Synthesis of new Conjugates of some NSAIDs with Sulfonamide as Possible Mutual Prodrugs using Tyrosine Spacer for Colon Targeted Drug Delivery

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Abstract

The purpose of this research work is to synthesize conjugates of some NSAIDs with sulfamethoxazole as possible mutual prodrugs to overcome the local gastric irritation of NSAID with free carboxyl group by formation of ester linkage that supposed to remain intact in stomach and may hydrolyze in intestine chemically or enzymatically; in addition to that attempting to target the synthesized derivative to the colon by formation of azo group that undergo reduction only by colonic bacterial azo reductaze enzyme to liberate the parent compound to act locally (treatment of inflammation and infections in colon).

Key words: Mutual prodrug, Ester linkage, Azo bond, Colon targeting

تحضير مشتقات جديدة لبعض مضادات الالتهاب الغير ستيرويدية مع السلفونمايد كمقدمات ادوية محتملة باستخدام التايروسين كذراع لتوجيهها الى القولون

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الخلاصة

الهدف من البحث هو تحضير مشتقات لبعض مضادات الالتهاب الغير ستيرويدية مع مركبات السلفونمايد كمقدمات ادوية محتملة للتقليل من التأثيرات المعوية لمضادات الالتهاب الغير ستيرويدية الناتجة من وجود مجموعة الكاربوكسيل الحرة بتحويلها الى مجموعة استير التي من المفترض عدم تحللها في المعدة وامكانية تحللها في الامعاء كميائيا او انزيميا" بالاضافة الى محاولة توجيه المشتق المحضر الى القولون من خلال تحضير اصرة الازو والتي يحصل لها اختزال بواسطة انزيم الازو ريدكتيز المتحرر من بكتيريا القولون وبذلك يتحرر المركب الاصلي ليعالج موضعيا الالتهاب في القولون.

الكلمات المفتاحية: مقدمات دواء متبادل، ارتباط الاستير، اصرة الازو، التوجيه الى القولون

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly prescribed drugs for the treatment of pain, inflammation and fever [(1)]. However gastric irritation caused by most of the NSAIDs used today restricts their use. The pharmacological activity of NSAIDs is related to the blocking of prostaglandin H_2 (PGH_2) biosynthesis from arachidonic acid by inhibiting the activity of cyclooxygenases (COXs). The COX enzyme exists in two isoforms: a constitutive isoform, COX-1, found in most tissues including stomach, kidney, and platelets, and an inducible isoform, COX-2, expressed at the site of inflammation [(2)]. Classical NSAIDs, such as ibuprofen, flufenamic acid, diclofenac, and aspirin, preferentially inhibit COX-1, thus suppressing the biosynthesis of prostaglandins that maintain gastric mucosal integrity and leading to gastrointestinal (GI) side effects, including ulceration and hemorrhage (systemic effect)[(3,4)].Topical irritation by the free carboxylic group of the NSAIDs is considered an important factor in establishing superficial stomach erosion[(5, 6)]. Since the introduction of specific COX-2 inhibitors, which are less harmful to the GI tract; the use of conventional NSAIDs have declined. However, the safety profile of COX-2 inhibitors has been questioned due to the risk of ulcer complications in high – risk individuals and to cardiovascular adverse effects [(7, 8)]. Thus, the need for NSAIDs with improved GI tolerability still exists.

One approach that has been used to decrease NSAID induced GI toxicity without adversely affecting their anti-inflammatory activity is to mask the carboxylic acid group by synthesizing the corresponding ester prodrugs[(9)]. A Prodrug is a chemically modified inert drug precursor which upon biotransformation liberates the pharmacologically active parent compound [(10)]. A major requisite for these prodrugs is that they must be readily hydrolyzed, enzymatically or chemically, after oral absorption to quantitatively release the parent drug [(11)]. Amino acids have been considered the ideal carriers for the development of prodrugs; and some of them have marked antiinflammatory activity of their own [(12)].Mutual prodrug, where the carrier used is another biologically active drug instead of some inert molecule [(13)].

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Site specific drug delivery

A drug, after its absorption into systemic circulation, gets distributed to target site as well as non-targeted tissues. The distribution of drug to non-targeted tissues may lead to undesirable toxic effects in those tissues and insufficient concentration in the target site to evoke any therapeutic response. If the drug needs a long time to reach to the target site, it may get eliminated without reaching such a site; and even if the drug reaches the targeted area in sufficient concentrations, it may have such a low penetration power that it may not penetrate the target cells at all[14]. Targeting the drug to its site of action through prodrug concept has been utilized to overcome these problems. While designing the prodrug, one must take into account the enzymes that are specifically present in that organ or tissue or specific pH of that area which is different from physiological pH so that the prodrug releases the drug only in the targeted organ[15].

Colon targeted drug delivery

The colon has some unique features, which make this organ attractive for site-specific drug delivery. There is a considerable interest in the colon specific drug delivery in order to treat diseases of the large intestine, such as colitis, colon cancer, constipation, irritable bowel syndrome, and infectious diseases[16]. To achieve successful colonic delivery, the drug needs to be protected from absorption and /or the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs[17].

The presence of azo reductase enzyme in colon from colonic microflora, which is not present in the stomach or small intestine, plays an important role in the release of drug from azo bond prodrug. This enzyme causes reduction, and thus cleavage, of the azo bond[18, 19].

Sulfonamides are one of the least expensive drugs and this factor largely accounts for their greater extent of use in developing countries. These drugs are considered useful for gastrointestinal (GI) tract infections. An infection always leads to inflammation therefore sulfa drugs can be coupled with NSAIDs so that these mutual prodrugs can be used for infections as well as for inflammation[20].

Materials and Methods

Ibuprofen, naproxen, and sulfametho-xazole were purchased from SDI (Iraq); ketoprofen was purchased from (India); Boc-tyrosine was purchased from Fluka (Switzerland). All chemicals were reagent grade and obtained from commercial sources. Elemental microanalysis was performed using CHN analyzer (Jordan); melting points were measured on Barnstead Electrothermal melting point apparatus (USA) and are uncorrected; infra red spectra were recorded as KBr disks on FTIR spectrophotometer (College of Pharmacy, University of Al-Mustanseriya).UV spectra were done using UV spectrophotometer (College of pharmacy, University of Baghdad).

Chemical synthesis

Synthesis of compound 1A (diazonium salt formation [21])

Sulfamethoxazole (2.53g, 10 mmole) was dissolved in a mixture of equal quantities (12.5ml) of each of conc. HCl and water in a suitable beaker; the resulting solution was stirred and cooled by immersing in a bath of crushed ice; throughout the reaction the temperature was kept below 5ºC. A cold solution of (0.75g, 11 mmole) sodium nitrite in(5ml) water was placed in a dropping funnel which was cooled using crushed ice, then it was added dropwise into the first solution in the ice bath with continuous stirring ; the temperature should not be allowed to rise above 10ºC.The last quantity of the sodium nitrite solution was added more slowly and after stirring for 3-4 minutes, the solution was tested for excess sodium nitrite using potassium iodide-starch paper. A solution of sulfamic acid (1.5ml) of 2% was added and stirring was continued for 20 minutes. The diazonium salt formed was used immediately in the following step.

Synthesis of compound1B (Azo bond formation)[21]

Boc- tyrosine (2.8 g, 10 mmole) was dissolved in (8 ml) of (10 %) NaOH in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5ºC by the addition of crushed ice. The cold diazonium salt solution from the previous step (compound 1A) was placed in a dropping funnel, then it was added drop by drop to the cooled, stirred Boc-tyrosine solution; an orange color was developed and orange crystals soon separated. At the end of the addition the mixture was stirred for 3hours in the ice bath. Then the solution was filtered through a Buchner funnel with gentle suction, washed well with water, and recrystallized from ethanol: water mixture (1:5) to obtain (37 %) of compound 1B.

Synthesis of compound 1C [22]

To a suspension of compound 1B (2.73 g, 5 mmole) in methanol which was cooled to -10ºC, thionyl chloride (0.7 ml, 10 mmole) was added dropwise with continuous stirring during which the temperature of the reaction mixture

After completion of the addition, the temperature of the mixture was allowed to rise and kept at 40ºC for 3 hours followed by refluxing for further 3 hours, then left at room temperature overnight. The solvent was evaporated to dryness in vacuum. Red powder appeared which was re-dissolved in methanol and evaporated several times to ensure the complete removal of excess thionyl chloride.The residue was then collected and recrystallized from methanol: diethylether mixture (1:5) to obtain (90 %) of compound 1C as brown residue.

Synthesis of compounds 1D, 2D, 3D [23]

Ibuprofen 1g, 5mmole (or naproxen 1.17g, 5mmole or ketoprofen 1.27g, 5mmole) was dissolved in chloroform (20ml) in a round bottom flask; to it few drops of DMF were added, the mixture was stirred inside an ice bath where the temperature should be below 0ºC. A slight excess of thionyl chloride (0.7 ml, 10 mmole) was added drop wise over a period of 15-20 minutes with continuous stirring. After complete addition of thionyl chloride the temperature was allowed to rise gradually then refluxing for 8 hours. The solvent and the excess thionyl chloride were evaporated under vacuum followed by re-dissolving in chloroform and re-evaporation several times. The acid chloride was obtained as yellow oily residue and used immediately in the following step.

Synthesis of compound I, II, III [24]

A suspension of compound 1C (2.8 g, 5 mmole) and TEA (1.4ml, 10 mmole) in dry THF (100ml) was stirred in an ice bath. Followed by a dropwise addition of a solution of compound 1D or 2D or 3D (in dry acetone) (5 mmole) over a period of 1 hour, the temperature of the mixture was kept below -5ºC during the addition. After that the mixture was stirred for 72 hours at room temperature. The solvent was evaporated then the residue was dissolved in chloroform followed by filteration to remove solids. The chloroform layer was shaken with 1M sodium carbonate solution for 15 minutes (3 x 25ml), D.W. (3 x 25ml), 0.05N HCl (3 x 25ml), D.W. (3 x 25ml), and finally with (25ml) brine solution (saturated NaCl solution).The chloroform extract was dried over anhydrous magnesium sulfate. The residue, after evaporation of solvent, was collected and recrystallized from chloroform: petroleum ether (40-60) mixture (1:5) to obtain (20 %) yield red residue compound I; (46%) yellow residue compound II; (60%) yellow residue compound III.

Results and Discussion

Primary aromatic amines react with nitrous acid in the presence of HCl (or other mineral acid) at about 0ºC to yield diazonium salts. Coupling reaction is an electrophilic aromatic substitution with the diazonium ion acting as the electrophile which reacts at the position of greatest electron availability (the position para or ortho to the electron releasing group)[25]. Conversion of acid chloride into ester ; on treatment with the appropriate nucleophile, an acid chloride can be converted to an ester by nucleophilic acyl substitution mechanisms. Nucleophilic acyl substitution reactions take place in to steps:

1. Addition of the nucleophile.
2. Elimination of a leaving group.[26]

Physical appearance, percentage of yield, melting points, R_f values, and the molar extinction coefficients of intermediates and final compounds are presented in table (1).

Table (1): Physical appearance, percentage of yield, melting points, R_f values of intermediates and final compounds

compound	Physical appearance	Yield%	Melting Point (ºC)	R_f * value		Ɛ at 332 nm
				A	B	
1B	Orange powder	37%	124-126	0.43	0.26	-
1C	brown powder	90%	152-155	0.82	0.39	-
I	Red powder	20%	144-146	0.94	0.54	8555.5
II	Yellow powder	46%	139-142	0.96	0.55	3673.9
III	Yellow powder	60%	120-123	0.9	0.42	9868.6

***** A. Chloroform: ethanol (8:2), B. Toluene: ethanol (8:2)

Table (2): Elemental microanalysis results of the final compounds

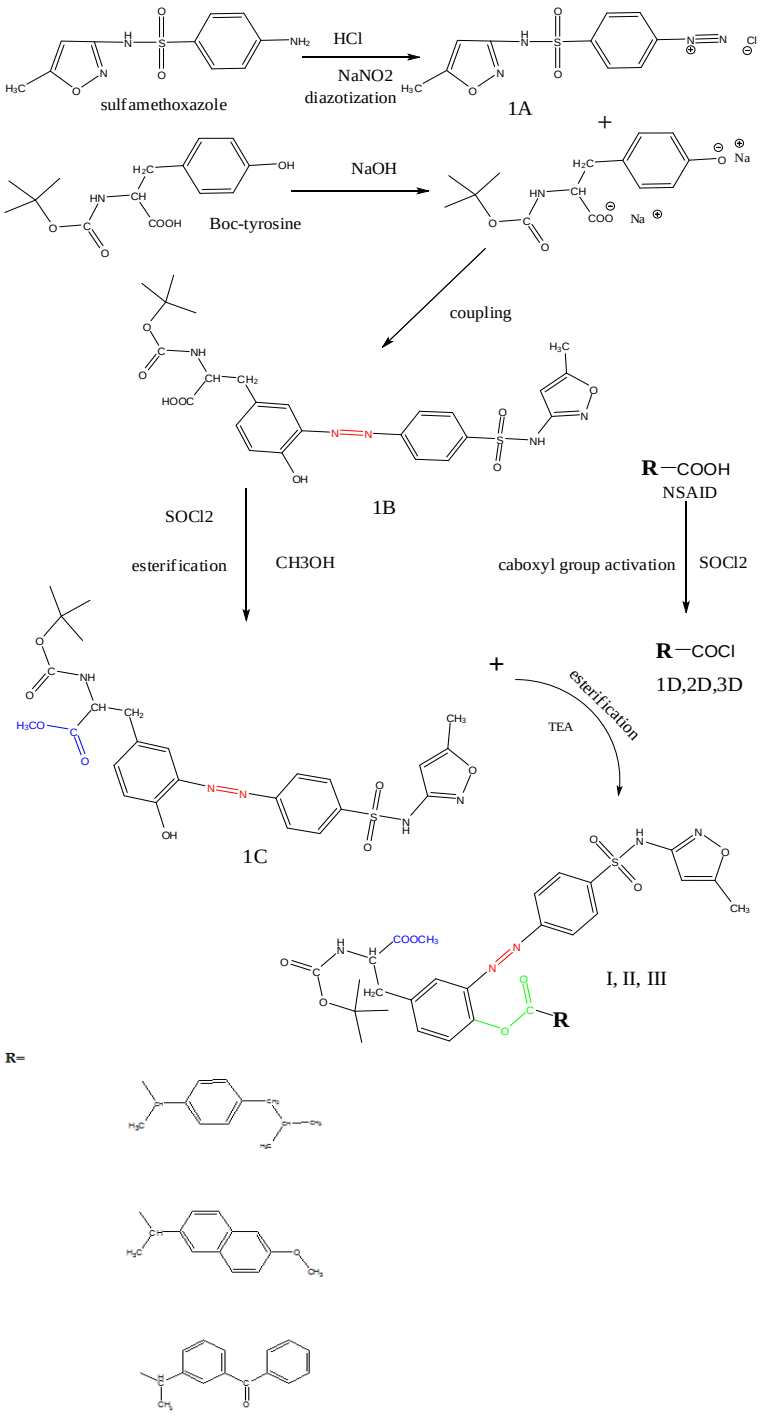
Compound	Molecular formula	Molecular weight	Elemental microanalysis%		
			Element	Calculated	Observed
I	$C_{38}H_{45}N_5O_9S$	747.86	C H N S	61.03 6.06 9.36 4.29	61.096 6.061 9.456 4.755
II	$C_{39}H_{41}N_5O_{10}S$	771.84	C H N S	60.69 5.35 9.07 4.15	61.505 6.664 9.412 3.721
III	$C_{41}H_{41}N_5O_{10}S$	795.86	C H N S	61.88 5.19 8.80 4.03	62.34 5.312 9.545 4.412

Table (3): FT IR characteristic bands of the synthesized compounds

Compound	Band (cm^{-1})	Interpretation
Compound 1B	3352 3263 3095 2924, 2854 1716 1689 1618,1502 1467 1467,1396 1423 1271 1174, 1342 1174 786,933	N-H stretching of amide O-H stretching Aromatic C-H stretching Asymmetric and symmetric C-H stretching of CH_3 , CH_2 Carboxylic C=O stretching Amide C=O stretching Aromatic C=C stretching N=N stretching C-H bending of CH_3, CH_2 O-H in plane bending C-O stretching of carboxylic acid O=S=O sulfonamide two bands C-O stretching of phenol Aromatic C-H bending
Compound1C	3412 3265 2956, 2858 1749 1616,1502 1464,1396 1464 1278 1396,1170 790, 636	N-H stretching of amide O-H stretching of phenol and N-H stretching of sulfonamide Asymmetric and symmetric C-H stretching of CH_3, CH_2 C=O stretching of ester Aromatic C=C stretching C-H bending of CH_3 ,CH_2 N=N stretching C-O stretching of ester O=S=O sulfonamide two bands Aromatic C-H out of plane bending

Table (3): Continued FT IR characteristic bands of the synthesized compounds

Compound	Band (cm^{-1})	Interpretation
Compound I	3298, 3254	N-H stretching of amide and sulfonamide
		Aromatic C-H stretching
	3095	Asymmetric and symmetric C-H stretching of
	2955, 2870	CH_3, CH_2
	1734	C=O stretching of ester
	1653	C=O stretching of amide
	1616, 1512	Aromatic C=C stretching
	1589	N-H bending (amide II band)
	1464	N=N stretching
	1464, 1398	C-H bending of CH_3 ,CH_2
	1398,1170	O=S=O of sulfonamide
	1276	C-O stretching of ester
	785, 638	Aromatic C-H out of plane bending
Compound II	3309, 3215	N-H stretching of amide and sulfonamide
	3063	Aromatic C-H stretching
	2958	Asymmetric C-H stretching
	1739	C=O stretching of ester
	1643	C=O stretching of amide
	1612, 1502	Aromatic C=C stretching
	1537	N-H bending (amide II band)
	1392, 1346	C-H bending of CH_3 ,CH_2
	1346, 1172	O=S=O of sulfonamide
	1271	C-O stretching of ester
	715, 636	Aromatic C-H out of plane bending
Compound III	3327	N-H stretching of amide
	3036	Aromatic C-H stretching
	2928, 2850	Asymmetric and symmetric C-H stretching of CH_3, CH_2
	1743	C=O stretching of ester
	1627	C=O stretching of amide
	1573 ,1535	Aromatic C=C stretching and N-H bending (amide II band) could be in this region
	1440	N=N stretching
	1273	C-O stretching of ester
	642	Aromatic C-H out of plane bending



Scheme of synthesis of compound I, II, III

Determination of λ max

Scanning the solutions of compounds I, II, III in chloroform (25µg /ml) by UV/visible spectrophotometer at 200-800 nm gave different peaks with λ max at 332nm; see figures (1), (2), (3).

The molar extinction coefficient for compounds I, II, III were determined at λ max = 332 nm and presented in table (1).

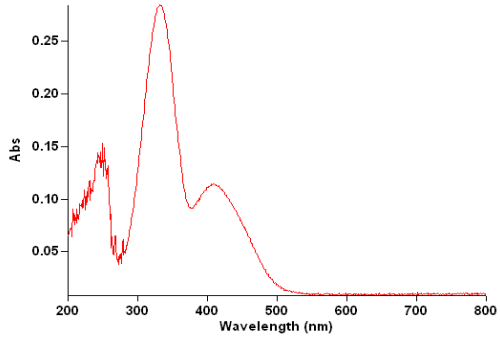


Figure (1): UV spectrum of compound I shows 3 peaks; 246, 332, and 409 nm; λ max is 332 nm.

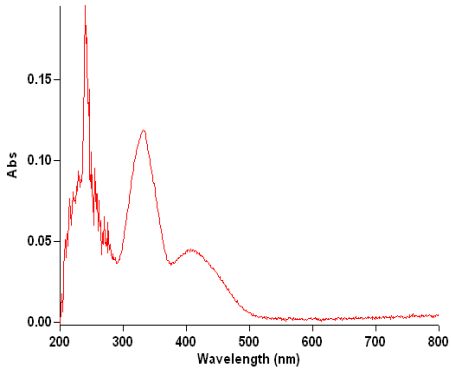


Figure (2): UV spectrum of compound II shows 3 peaks; 241, 332, and 409 nm; λ max is 332 nm.

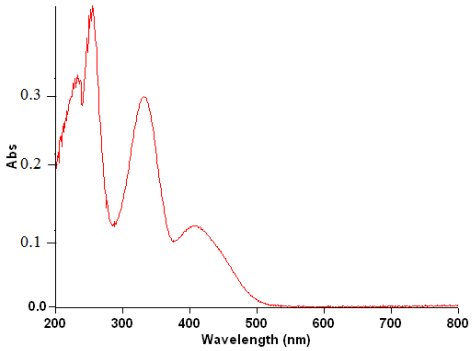


Figure (3): UV spectrum of compound III shows 4 peaks; 236, 255, 332, and 409 nm; λ max is 332 nm.

Preparation of calibration curve

Calibration curve of compound III was constructed in chloroform using different concentration solutions (20, 40, 60, 80, 100 µg/ml) at λ max (332 nm) and presented as straight line; see figure (4).

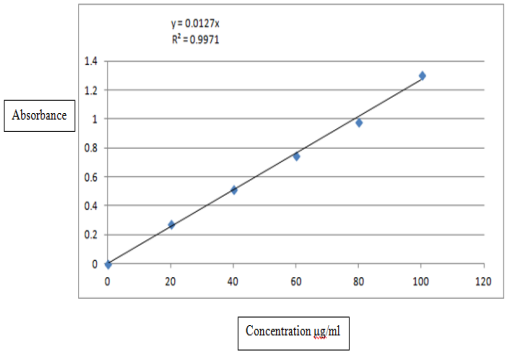


Figure (4): The calibration curve of compound III

Determination of partition coefficient *(27)*

A drug partition coefficient is a measure of its distribution in a lipophilic/hydrophilic phase system, and is indicative of its ability to penetrate biological multiphase system. The partition coefficient of compound III was determined in two systems: n-octanol/HCl buffer (PH=1.2) where the value was 35.03 and n-octanol/ phosphate buffer (PH=7.4) where the value was 38.98. This indicates that the compound is highly lipophilic.

Conclusion

The synthesis of the designed compounds has been successfully achieved and the structural formula for these compounds was characterized using IR spectroscopy, elemental microanalysis, melting points, UV spectra, and R_f values. From the results compound III is highly lipophilic.

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