) isoform of the cox.[7]

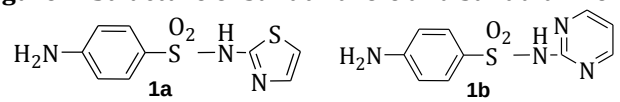
Two principal types of COX can be distinguished:
COX-1 is constitutive, that is, always present and active; it contributes to the physiological function of organs.[8]

COX-2 is induced by inflammatory processes and produces prostaglandins that promote inflammation by causing vasodilation and an increase in vascular permeability. However, in some organs, COX-2 is also expressed constitutively (kidney, vascular endothelium, uterus, and CNS).[9]

Recently, a research group described a third enzyme with COX activity, named **COX-3** that has been found in canine and human cerebral cortex.[10]

An infection always leads to inflammation.[11] Therefore sulfa drugs can be coupled with NSAIDs. In this way the carrier drug (sulfonamides) may be useful to overcome some side effects of parent drug particularly gastrointestinal (GI) irritation. Sulfonamides are anti bacterial agents interfere with the synthesis or action of folate, the action of sulfonamide is to inhibit growth of bacteria not to kill them so it is bacteriostatic.[12] Sulfonamide with varying physical, chemical, pharmacologic and antibacterial properties are produced by attacking substituents to the amido group (-SO_2-NH-R-) or the amine group (-NH_2) of the sulfanilamide nucleus[13] examples sulfathiazole (Figure **1a**) and sulfadiazine (Figure **1b**).

Figure 1. Structure of sulfathiazole and sulfadiazine



1a **1b**

Ibuprofen (Figure **2a**) was the first in the class of arylpropionic acid analogues to become available in the world. It has been joined by flurbiprofen, ketoprofen and naproxen (Figure **2b**). They might offer significant advantages over aspirin and indomethacin for many patients since they are usually better tolerated.[14]

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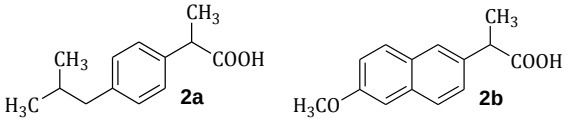
Figure 2. Structure of ibuprofen and naproxen



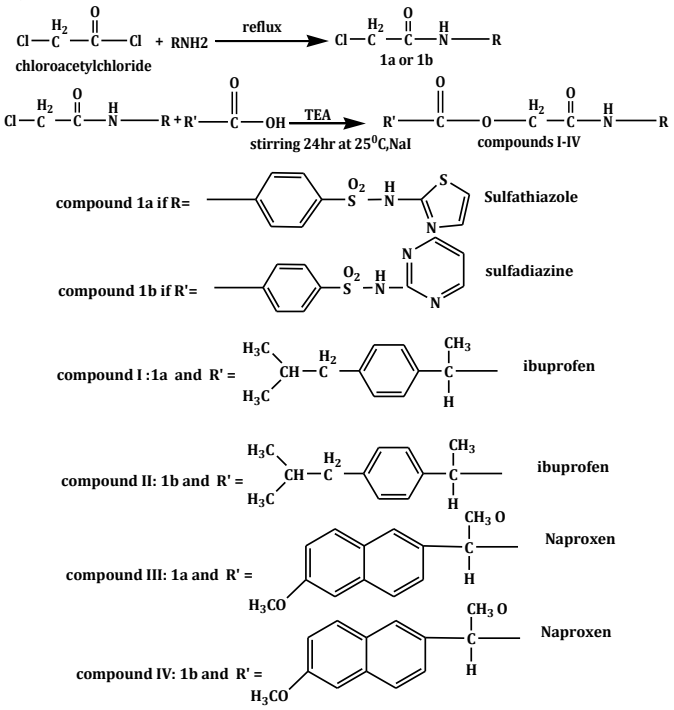
The pro-drug designing is one of the several strategies used to overcome this drawback.[15] Mutual prodrug is a type of carrier-linked, where the carrier used is another biologically active drug instead of some inert molecule. A mutual pro-drug consists of two pharmacologically active agents coupled together so that each acts as a pro-moiety for the other agent and vice versa.[16]

In the view of this background, the present study was conducted to design and synthezise mutual prodrugs of NSAIDs (ibuprofen and naproxen) with different sulfa drugs (sulfathiazole and sulfadiazine) using glycolic acid spacer (-OCH2COO-), to devoid the ulcerogenic side effects of NSAIDs, this is obtained by masking the free carboxyl group of the NSAIDs by ester formation in order to decrease the local irritation. On the other hand an infection usually leads to inflammation, therefore sulfa drugs can be coupled through amide linkage with glycolic acid (we used chloroacetyl chloride),in this way these pro-drugs may be used for infection as well as for inflammation.

CHEMISTRY

Steps of preparation of all compounds are represented in scheme **1**. Coupling reaction of sulfa drugs with chloroacetylchloride resulted in synthesis of compound **1a** and **1b**. Coupling reaction of NSAIDs with compounds **1a** and **1b** results in synthesis of compound **I-IV**.

Scheme 1. steps of preparation of compounds 1a, 1b, I, II, III and IV



Preliminary kinetic study of compound I and compound III

Hydrolysis in aqueous buffer solutions (at pH 1.2 and 7.4): The hydrolytic stability of the synthesized compounds was studied in near physiological conditions in HCl (simulated gastric fluid, SGF, pH 1.2) and phosphate buffer (simulated intestinal fluid, SIF, pH 7.4).[17]

The reactions were monitored by using UV spectrophotometic method and the result indicated that the synthesized compounds were sufficiently stable in pH 1.2 and pH 7.4 at 37°C.

Hydrolyses in plasma (enzymatic stability): The hydrolysis rates of target compounds **I** and **III** 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 target compound 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.[17]

RESULTS AND DISCUSSION

All the compounds were evaluated for chemical and enzymatic hydrolysis using UV method, relevant for studying the hydrolysis of synthesized derivatives.

Under experimental conditions used the hydrolysis of the compounds **I** and **III** 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 **3** and **4** are representive graphs for compounds **I** and **III** respectively; while table **1** shows the pH values, the corresponding K_{obs} and half-life of the hydrolysis of compounds **I** and **III**.

Figure 3. The hydrolysis of compound I in 0.1M HCl and phosphate buffer of pH 1.2 and 7.4 at 37°C (μ=1), (R^2 =0.995 and 0.996 respectively)

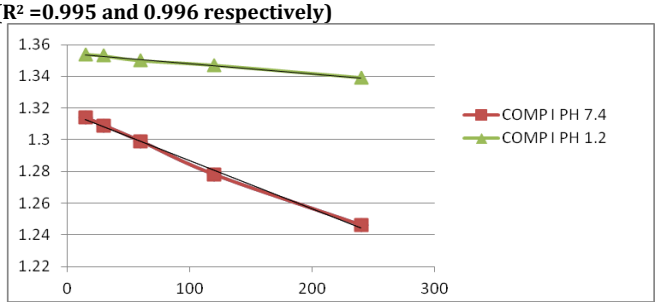


Figure 4. The hydrolysis of compound III in 0.1M HCl and phosphate buffer of pH 1.2 and 7.4 at 37°C (μ=1), (R^2 =0.995 and 0.990 respectively)

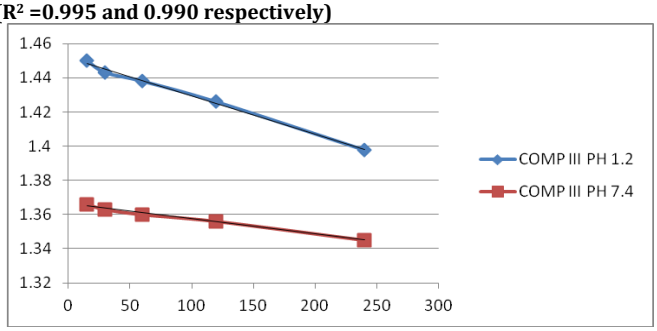


Table 1. The rate constant of hydrolysis of compounds I and III at pH 1.2 and pH 7.4 at 37°C, and μ=1

compound	pH	Kobs(min^{-1})	t½(min)
I	1.2	6.513×10^{-4}	1064
	7.4	6.978×10^{-3}	993
III	1.2	1.171×10^{-3}	591
	7.4	2.072×10^{-3}	334

The half-life was calculated using equation **1**, which derives from the first order kinetic law.[18]

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

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.[17] Incubation of compounds (**I** and

III) in plasma shows significant hydrolysis of the target compounds, as shown in table **2**.

Table 2. Percent hydrolysis of compounds I and III in 80% plasma

Compound	Hydrolysis (%) in plasma				
	15min	30min	60min	20min	240min
I	37.53	54.87	69.33	78.52	83.79
III	40.21	59.76	70.97	81.34	87.23

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).

Preliminary kinetic study for compounds **I** and **III** revealed that these compounds were stable at pH 1.2 and pH 7.4 and not undergo chemical hydrolysis at gastrointestinal fluid, while undergo significant enzymatic hydrolysis in 80% diluted plasma.

EXPERIMENTAL

Chemistry

All reagents and anhydrous solvents were of analytical grade and were used as received from the commercial supplier (Reidal Dehean Germany; Sigma-Aldrich Germany; BDH England). Melting points (uncorrected) were determined by capillary method on Thomas hoover apparatus (England). Determination of infrared spectra by using F.T.IR-spectrophotometer, were done at the College of Science, University of Al-Mustanseria, and the determination of the spectra taken were performed as KBr discs. 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 by using CHNS analyzer Euro-vector EA3000A (Italy). The analysis was carried out at institute of earth and environmental sciences, AL al-Bayt University, Jordan. The identification of compounds was done using iodine vapor and the chromatograms were eluted by: chloroform: ethyl acetate: ether (10:5:1).[19]

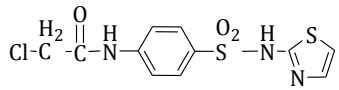
Coupling reaction of sulfa drugs with chloroacetylchloride: The mixture of 0.02 M of sulfathiazole, (30 ml) benzene and (3ml) of triethylamine (TEA), the mixture was stirred on water bath or ice bath, chloroacetylchloride (CAC) 0.02 M (1.6ml in 30ml of benzene) was added gradually (drop wise) and the mixture refluxed for 2 hours. The excess of benzene was evaporated under vaccume and the precipitate was washed with sodium carbonate (2%), HCl (5%) and disilled water; then recrystallized from ethanol[20], white-brown crystalline product was obtained.

Coupling reaction of NSAIDs with compounds 1a and 1b: mixture of compound **1a** or **1b** (0.01mole), NSAIDs (0.01mole), TEA (0.01mole), sodium iodide (0.01 mole) in DMF (25ml) was stirred over night at room temperature. The mixture was poured into finely crushed ice with stirring and extracted with chloroform (4*25ml). The combined organic layer was washed with sodium thiosulphate (2%, 3*50ml), HCl (5%, 3*50ml), sodium hydroxide (5%, 3*50ml) and finally with brine solution (2*25ml). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure to obtain the product.[21] The percentage yield, physical data and Rf values of compound **1a**, **1b** and **I-IV** are given in table **3**.

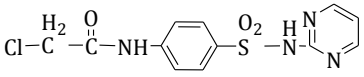
Table 3. physical data, percentage yield and Rf values of the compounds 1a, 1b, I, II, III and IV

Compounds and intermediates	Empirical formula	Molecular weight	Description	% yield	Melting point °C	R_f value
1a	C11H10ClN3O3S2	331.80	White-brown crystals	68	217 decomposed	0.82
1b	C13H15ClN4O3S	342.8	White crystals	60	195-198	0.90
I	C24H27N3O5S2	501.62	White-brown crystals	57	212-216	0.82
II	C25H23N3O6S2	525.6	White crystals	62	188-192	0.87
III	C25H28N4O5S	496.58	White-brown crystals	58	204 decomposed	0.92
IV	C26H24N4O6S	520.62	White crystals	67.5	202-206	0.84

- **Compound 1a:** IR (KBr): 3332 Secondary amide, 1627 C=O of secondary amide, 1534, 1437 N-H bending vibration of secondary amide, 1313 SO_2 cm^{-1}.



- **Compound 1b:** IR (KBr): 3230 Secondary amide, 1672 C=O of secondary amide, 1446 N-H bending vibration of secondary amide, 1315 SO_2, 1541 C=C of aromatic cm^{-1}.



- **Compound I:** IR (KBr): 3333 N-H secondary amide, 1640 C=O of secondary amide, 1746 C=O of ester, 1572 C=C, 1319 SO_2 cm^{-1}. CHNO Calculated: C, 57.47; H, 5.43; N, 8.38; S, 12.78. Found: C, 57.77; H, 5.46; N, 8.24; S, 12.29.
- **Compound II:** IR (KBr): 3329 N-H secondary amide, 1687 C=O of secondary amide, 1751 C=O of ester, 1616 C=C aromatic, 1365 SO_2 cm^{-1}. CHNO Calculated: C, 57.13; H, 4.41; N, 7.99; S, 12.2. Found: C, 57.36; H, 5.57; N, 8.04; S, 11.54.
- **Compound III:** IR (KBr): 3392 N-H secondary amide, 1635 C=O of secondary amide, 1745 C=O of ester, 1635 C=C aromatic, 1363 SO_2 cm^{-1}. CHNO Calculated: C, 60.47; H, 5.68; N, 11.28; S, 6.46. Found: C, 60.79; H, 5.42; N, 11.10; S, 11.10.
- **Compound IV:** IR (KBr): 3483 N-H secondary amide, 1680 C=O of secondary amide, 1726 C=O of ester, 1627 C=C aromatic, 1340 SO_2 cm^{-1}. CHNO Calculated: C, 59.99; H, 4.65; N, 10.76; S, 6.16. Found: C, 60.19; H, 4.47; N, 10.35; S, 5.62.

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