Synthesis and kinetic studies of mutual azo prodrugs of ٥-aminosalicylic acid with sulfamethoxazole and trimethoprim as models for colon targeting

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ABSTRACT

In this study, two mutual azo prodrugs were synthesized for colon targeting in a treatment of colonic diverticular disease. The first was synthesized by coupling sulfamethoxazole with salicylic acid; the second was synthesized by coupling one mole of trimethoprim with two moles of salicylic acid. In vitro kinetic studies of these mutual prodrugs in hydrochloric acid buffer (pH ١.٢) and in phosphate buffer (pH ٧.٤) were monitored. Hydrolysis of these mutual prodrugs was established in rat fecal matter. The release of ٥-aminosalicylic acid and sulfamethoxazole or trimethoprim from these mutual prodrugs was almost complete and it followed first order kinetics. The prodrug approach to drug physico-chemical properties modification based on enzyme specifications may offer a new approach for improving drug product efficacy and reducing most of its adverse effects.

الخلاصة

في هذا الدراسة، تم تحضير اثنين من بادئ الدواء التبادلي من نوع الآزو لغرض استهداف القولون لعلاج مرض الردب القولوني. الأول صنع باقتران السلفاميثوكسازول مع حامض الساليسيليك؛ الثاني صنع باقتران مول واحد من الترايميثوبريم مع مولين من حامض الساليسيليك. تمت الدراسات الحركية خارج جسم الكائن الحي لبادئي الدواء التبادلي في محلول حامض الهايدروكلوريك البفري (درجة حموض١،٢) وفي محلول الفوسفيت البفري (درجة حموضة ٧،٤) وتم التأكد من تحلل بادئي الدواء التبادلي في مادة براز الجرذان. إن تحرير ٥- أمينو حامض الساليسيليك والسلفاميثوكسازول أو الترايميثوبريم من بادئي الدواء التبادلي كان شبه كامل متبعا حركيات الرتبة الأولى. إن استخدام نهج بادئ الدواء لتغير الخواص الفيزيوكيميائية والذي يعتمد على خصوصية الإنزيمات لتحرير الدواء قد يشكل طريقا جديدا لتحسين الأداء العلاجي للدواء وتقليل معظم أثاره الجانبية.

Diverticulitis of colon is quite frequent in developed countries and its prevalence rises with age.[١] Perforated colonic diverticular disease is a condition associated with high mortality and morbidity. Medical and surgical management of this condition has improved over the last two decades, but the mortality rate is largely unchanged.[٢]

Diverticula most commonly affect the sigmoid and descending colon in more than (٩٠%) of patients.[٣, ٤] When the diverticula become inflammed, the condition is called diverticulitis.[٥] Most of the inflammed diverticula are infected by bacteria, the bacterial infections may aggravate the symptoms and prolong the clinical course of the disease.[٦] Gram negative aerobes are the common causative bacteria.[٧] ٥-aminosalicylic acid (٥-ASA) is among the oldest anti-inflammatory agents in use today for the treatment of inflammatory bowel diseases.[٨]

When ٥-ASA is taken orally as poorly absorbed formulations, it acts locally in the colon and it will be absorbed by colonic epithelial cells.[٩] The effectiveness of this drug is related to its mucosal concentration.[١٠]

٥-ASA appears to have advantage in obtaining symptomatic relief in an uncomplicated diverticular disease and in reducing the incidence of primary complications.[١١] The rationale for the use of ٥-ASA in the treatment of diverticular disease involves its anti-inflammatory activity. ٥-ASA inhibits some key factors of the inflammatory mediator cascade

(cyclooxygenase, platelet activating factor synthetase, thromboxane synthetase)[١٢], inhibits the production of interleukin(I L)-١ and free radicals[١٣] and has intrinsic antioxidant activity.[١٤]

Poorly absorbed antibacterial agents may play a specific role in management of diverticulitis.[١٥] Trimethoprim and Sulfamethoxazole have occupied important roles in treatment of various commonly encountered infections[١٦, ١٧] and they play an important role in the management of many intra-abdominal infections including diverticulitis.[١٨]

During the last decade there has been interest in developing site-specific formulations for targeting drug delivery to colon.[١٩] The colon is a site where both local and systemic drug delivery can take place.[٢٠]

Colonic drug delivery has gained increased importance not just for the delivery of drugs for the treatment of local diseases associated with the colon but also for the potential it holds for systemic delivery of proteins and therapeutic peptides.[٢١] The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine.[٢٢]

Due to the distal location of colon in the gastrointestinal tract, a colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and effect an abrupt onset of drug release upon entry into the colon. Such a system can be formulated by utilizing some specific conditions existing in the colon in comparison to other parts of the gastrointestinal tract.[٢٣]

The presence of colonic microflora has formed a basis for development of colon-specific drug delivery systems through many mechanisms of activation; such as: azo-reduction and glycosidic bond hydrolysis.[٢٤, ٢٥]

Well known azo-drugs that exploit azo-reductase for site-specific drug release are the ٥-ASA prodrugs (e.g. olsalazine). These pass unaffected through the intestine where they are poorly absorbed, but are reduced by azo-reductase secreted from colonic microflora.[٢٦, ٢٧]

Materials and Instruments

Materials

The parent compounds (sulfamethoxazole and trimethoprim) were supplied from Nenevah Drug Industry (Iraq). All other chemicals used in preparations were supplied from Fluka-company (Germany).

Instruments

Melting points were determined on electrothermal CIA ٩٣٠٠ melting point apparatus and they are uncorrected.

The ultraviolet spectra were obtained via Carrywinn UV Varian UV–visible spectrophotometer (Australia).

The infrared absorbance was recorded by Buck ٥٠٠ scientific IR spectrophotometer (USA).

Structures were drawn by Chemdraw Office ٢٠٠١ software.

Thin-layer chromatography (TLC) was carried out on TLC plastic sheets silica gel ٦٠ F٥ pre-coated, ٢٠ ×٢٠ cm, layer thickness ٠.٢ mm. The spots on the chromatograms were localized using UV light (٣٦٦ nm) (Whatmann). The solvent system employed for separation was (TA) which is composed from methanol: strong ammonia solution (٩٨.٥:١.٥).

Experimental Methods

Synthesis of compound I

Sulfamethoxazole (١٢.٦٥ g, ٠.٠٥ mole) was dissolved in a mixture of equal quantity (١٦ ml) of each of conc. HCl and water in a beaker; the resulting solution was cooled by immersing in a bath of crushed ice; throughout the reaction, the reaction temperature was kept below ٥°C.

The cold solution of sodium nitrite (٤ g, ٠.٠٥٧ mole) in ٢٠ ml water was placed in a dropping funnel and then added drop by drop into the stirred solution of sulfamethoxazole in an ice bath; the reaction temperature was kept below ١٠°C by adding few grams of crushed ice when necessary.

After the last addition, the resulting solution was stirred for ٥ minutes in an ice bath. A drop of the solution diluted with (٤ drops) of water was tested with potassium iodide-starch paper; if no immediate blue color was obtained at the point of contact with paper, a further ١ ml of sodium nitrite solution was added, and the solution tested again after ٥ minutes.

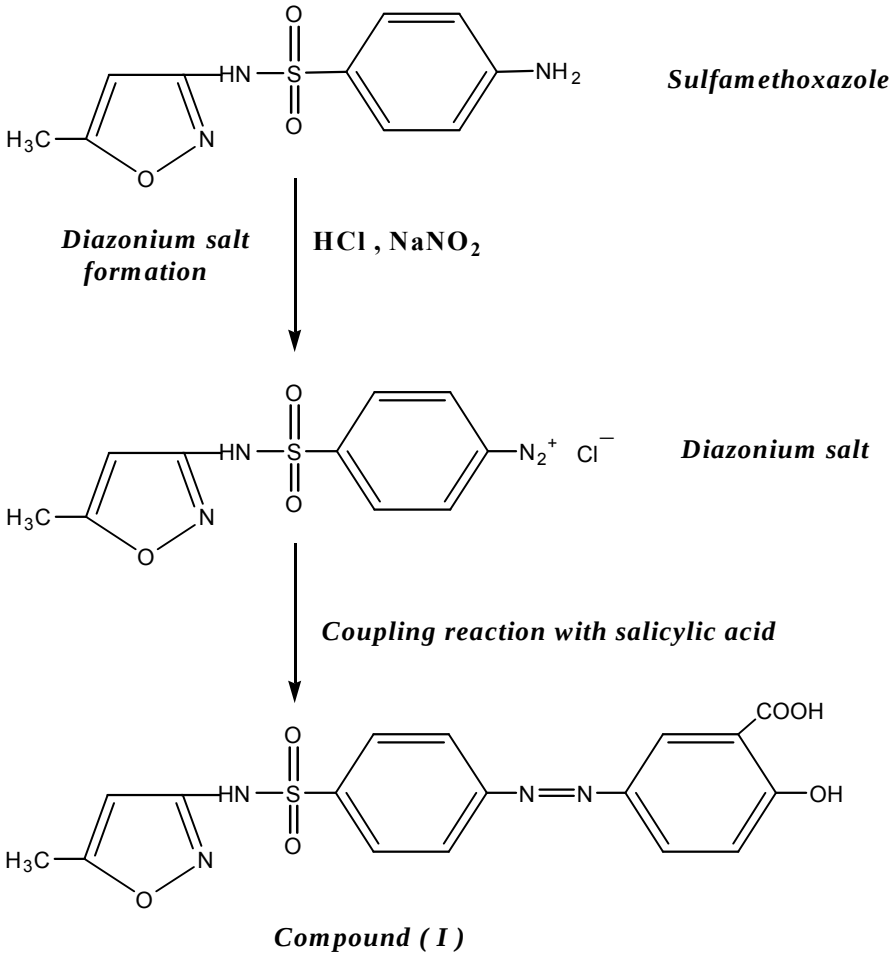
Further adding and testing were continued until an immediate blue color was obtained.

The salicylic acid solution was prepared by mixing salicylic acid (٦.٩ g, ٠.٠٥ mole) with (٤٥ ml) of ١٠% NaOH in a beaker immersed in the ice bath. The solution was vigorously stirred and the temperature kept below ٥°C; this was assisted by a direct addition of crushed ice.

The cold diazonium salt solution with few grams of crushed ice were placed in a dropping funnel and added drop by drop to the stirred salicylic acid solution in an ice bath;

orange color was developed and orange crystals soon separated giving compound I.

After the diazonium salt solution was added; the mixture was allowed to stand in ance bath for ٣٠ minutes; filtered off and the crystals washed three times with cold water, then re-crystallized from ethanol. The



4-(salicylic acid-5"-azo-yl)-N-(5'-methyl-3'-isoxazolyl)benzene-sulfonamide

Scheme ١. Synthesis of compound I

Compound I has the following data: the melting point was (١٨٥-١٨٧°C), the percentage of yield was (٨٥%), the molecular formula was established as ($C_{١٧}H_{١٤}N_{٤}O_{٦}S$) and the Rf value was (٠.٤٦).

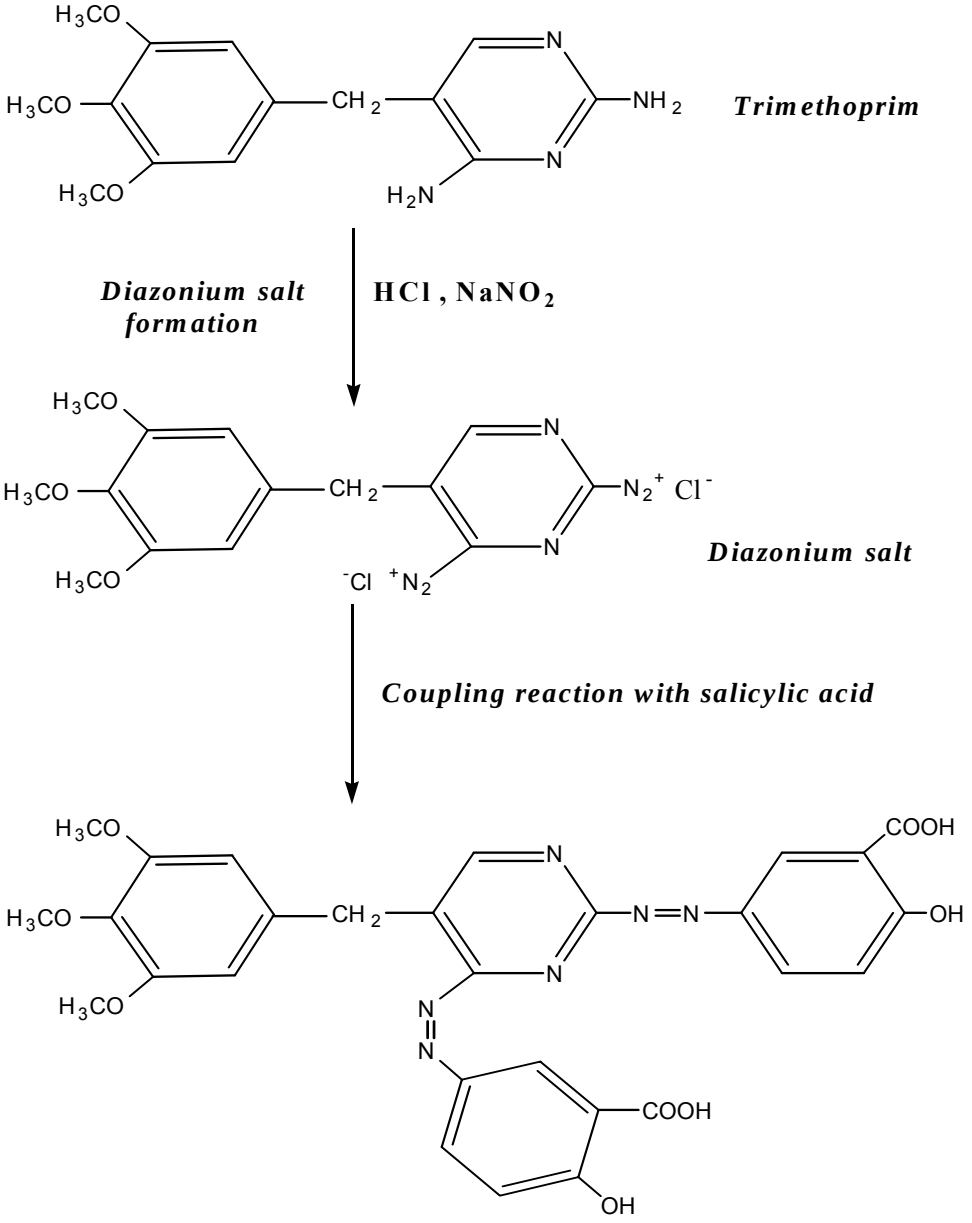
compound purity was established by thin-layer chromatography using TA solvent and the TLC result showed that only a single spot was observed.

Synthesis of compound II

Trimethoprim (١٤.٥ g, ٠.٠٥ mole) was dissolved in a mixture of equal quantity (٣٢ ml) of each of conc. HCl and water in a beaker; the resulting solution was cooled by immersing in a bath of crushed ice; throughout the reaction, the temperature was kept below ٥°C. The cold solution of sodium nitrite (٧.٨٧ g, ٠.١١٤ mole) in ٤٠ ml water was placed in a dropping funnel and then added drop by drop into the stirred solution of trimethoprim in an ice bath; the reaction temperature was kept below ١٠°C by adding few grams of crushed ice when necessary.

After the last addition, the resulting solution was stirred for ٥ minutesin an ice bath. A drop of the solution diluted with ٤ drops of water was tested with potassium iodide-starch paper; if no immediate blue color was obtained at the point of contact with paper, a further (١ ml) of sodium nitrite solution was added, and the solution tested again after ٥ minutes. Further adding and testing were continued until an immediate blue color was obtained.

The salicylic acid solution was prepared by mixing salicylic acid (١٣.٨ g, ٠.١ mole) with (٩٠ ml) of ١٠% NaOH in a beaker immersed in the ice bath, this solution was vigorously stirred and the temperature kept below ٥°C; this was assisted by a direct addition of crushed ice.
The cold diazonium salt solution with few grams of crushed ice were placed in a dropping funnel and added drop by drop to the stirred salicylic acid solution in an ice bath; red color was developed and red crystals soon separated giving compound II. After the diazonium salt solution was added; the mixture .



Compound (II)

2,4--di(salicylic acid-5"-azo-yl)-5-(3',4',5'-trimethoxybenzyl)pyrimidine

Scheme ٢. Synthesis of compound II Compound II has the following data: the melting point was (٢٣٥-٢٣٧°C), the percentage of yield was (٦٨%), the molecular formula was established as C٢٨H٢٨N٦O٩ and the Rf value was ٠.٥٧.

was allowed to stand in an ice bath.for ٣٠ minutes; filtered off and the crystals washed three times with cold water, then re-crystallized from ethanol.

The compound purity was established by thin-layer chromatography using TA solvent and the TLC result showed that only a single spot was observed.

In vitro stability studies

In order to obtain information on the stability of compounds I and II in (٠.٠٥ M) hydrochloric acid buffer (pH ١.٢) and in ٠.٠٥ M phosphate buffer (pH ٧.٤), the following procedure was used: ٢٨A sample (٠.٣٤ mole) of mutual prodrug was introduced into a conical flask containing (١٠٠٠ ml) of HCl buffer; the resulting solution was kept at a constant temperature (٣٧ ± ١°C) using a water bath with gentle stirring. The absorbance of this solution was determined by using ٢ cm quartz UV cell. The following formula was applied[٢٩] to calculate the absorbance coefficient (ε) of the solution:

Table ١. Kinetic data obtained from the stability studies

Compound number	A	λ_{max}	a (mole×١٠^{٦})	ε
Compound (I)	٠.٧٦٥	٣٠٢ nm	٦٨٠.٣٣	٥٦١.٩٤
Compound (II)	٠.٧٤٤	٤٠٨ nm	٦٨٠.٣٣	٥٤٦.٥٢
Compound (II)	٠.٧٩٢	١٨٣ nm	٦٨٠.٣٣	٥٨٢.١٦

$A = \varepsilon l a$ where a = Conc. of mutual prodrug at zero time.

Since the magnitude of absorbance coefficient was known, it is possible to determine the conc. of the compound I remaining at different time intervals using the following formula:

$A = \varepsilon l x$ where x = Conc. of mutual prodrug remaining.

A sample (٢ ml) was taken from the reaction flask, shaken with an equal volume of chloroform in order to remove the interfering products which could be released from hydrolysis of the mutual prodrug. The aqueous portion was estimated on UV spectrophotometer to determine the amount of mutual prodrug remaining. Every ٣٠ minutes, the above procedure was preceded for three hours and the decrease in the prodrug concentration with the time was monitored.

To examine the stability of mutual prodrug in phosphate buffer, the same procedure used to determine the stability in HCl buffer was followed; except that the phosphate buffer replaced the HCl buffer and the UV data were taken every ٣٠ minutes for six hours. The magnitudes of the solution absorbance at time zero and the absorbance coefficient of both compounds are summarized in table ١.

Release study in rat fecal matter

A sample (٠.٢٢٧ g, ٥٦٥.٩×١٠-٦ mole) of compound I was dissolved in sufficient volume of phosphate buffer (pH ٧.٤) to achieve a final concentration of ٣٠٠ µg /ml. Fresh fecal material of rats weighed about ١ g was placed in test tubes.

To each test tube, ١ ml of compound I solution was added; diluted to ٥ ml with phosphate buffer to achieve a final concentration of ٦٠ µg /ml. the test tubes were incubated at a constant temperature (٣٧ ± ١°C) using a water bath. Every ٣٠ minutes, one test tube was removed from a water bath and its contents were shaken with ٥ ml of chloroform. The aqueous portion was estimated on UV spectrophotometer to determine the amount of compound I remaining; this procedure proceeded for seven hours.[٣٠]

The above procedure was used to detect the hydrolysis of azo linkage para to methyl pyrimidine of compound II in rat fecal matter by using ٠.٣٥٤ g, ٥٩٧.١٩×١٠-٦ mole. In addition, the same procedure was used to detect the hydrolysis of azo linkage ortho to methyl pyrimidine of compound II in rat fecal matter by using ٠.٣٢٦ g, ٥٥٠.٢٥×١٠-٦ mole.

The calculations of the release study were carried out as for the stability study. The results obtained from the release study are listed in tables ٣, ٤ and ٥. All the kinetic procedures (stability studies in HCl buffer; in phosphate buffer and release study in rat fecal matter) were carried out three times.

Results and Discussion

Trimethoprim and sulfamethoxazole may be active against many aerobic gram-negative bacteria which represents the common causative micro-organism in a diverticulitis.[٣١] A variety of adverse effects were reported with the using of trimethoprim and sulfamethoxazole; such as: hyper-sensitivity reactions,[٣٢] hematological side effects and crystalluria.[٣٣]

٥-ASA is very effective in diverticulitis but it is absorbed mainly in the upper gastrointestinal tract that it usually fails to reach the colon leading to significant adverse effects. Therefore, to overcome this problem, colonic drug delivery has been developed for the topical treatment of diverticulitis.[٣٤]

In this study, a mutual azo prodrug of sulfamethoxazole with salicylic acid and a mutual azo prodrug of trimethoprim with salicylic acid were synthesized. The azo linkages in both mutual prodrugs were proposed to be broken in colon by the action of azo-reductase produced by colonic microflora.
In the first mutual prodrug, the hydrolysis of azo linkage leads to release of the sulfamethoxazole and ٥-ASA. While in the second mutual prodrug, the hydrolysis of azo linkages leads to release one mole of trimethoprim and two moles of ٥-ASA. Trimethoprim or sulfamethoxazole may be used to treat aerobic gram-negative bacterial infection in a diverticulitis, while ٥-ASA may reduce the inflammation in this disease.

Synthesis of compound I

A primary aromatic amine (sulfamethoxazole) was dissolved in a cold aqueous mineral acid solution then treated with sodium nitrite to form a diazonium salt, this process called a diazotization of primary amine.

Two important points must be taken into consideration in the preparation of diazonuim salt; which are: the amine is comparatively a weak base, so that, a certain amount of amine will be produced by salt hydrolysis unless an excess of acid is present; and the reaction mixture must be kept very cold during the process (the reaction is exothermic); otherwise, the diazonium salt may be partially converted into the corresponding hydroxyl compound.[٣٥]

The aromatic ring undergoing attack by the diazonium ion must in general contain a powerful electron-donating group (OH, NH٢, NHR). Substitutÿÿÿÿusually occurs at para position to the activating group. The aromatic ring of salicylic acid is usually attacked by the diazonium ion at position para to OH, which is the same position meta to carboxylic acid group.

The principal peaks of IR spectrum of compound I are listed in table ٢. The disappearance of absorbance band at ٣٤٣٦ cm-١ of primary amine of sulfamethoxazole and the appearance of a weak absorbance band at ١٤٨٣ cm-١ of azo group (unsymmetrical p-substituted azo benzene) confirmed the formation of azo compound, these data were quite in agreement with the structure of compound I.
The U.V. spectrum of compound I in an aqueous acidic solution (pH ١.٢) shows two maximum absorbencies, the first λmax at ٣٢٨ nm and the second λmax at ١٦١ nm.

Synthesis of compound II

One mole of trimethoprim was dissolved in a cold aqueous mineral acid solution then treated with two moles of sodium nitrite to form an intermediate having two diazonium ions according to a diazotization of primary amine.

The diazonium ions may be attacked by two moles of salicylic acid to form compound II. The substitution usually occurs at position (٥) of salicylic acid because of this position is para to OH group and meta to carboxylic acid group.

The principal peaks of IR spectrum of compound II are listed in table ٢. The disappearance of absorbance bands at ٣٤١٢, ٣٤٧٨ cm-١ of the two primary amines of trimethoprim and the appearance of weak absorbance bands at ١٤٩٤, ١٥٤٠ cm-١ of azo groups confirmed the formation of diazo compound; these data were quite in agreement with the structure of compound II.

The UV spectrum of compound II in an aqueous acidic solution (pH ١.٢) shows two maximum absorbencies, the first λmax at ٣٠٩ nm and the second λmax at ٢٧٠ nm.

In vitro kinetic studies

Compound I in ٠.٠٥ M hydrochloric acid buffer (pH ١.٢) showed negligible release of sulfamethoxazole and ٥-ASA. Whereas in phosphate buffer (pH ٧.٤), only (١٦.٨٢ %) release was observed over a period of six hours. The objective of bypassing the upper gastrointestinal tract with minimum prodrug release was achieved.

Further study in rat fecal matter was carried out to confirm the colonic reduction of azo

Table ٢. The I.R. spectra of compounds I and II

Compound number	Phenolic OH stretching (H-bonded)	Carboxylate anion stretching	(-N=N-) stretching	(C-N) stretching	(C-H) bending -١,٢,٥ trisubstituted benzene
Compound (I)	٣٢٣٨	١٥٨٠, ١٤٠٥	١٤٨٣	١٠٨٩	٨٥٠, ٦٩٨
Compound (II)	٣٣٥٧ ٣١٩٥	١٦١٢, ١٣٨٤ ١٥٩١, ١٣٧٨	١٥٤٠ ١٤٩٤	١٠٨٦ ١٠٦١	٨٨٧, ٧١٤ ٨٢٤, ٦٧٢

Table ٣. Kinetic data for release study of compound I in rat fecal matter

Absorbance	Time (min.)	x (mole×١٠^{٦})	(a-x) (mole×١٠^{٦})	a/(a-x)	ln (a/a-x)
٠.٥٣٦	٣٠	٤٧٦.٩٢	٨٨.٩٨	٦.٣٦	١.٨٥
٠.٥٢٣	٦٠	٤٦٥.٥٦	١٠٠.٣٤	٥.٦٤	١.٧٣
٠.٥٠٤	٩٠	٤٤٨.٢٥	١١٧.٦٥	٤.٨١	١.٥٧
٠.٤٧٣	١٢٠	٤٢٠.٨٠	١٤٥.١٠	٣.٩٠	١.٣٦
٠.٤٥٩	١٥٠	٤٠٨.٧١	١٥٧.١٩	٣.٦٠	١.٢٨
٠.٤٣٣	١٨٠	٣٨٥.١٠	١٨٠.٨٠	٣.١٣	١.١٤
٠.٤١٤	٢١٠	٣٦٨.٠٣	١٩٧.٨٧	٢.٨٦	١.٠٥
٠.٣٥٠	٢٤٠	٣١١.٥٦	٢٥٤.٣٤	٢.٢٣	٠.٨٠
٠.٣٢٠	٢٧٠	٢٨٤.٣٦	٢٨١.٥٤	٢.٠١	٠.٧٠
٠.٢٦٦	٣٠٠	٢٣٦.٨٩	٣٢٩.٠١	١.٧٢	٠.٥٤
٠.٢٤٣	٣٣٠	٢١٦.٥٨	٣٤٩.٣٢	١.٦٢	٠.٤٨
٠.١٣٠	٣٦٠	١١٥.٨٣	٤١٠.٠٧	١.٣٨	٠.٣٢
٠.٠٨٨	٣٩٠	٧٨.٠٦	٤٨٧.٨٤	١.١٦	٠.١٥
٠	٤٢٠	٠	٥٦٥.٩٠	١	٠

(a)= conc. of compound (I) at time zero and equal to (٥٦٥.٩×١٠$^{-٦}$ mole), (x) = conc. of compound I remaining for any time.

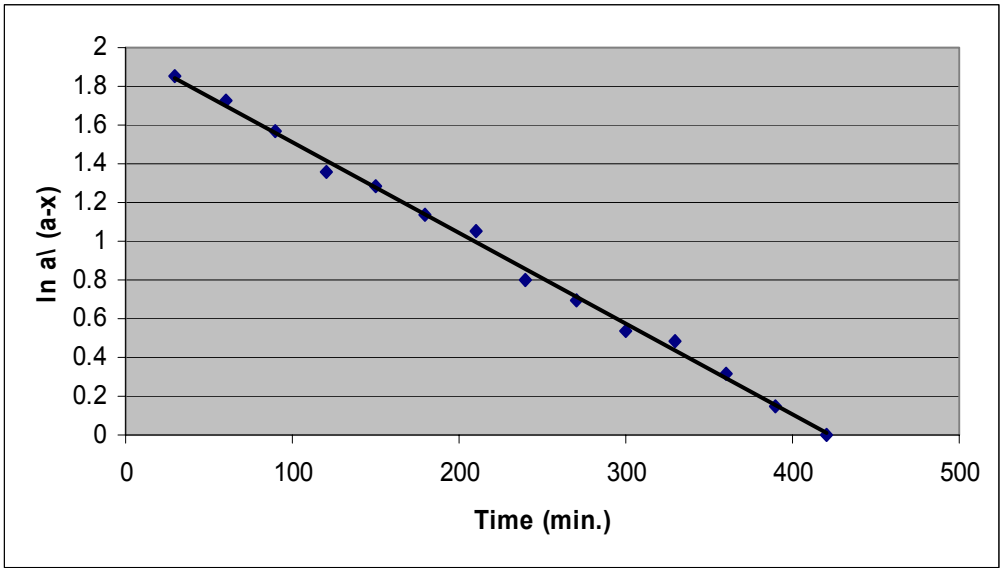


Figure ١. The slope of release study for compound I in rat fecal matter

prodrug over a period of seven hours, compound I showed (٨٣.١٨ %) cumulative release of sulfamethoxazole and ٥-ASA.The release study of compound I in rat fecal matter followed first order kinetics (Figure ١), the t.٥ (average of three trials) of compound I

Table ٣ shows the kinetic data obtained from the release study of compound I in rat fecal matter at ٣٧°C and λmax = ٣٠٢ nm was found to be ١٤٨.٤ minutes, whereas the rate constant (k) was found to be ٤.٦٧×١٠$^{-٣}$± ٠.٠٠٠١. Compound II, in ٠.٠٥ M hydrochloric acid buffer (pH ١.٢) showed negligible release of trimethoprim and ٥-ASA. Because of compound (II) has two azo groups may be hydrolyzed in phosphate buffer (pH ٧.٤), two kinetics may be followed. First is the kinetic of an azo group para to methyl pyrimidine; second is the kinetic of an azo group ortho to methyl pyrimidine. The last group is less stable than the first one. The hydrolysis of azo group para to methyl pyrimidine in phosphate buffer (pH ٧.٤) showed only ١٢.٢٢ % release over a period of six hours whereas in rat fecal matter over a period of seven hours, this group showed ٨٧.٧٨ % cumulative release of trimethoprim and ٥-ASA. Table ٤ shows the kinetic data obtained from the release study of azo group para to methyl pyrimidine of compound II in rat fecal matter at ٣٧°C and λmax = ٤٠٨ nm.

Table ٤. Kinetic data for release study of azo group para to methyl pyrimidine of compound II in rat fecal matter.

Absorbance	Time (min.)	x (mole×١٠^{٦})	(a-x) (mole×١٠^{٦})	a\(a-x)	ln (a\a-x)
٠.٥٤٥	٣٠	٤٩٨.٤٨	٩٨.٧١	٦.٠٥	١.٨٠
٠.٥٢٥	٦٠	٤٨٠.٠٩	١١٧.١٠	٥.١٠	١.٦٣
٠.٥٠٧	٩٠	٤٦٣.٨٩	١٣٣.٣٠	٤.٤٨	١.٥٠
٠.٤٩٢	١٢٠	٤٥٠.١٠	١٤٧.٠٩	٤.٠٦	١.٤٠
٠.٤٧٨	١٥٠	٤٣٧.٥١	١٥٩.٦٨	٣.٧٤	١.٣٢
٠.٤٥٦	١٨٠	٤١٧.٣١	١٧٩.٨٨	٣.٣٢	١.٢٠
٠.٤١٣	٢١٠	٣٧٧.٦٣	٢١٩.٥٦	٢.٧٢	١
٠.٣٧٤	٢٤٠	٣٤١.٩٨	٢٥٥.٢١	٢.٣٤	٠.٨٥
٠.٣١٨	٢٧٠	٢٩٠.٩٤	٣٠٦.٢٥	١.٩٥	٠.٦٧
٠.٢٩٨	٣٠٠	٢٧٢.٦٣	٣٢٤.٥٦	١.٨٤	٠.٦١
٠.٢٣٧	٣٣٠	٢١٦.٨١	٣٨٠.٣٨	١.٥٧	٠.٤٥

٠.١٩٦	٣٦٠	١٧٩.٥٧	٤١٧.٦٢	١.٤٣	٠.٣٦
٠.١١٣	٣٩٠	١٠٣.٦٤	٤٩٣.٥٥	١.٢١	٠.١٩
٠	٤٢٠	٠	٥٩٧.١٩	١	٠

(a) = conc. of compound II) at time zero and equal to (٥٩٧.١٩×١٠$^{-٦}$ mol), (x) = conc. of compound II *remaining* for any time.

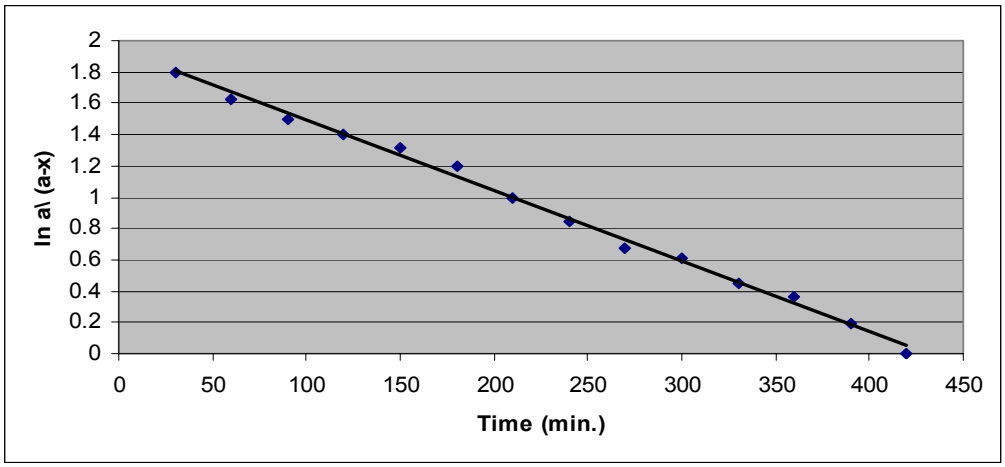


Figure ٢. The slope of release study of azo group para to methyl pyrimidine of compound II in rat fecal matter.

This release study followed first order kinetics (Figure ٢), the t.٥ (average of three trials) of compound II was found to be ١٥٧.٥ minutes, whereas the rate constant (k) was found to be ٤.٤×١٠$^{-٣}$ ± ٠.٠٠٠١.

The hydrolysis of azo group ortho to methyl pyrimidine in phosphate buffer (pH ٧.٤) showed only ١٩.١٢ % release over a period of six hours whereas in rat fecal matter over a period of seven hours, this group showed ٨٠.٨٨ % cumulative release of trimethoprim and ٥-ASA.Table ٥ shows the kinetic data obtained from the release study of azo group ortho to methyl pyrimidine of compound II in rat fecal matter at ٣٧°C and λmax = ١٨٣ nm.

Table ٥. Kinetic data for release study of azo group ortho to methyl pyrimidine of compound II in rat fecal matter.

Absorbance	Time (min.)	x (mole×١٠^{٦})	(a-x) (mole×١٠^{٦})	a\(a-x)	ln (a\a-x)
٠.٥٤٨	٣٠	٤٧٠.٣٩	٧٩.٨٦	٦.٨٩	١.٩٣
٠.٥٣٩	٦٠	٤٦٢.٩١	٨٧.٣٤	٦.٣٠	١.٨٤
٠.٥١٧	٩٠	٤٤٣.٦١	١٠٦.٦٤	٥.١٦	١.٦٤
٠.٤٩٥	١٢٠	٤٢٤.٩١	١٢٥.٣٤	٤.٣٩	١.٤٨
٠.٤٧٦	١٥٠	٤٠٩.١٦	١٤١.٠٩	٣.٩٠	١.٣٦
٠.٤٤٠	١٨٠	٣٧٧.٧٦	١٧٢.٤٩	٣.١٩	١.١٦
٠.٤١٧	٢١٠	٣٥٧.٨٥	١٩٢.٤٠	٢.٨٦	١.٠٥
٠.٣٧٨	٢٤٠	٣٢٤.٧٤	٢٢٥.٥١	٢.٤٤	٠.٨٩
٠.٣١٩	٢٧٠	٢٧٣.٧٤	٢٧٦.٥١	١.٩٩	٠.٦٩
٠.٢٨٣	٣٠٠	٢٤٢.٨٥	٣٠٧.٤٠	١.٧٩	٠.٥٨
٠.٢٣٣	٣٣٠	١٩٩.٧٧	٣٥٠.٤٨	١.٥٧	٠.٤٥
٠.١٨٠	٣٦٠	١٥٤.٣٩	٣٩٥.٨٦	١.٣٩	٠.٣٣

٠.٠٨٤	٣٩٠	٧١.٧٧	٤٧٨.٤٨	١.١٥	٠.١٤
٠	٤٢٠	٠	٥٥٠.٢٥	١	٠

(a) = conc. of compound(II) at time zero and equal to (٥٥٠.٢٥×١٠$^{-٢}$ mole), (x) = conc. of compound (II) remaining for any time.

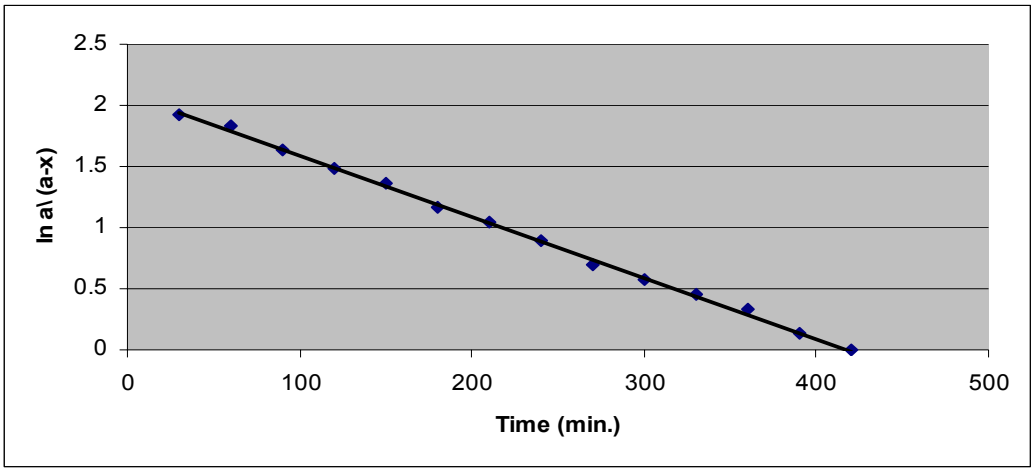


Figure ٣. The slope of release study of azo group ortho to methyl pyrimidine of compound II in rat fecal matter

This release study followed first order kinetics (Figure ٣), the t.٥ (average of three trials) of compound II was found to be ١٣٨.٦ minutes whereas the rate constant (k) was found to be ٥×١٠$^{-٣}$ ± ٠.٠٠٠١.

Conclusion

This study reports the synthesis and in vitro kinetic studies of two mutual azo prodrugs. The first of equimole of sulfamethoxazole and ٥-ASA; the second of one mole of trimethoprim and two moles of ٥-ASA for their colon targeting delivery to treat a diverticulitis.

Introduction of azo linkages in these mutual prodrugs has enhanced the aqueous solubility of ٥-ASA, sulfamethoxazole and trimethoprim, so that with minimum absorption in upper gastrointestinal tract. These mutual prodrugs would be directly delivered to colon and release the active compounds by the reducing action of azo reductase secreted by the colonic microflora.

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