ijlpr 2023; doi 10.22376/ijlpr.2023.13.1.P56-72

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Azo Prodrugs for Colon Target

Synthesis and Evaluation of Mutual Azo Prodrugs of Sulfamethoxazole and Salicylic Acid Derivatives for Colon Targeted Release

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Abstract: Several adverse effects are related to medications that treat moderate to severe inflammatory bowel diseases (IBDs). Targeted drug administration is necessary to treat inflammatory bowel disease to maximize efficacy and minimize toxicity. This study investigated the prodrug approach for antibacterials that target the colon. We chose sulfonamide (Sulfamethoxazole) as an antibiotic to target the colon. The prodrug approach is one of the most effective treatments for inflammatory bowel disease (IBD), and this mutual azo prodrug might serve the same function. This investigation aims to synthesize mutual azo prodrugs (S1-S5) and evaluate the colon-targeted release (in-vitro and in-vivo release studies). The objective of synthesizing mutual azo prodrugs (S1-S5) is that due to the high molecular weight of synthesized prodrugs (>500), they will enter the colon intact and not be absorbed in the upper GIT (Lipinski rule of 5). A coupling reaction between the sulfamethoxazole diazonium salt and salicylic acid derivatives allowed for synthesizing of mutual azo prodrugs (S1-S5). By monitoring the chemical reactions, the purity of the synthesized prodrugs was assessed, and by using FTIR, NMR (^{1}H and ^{13}C), Mass Spectrometry (MS), and Elemental Analysis, the structures of newly synthesized mutual azo prodrugs (S1-S5) were analyzed. Studies on the in-vitro stability of synthesized prodrugs showed less release after 6 hours in HCl buffer (pH 1.2), and only 10% release after 6 hours in phosphate buffer (pH 7.4) was observed. The sensitivity of the synthesized azo prodrug to the bacterial enzyme azoreductase was shown by incubating the azo prodrugs with the cecal contents of a rat; the release results showed that the release of free drugs from the azo prodrugs was more than 80%. Using trinitrobenzene sulfonic acid (TNBS)-induced colitis rats model, the in-vivo study was evaluated, and results revealed that mutually synthesized azo prodrugs are effective as 5-aminosalicylic acid in ulcerative colitis. Based on the release studies results, it is concluded that the azo prodrugs are a potential therapeutic target for ulcerative colitis.

Keywords: IBDs, Ulcerative Colitis, Mutual Azo Prodrug Approach, Enzyme Degradation, and Colon Targeted Drug Delivery.

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Received On 22 August, 2022
Revised On 1 November, 2022
Accepted On 10 November, 2022
Published On 2 January, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Anoop Kumar and Ashutosh Mishra , Synthesis and Evaluation of Mutual Azo Prodrugs of Sulfamethoxazole and Salicylic Acid Derivatives for Colon Targeted Release.(2023).Int. J. Life Sci. Pharma Res.13(1), P56-72
http://dx.doi.org/10.22376/ijlpr.2023.13.1.P56-72

1. INTRODUCTION

A prodrug is a drug or substance metabolized (regenerated in the body) into a pharmacologically active medication after administration. When a medicine is poorly absorbed from the gastrointestinal tract, prodrugs boost bioavailability. [1-2] Adrien Albert used the term prodrug in 1958 to describe substances that are physiologically inert derivatives of the drug molecule active type. [3] The prodrug may also transport proteins and peptides susceptible to enzymes in the stomach and intestines (intestine). [4] To introduce medicine into the colon, there are two approaches: covalently attaching the drug to a carrier and (ii) intact drug delivery to the colon. The covalent drug-carrier attachment comprises the prodrug approach, azo conjugates, glucuronide conjugates, cyclodextrin conjugates, amino acid conjugates, and polymer prodrug approaches. The intact type of medicine is delivered by covering it with pH-sensitive polymers, biodegradable polymers, and chemically reactive polymers and embedding it in biodegradable matrices and hydrogels, among other things. [5-7] The most successful approaches have resulted in the delivery of specific medications in the colon. [8] The colon has been a popular therapeutic site for colonic disorders such as Crohn's disease, ulcerative colitis, inflammatory diseases of the colon, and cancer. [9-11] To guarantee successful drug delivery to the target site, the drug in the colon must be shielded from the gastrointestinal tract's environment or absorption in the upper gastrointestinal tract and transported to the target site.[12] We know that the colonic microflora contains more anaerobic bacteria and enzymes such as glucosidase, glucuronidase, nitroreductase, nitrate reductase, and azoreductase than the rest of the gastrointestinal tract (10^8-10^9 microorganism/g gut content in rats and 10^{10}-10^{12} microorganism/g gut content in humans)[13-14]. Numerous enzyme-based prodrugs are already available, but none have attained the same therapeutic value as azo prodrugs of 5-aminosalicylic acid (5ASA), such as sulfasalazine[15] and its analogs balsalazide, ipsalazine, and olsalazine, [16-17] which are used to treat inflammatory bowel disease. The success of 5ASA derivatives is attributed to azo linkages within these molecules, particularly susceptible to attack by colonic bacteria in the body cavity (cecum) and human and rat feces. [18] Compounds with the general formula RN = NR' or ArN = NAr' are AZO compounds. The azo compound establishes a new class in synthetic organic chemistry with numerous applications, primarily as dyes and pigments. [19] Azo compounds are metabolized in the colon, where they are converted into two aromatic amines by azo reduction (- N = N-) with the aid of the colonic microflora-secreted enzyme azoreductase. Additionally, azo compounds are a good tool for developing medicines that target the colon, where a bacterial infection can be minimized. IBD and Crohn's disease are currently treated with azo chemicals (prodrugs). Sulfonamides are among the least expensive medications, which largely explains their growing use in developing nations. [20] It is hypothesized that these medications can treat gastrointestinal (GI) illnesses. Infections cause inflammation; therefore, sulfonamides can combine with salicylic acid derivatives to generate azo prodrugs (S1-S5) that, following biotransformation, liberate SM, 5ASA, and antimicrobial drugs such as MTZ, OZ, CF, and NF for use against infections and inflammations. [21] Sulfonamide prodrugs were produced by Nazeruddin et al. by combining ibuprofen (NSAID) with an antibacterial agent (sulfa drugs), [22] ShaymaLuayAbdulhadi et al. developed NSAIDs with sulfonamide conjugates as mutual prodrugs. [23] Yasser Fakri Mustafa produced 5-aminosalicylic acid with sulfa drug (Sulfamethoxazole) and trimethoprim as colon targeting models. [24] Koshti et al. synthesized prodrugs of sulfonamides as azo derivatives of carvacrol to be used for infection and inflammation[25]. Entamoeba histolytica, a protozoan parasite, causes intestinal amoebiasis, a contagious disease that is a serious public health concern in underdeveloped nations. According to the World Health Organization, Entamoeba histolytica is the third highest cause of death from this parasite. [26] The trophozoite form of Entamoeba histolytica invades colon tissue and causes amoebic colitis. Entamoeba histolytica infection can also result in amoebic brain abscesses. [27] For the treatment of amoebiasis, giardiasis, trichomoniasis, and other anaerobic and bacterial infections, the medicine of choice is a nitroimidazole derivative such as metronidazole (MTZ) or ornidazole (OZ). [28, 29] MTZ enters bacterial cells, where its nitro group is reduced by an electron transporter protein with a low redox potential, giving a covalent product (adduct) with guanine or cytosine in the DNA strand that has an antibacterial effect. [30, 31] Sulfamethoxazole (SM), metronidazole (MTZ), ornidazole (OZ), ciprofloxacin (CF), and norfloxacin (NF) were employed as model drugs in this study. All of these medicines have been used for IBDs (Ulcerative colitis and Crohn's disease) alone or in combination with other drugs; however, due to higher upper gastrointestinal absorption and lower oral bioavailability, they cannot be called safer drugs for IBD patients. As a result, colon-specific mutual azo prodrugs of S1-S5 are produced by the coupling reaction between sulfamethoxazole diazonium salts and salicylic acid derivatives of antimicrobial drugs such as MTZ, OZ, CF, NF, and SM and explored for further studies.

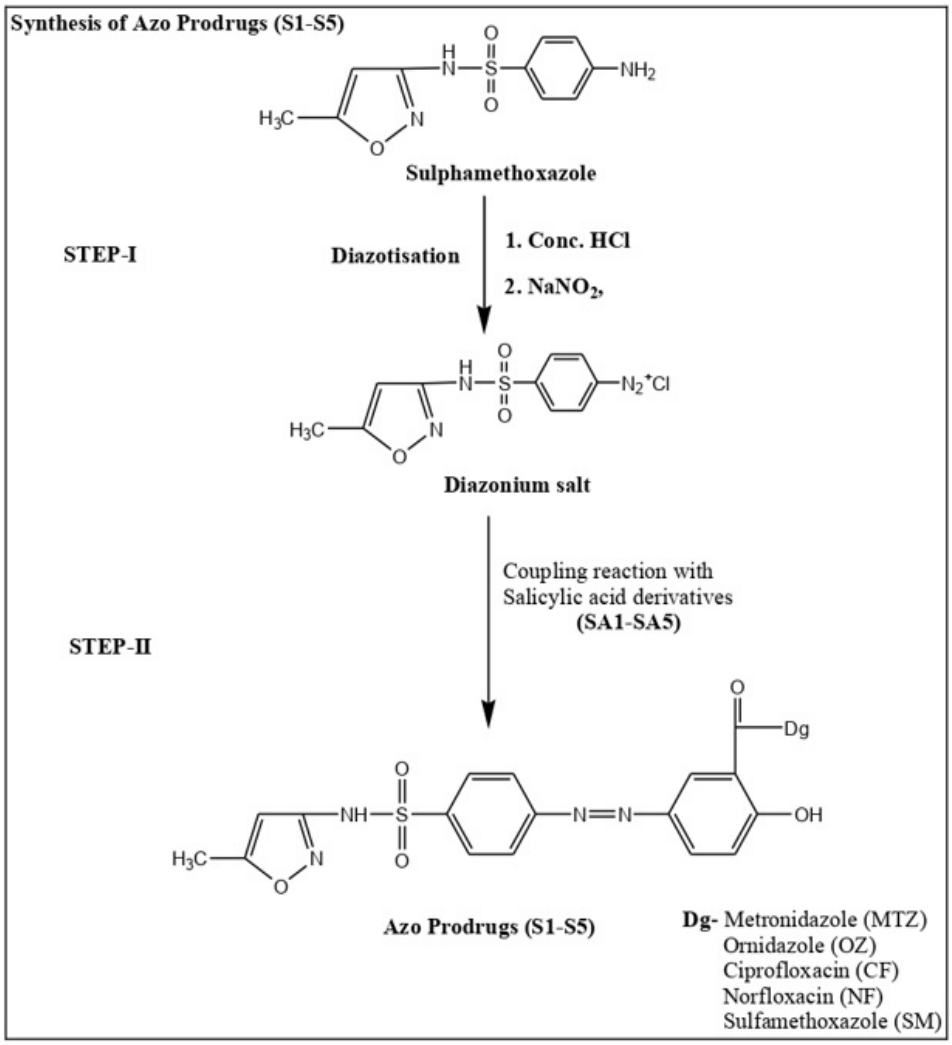
2. MATERIALS AND METHODS

2.1 Materials

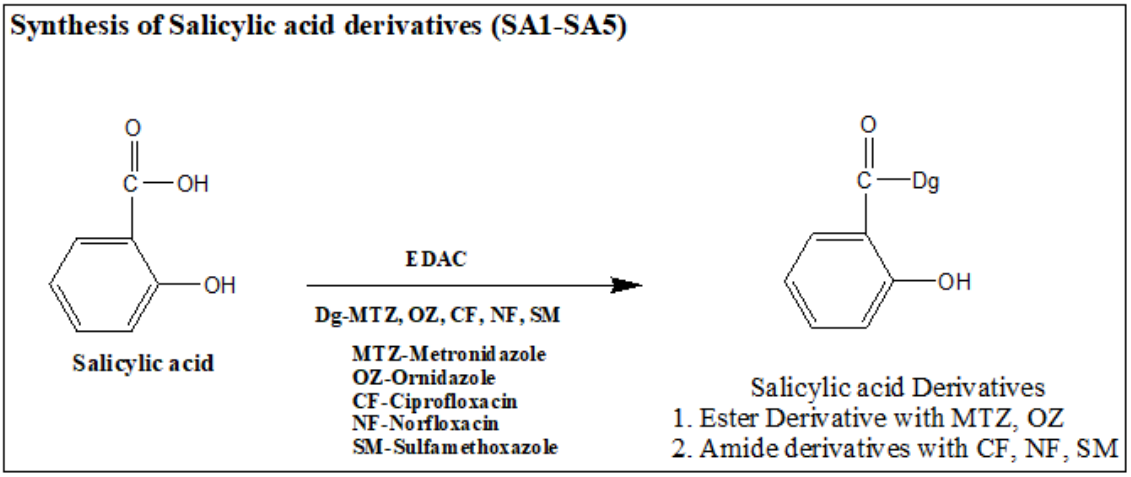
All the reactions were carried out in laboratory conditions. All the synthesis was done with laboratory-grade chemicals and analytical-grade solvents purchased from Aldrich Sigma, Hi Media, and CDH. Metronidazole IP, Ornidazole IP, Ciprofloxacin IP, Norfloxacin IP, and Sulfamethoxazole IP were given as free samples by Aishwarya Healthcare in Baddi, Himachal Pradesh. A Thiele tube and a digital melting point apparatus were used to determine melting points. FTIR, NMR (^{1}H and ^{13}C), mass spectroscopy, and elemental analyses were used to describe the synthesized compounds. The IR spectra were recorded on a SHIMADZU FTIR in KBr discs (cm-1) at GLA University Mathura, UP, INDIA, and the NMR (^{1}H and ^{13}C) spectra were measured in DMSO-d6 as the solvent on a Bruker Avance Neo 500 MHz NMR spectrometer with TMS (tetramethyl silane) as the internal standard. The chemical shift values are expressed in ppm relative to TMS (=0). The mass spectrum was recorded using the mass spectrometer SYNAPT-XS. Thermo Finnigan analyzer was used for the elemental analysis. All spectral data (NMR, MASS, and Elemental analysis) were obtained from SAIF Punjab University, Chandigarh PUNJAB, INDIA. Purification of the compounds was accomplished through column chromatography, and the progress of the reaction was confirmed through thin layer chromatography (TLC) with chloroform: methanol (2:2) and ethyl acetate:chloroform: methanol (2:2:1) as the mobile phases, and spots were visualized in the UV lamp and iodine chamber. All synthesized azo prodrugs (S1-S5) were screened for release and ulcerative colitis activity.

2.2 *Synthesis*

We report the synthesis of azo prodrugs because colon-specific prodrugs are so important. Sulfamethoxazole is converted into a diazonium salt. A coupling reaction occurs between the diazonium salt and salicylic acid derivatives (SCHEME-II, SA1-SA5, ester derivatives of Metronidazole, Ornidazole and amide derivatives of Ciprofloxacin, Norfloxacin and Sulfamethoxazole). To get the end products, the solution was stirred for another three hours at 0–5 ^{0}C. (SCHEME-I)



SCHEME I-: Synthesis of Azo Prodrugs (S1-S5) of Sulfamethoxazole with Salicylic acid derivatives



SCHEME-II: Synthesis of Salicylic acid derivatives (SA1-SA5)

2.3 Procedures

2.3.1 Synthesis of Azo Prodrug of Sulfamethoxazole with Salicylic Acid Derivative of Metronidazole (S-1)

Step-I: Synthesis of diazonium salt of Sulfamethoxazole

A solution of Sulfamethoxazole (0.68 g, 1.25 mmol) in 5 ml of concentrated hydrochloric acid was chilled in an ice-salt bath and then diazotized with a cold solution of sodium nitrite (0.19 g, 2.7 m mol) in 2 ml of concentrated sulfuric acid. The reaction described above was agitated for two hours at a temperature of 0 to 5 °C. A drop of the solution was diluted with water and tested on potassium iodide starch paper; if an instantaneous blue color was not observed at the point of contact with the form, another sodium nitrite solution was added, and the answer was retested 10 minutes later. They were added and evaluated until a primarily blue color was achieved. [32,33]

Step II: Coupling reaction of diazonium salt of Sulfamethoxazole with Salicylic acid derivatives

This is an electrophilic aromatic substitution reaction; the mechanism is the same as benzene nitration (i.e., it is a two-step reaction, with the first step determining the rate). First, the cold sulfamethoxazole diazonium salt solution was added to the well-stirred solution of the coupling chemicals (0.58 g, 2 mmol, salicylic acid-metronidazole ester conjugate SA-1) in 40 ml 10% NaOH. Next, the solution was agitated for three hours at 0-5°C, with alkaline conditions constantly maintained by adding the necessary amount of sodium bicarbonate. TLC was used to monitor the progress of the reaction, using chloroform: methanol (9:1) and chloroform:ethylacetate: methanol (2:2:1) as mobile phases. Next, the crude product was filtered, washed with hot water, dried, and recrystallized from ethanol. Finally, the purified product was vacuum dried [34-35,] and the percentage yield was 65 %. Table 1 displays the data of m.p. and % yield of the synthesized azo prodrugs S1-S5. The remaining compounds (S2 to S5) were synthesized similarly.

2.3.2 Synthesis of Salicylic Acid-Metronidazole (SA-MTZ) Conjugates (SA-1)

In aq. NaOH and salicylic acid (0.138 g, 1 mmol) was dissolved. After dissolving, add EDAC ((1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride, 0.19 g, 1 mmol, in water) solution to the salicylic acid solution with continued stirring at room temperature, the mixture was stirred at room temperature for 2 hours to activate the carboxyl group of salicylic acid, then metronidazole (0.21 g, 1.25 mmol)in methanol was added. The reaction mixture was stirred at room temperature for 5 days. The solvent was then suctioned out, and the residue was dialyzed against distilled water for 48 hours to purify the final product. Finally, with a 66% yield, the salicylic acid-metronidazole conjugate (SA-1) was produced as a white cotton powder. TLC on silica gel G was used to assess the purity of the produced conjugates using chloroform: methanol (4:1) and ethyl acetate:chloroform: ammonia (25:25:1) as phase mobile. The purity of the prodrugs is confirmed by the single spots obtained with Rf values of 0.63.[36-38.] The data of m.p. and the % yield (66-72) is shown in Table 2. The remaining compounds (SA2 to SA5) were synthesized similarly.

2.4 Analytical Data of Synthesized Azo Prodrugs (S1-S5)

2.4.1 Analytical Data of Synthesized Azo Prodrug of Sulfamethoxazole and Salicylic Acid Derivative of Metronidazole (S-1)

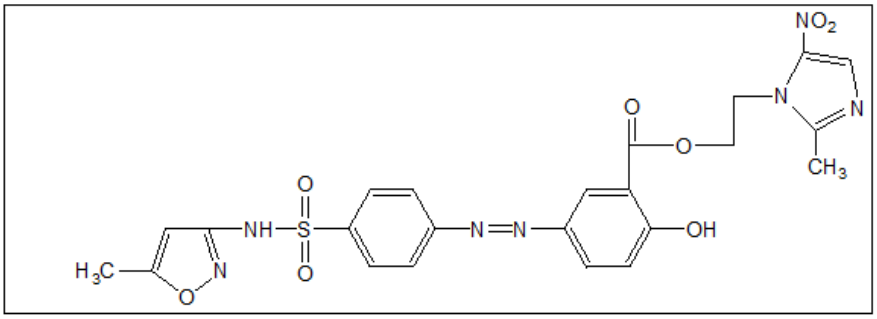


Fig. 1: - Azo prodrug of Sulfamethoxazole and Salicylic ester derivative of Metronidazole (S-1)

IR (KBr cm^{-1}): 3390 (NH*str*amine), 3298 (OH*str*, Aro), 3135 (C-H*str* ,Aro.), 2960 (CH*str*, Aliph.), 1738 (C=O*str*, Aro. ester), 1598 (C-C*str*Aro.), 1510 (N-O*str*Nitro), 1445 (-N=N-), 1365 (S=O*str*, Sulfo.), 1313 (C-O*str*Aro ester), 1160 (-C-N-).^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.31 (s, 3H, -CO=C-CH_3), 2.51 (s, 3H, -NC-CH_3), 4.69 (t 2H, -CH_2O) 4.79 (t, 2H, -CH_2N), 6.67 (d, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 8.07 (s 1H, -CNO_2, CN/ imidazole). ^{13}CNMR (500 MHz, DMSO-d6) δ ppm: 12.05 (-CH_3), 13.95 (-CH_3), 39.58 (-CH_2N-), 44.82 (-CN-), 62.97 (-CH_2O-), 95.81 (-C=C-O- Isoxazole), 112.76 (-C=C- Ar-C), 129.08 (-C=C- Ar-C), 153.40 (-CN=, Azo), 158.12 (-CN=Azo), 165.41 (-CO-O- ester), 169.93 (CO-N- Isoxazole), MS (70 eV) *m/z*: m/z 555, $(M+H)^+$. Anal. Calcd. for $C_{23}H_{21}N_7O_8S$: C, 49.73; H, 3.81; N, 17.65; S, 5.77; Found: C, 49.62; H, 3.72; N, 17.56, S, 5.68 %

2.4.2 Analytical Data of Synthesized Azo Prodrug of Sulfamethoxazole and Salicylic Acid Derivative of Ornidazole (S-2)

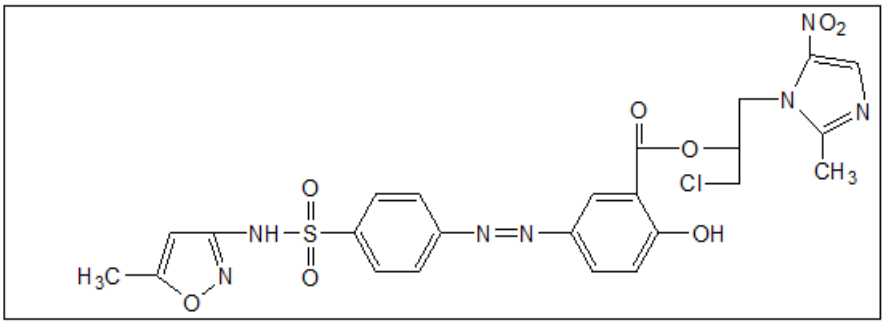


Fig. 2: - Azo prodrug of Sulfamethoxazole and Salicylic ester derivative of Ornidazole (S-2)

IR (KBr cm^{-1}): 3377 (NH*str*, amine), 3300 (OH*str*, Aro), 3120 (C-H*str*, Aro.), 2964 (CH*str*, Aliph.), 1742 (C=O*str*, Aro. ester), 1589 (C-C*str*Aro.), 1508 (N-O*str*Nitro), 1433 (-N=N-), 1370 (S=O*str*, Sulfo.), 1280 (C-O*str*Aro ester), 1234 (-C-N-).785 (C-Cl*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.29 (s, 3H, -CO=C-CH_3), 2.46 (s, 3H, -CN-CH_3), 3.72 (d, 2H, -Cl, -CHO), 4.22 (p, 1H, CH_2Cl, CH_2N), 4.57 (d, 2H, -CHO, CH_2Cl), 6.61 (d, 1H, Ar-H), 8.03 (s, 1H, -CNO_2, CN/ imidazole). ^{13}CNMR (500 MHz, DMSOd6) δ ppm: 11.94 (-CH_3), 14.27 (-CH_3), 39.62 (-CH_2N-), 46.55 (-CN-), 49.02 (-CH_2Cl), 69.03 (-CH_2O-), 95.22 (-C=C-O- Isoxazole), 112.54 (-C=C- Ar-C), 132.80 (-C=C- Ar-C), 153.20 (-CN=, Azo), 157.88 (-CN=Azo), 169.78 (CO-N- Isoxazole), 171.88 (-CO-O- ester), MS (70 eV) *m/z*: m/z 603, $(M+H)^+$. Anal. Calcd for $C_{24}H_{22}ClN_7O_8S$: C, 47.73; H, 3.67; Cl, 5.87; N, 16.23; O, 21.49; S, 5.31; Found: C, 47.62; H, 3.58; N, 16.12 %.

2.4.3 Analytical Data of Synthesized Azo Prodrug of Sulfamethoxazole and Salicylic Acid Derivative of Ciprofloxacin (S-3)

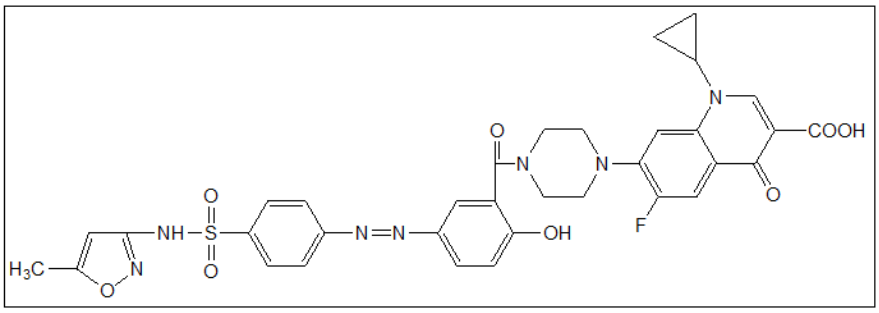


Fig. 3: - Azo prodrug of Sulfamethoxazole and Salicylic amide derivative of Ciprofloxacin (S-3)

IR (KBr cm^{-1}): 3300 (NH*str*, amine), 3220 (OH*str*, Aro), 3125 (C-H*str*, Aro.), 2860 (CH*str*, Aliph.), 2611 (OH*str*, Carb. acid), 1660 (C=O*str*, amide), 1602 (C-C*str*Aro.), 1490 (-N=N-) 1255 (C-F*str*), 1375 (S=O*str*, Sulfo.), 1180 (-C-N-*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.29 (s, 3H, -CO=C-CH_3), 3.35 (t, 2H, -CH_2-N/piperazine), 3.59 (t, 2H, -CH_2N/piperazine), 6.12 (s, 1H, -CN, -CN), 6.64 (d, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.97 (s, 1H, -C-CO, C-CO). ^{13}CNMR (500 MHz, DMSOd6) δ ppm: 8.32 (-CH_2-cyclopropane), 11.95 (-CH_3), 39.49, (-CN-), 95.25 (-C=C-O- Isoxazole), 112.56 (-C=C- Ar-C), 127.40 (-C=C- Ar-C), 153.24 (-CN=, Azo), 157.90 (-CN=Azo), 167.96 (-CO-N Amide), 169.81 (-COOH), 177.90 (-CO- Carbonyl), MS (70 eV) *m/z*: m/z 715, $(M+H)^+$. Anal. Calcd. for $C_{34}H_{30}FN_7O_8S$: C, 57.06; H, 4.22; F, 2.65; N, 13.70; O, 17.88; S, 4.48; Found: C, 57.01; H, 4.11; N, 13.62 %

2.4.4 Analytical Data of Synthesized Azo Prodrugs of Sulfamethoxazole and Salicylic Acid Derivative of Norfloxacin (S-4)

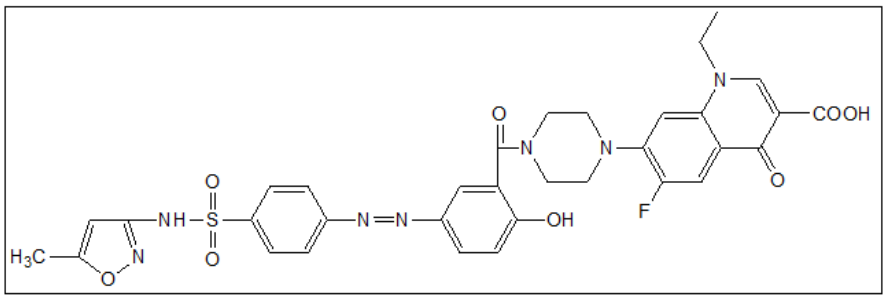


Fig. 4: - Azo prodrug of Sulfamethoxazole and Salicylic amide derivative of Norfloxacin (S-4)

IR (KBr cm^{-1}): 3342 (NH*str*, amine), 3300 (OH*str*, Aro), 3053 (C-H*str*, Aro.), 2981 (CH*str*, Aliph.), 2843 (OH*str*, Carb. acid), 1654 (C=O*str*, amide), 1598 (C-C*str*Aro.), 1485 (-N=N-), 1382 (C-F*str*), 1365 (S=O*str*, Sulfo.), 1160 (-C-N-*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 1.39 (t, 3H, -CH_2N-), 2.28 (s, 3H, -CO=C-CH_3), 2.50 (q, 2H, -CH_3), 3.38 (t, 2H, -CH_2N/ piperazine), 3.56 (t, 2H, -CH_2N/ piperazine), 6.10 (s, 1H, -CN-), 6.90 (q, 1H, Ar-H), 7.92 (d, 1H, Ar-H). ^{13}CNMR (500 MHz, DMSOd6) δ ppm: 11.97

(-CH3), 14.38 (-CH_3), 39.58, (-CN-), 95.28 (-C=C-O- Isoxazole), 112.60 (-C=C- Ar-C), 130.21 (-C=C- Ar-C), 153.26 (-C-N=, Azo), 157.94 (-C-N=Azo), 167.98 (-CO-N Amide), 172.17 (-COOH), 178.22 (-CO- Carbonyl), MS (70 eV) *m/z*: m/z 703, $(M+H)^+$. Anal. Calcd for $C_{33}H_{30}FN_7O_8S$: C, 56.32; H, 4.30; F, 2.70; N, 13.93; O, 18.19; S, 4.56; Found: C, 56.21; H, 4.20; N, 13.82 %

2.4.5 Analytical Data of Synthesized Azo Prodrug of Sulfamethoxazole and Salicylic Acid Derivative of Sulfamethoxazole (S-5)

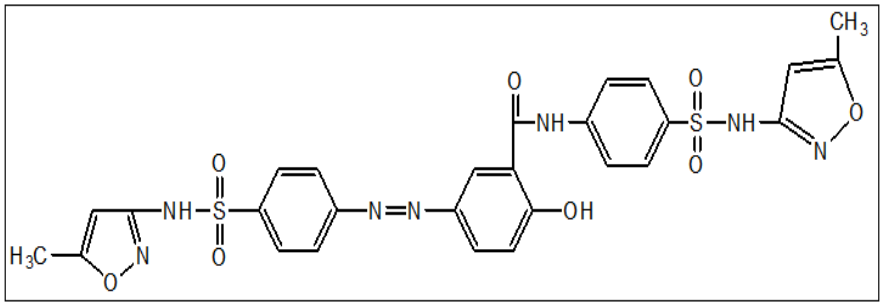


Fig. 5: - Azo prodrug of Sulfamethoxazole and Salicylic amide derivative of Sulfamethoxazole(S-5)

IR (KBr cm^{-1}): 3385 (OH*str* Azo deri.), 3305 (NH*str*, amide), 3145 (C-H*str*, Aro.), 2869 (CH*str*, Aliph.), 1654 (C=O*str*, amide), 1598 (C-C*str*Aro.), 1480 (-N=N-),1368 (S=O*str*, Sulfo.), 1158 (-C-N-*str*). ^{1}HNMR (500 MHz, DMSO-d6) δ ppm: 2.31 (s, 6H, -CO=C-), 7.84 (d, 1H, Ar-H), 7.97 (d, 1H, Ar-H), 8.05 (d, 1H, Ar-H). ^{13}CNMR (500 MHz, DMSO-d6) δ ppm: 11.99 (-CH3), 95.33 (-C=C-O- Isoxazole), 117.68 (-C=C- Ar-C), 128.17 (-C=C- Ar-C), 153.28 (-C-N=, Azo), 158.00 (-C-N=Azo), 161.20 (-C-OH Amide), 169.85 (-CO-NH, Amide), MS (70 eV) *m/z*: m/z 637, $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_7O_8S_2$: C, 50.86; H, 3.64; N, 15.38; O, 20.07; S, 10.06; Found: C, 5076; H, 3.54; N, 15.30 %

2.5 Analytical Data of Synthesized Salicylic Acid Derivatives (SA1-SA5)

2.5.1 Analytical Data of Synthesized Salicylic Acid-Metronidazole (SA-MTZ) Conjugates (SA-1)

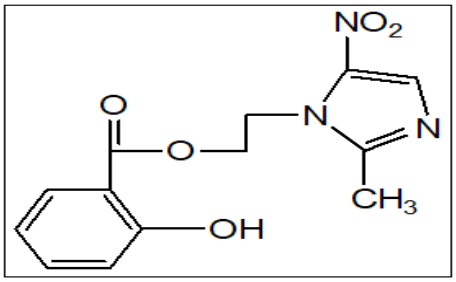


Fig. 6: - Salicylic Acid-Metronidazole (SA-1) conjugate

IR (KBr cm^{-1}): 3322 (OH*str*, Aro), 3125 (C-H*str*,Aro.), 2950 (CH*str*, Aliph.), 1735 (C=O*str*, Aro. ester), 1597 (C-C*str*Aro.), 1546 (N-O*str*), 1263 (C-O*str*Aro ester). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.46 (s, 3H, -CN=N-), 4.63 (t, 2H, -CH_2O) 4.73 (t, 2H, -CH_2N), 6.93 (p, 1H, Ar-H), 8.03 (s 1H, -CNO_2, CN/ imidazole). MS (70 eV) *m/z*: m/z 291, $(M+H)^+$.

2.5.2 Analytical Data of Synthesized Salicylic Acid-Ornidazole (SA-OZ) Conjugates (SA-2)

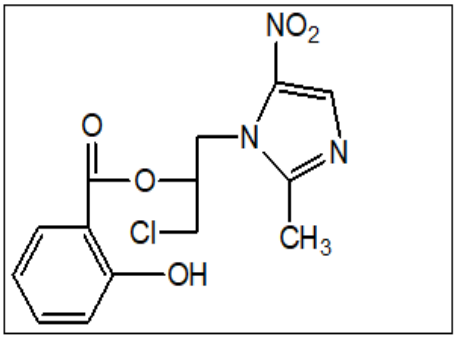


Fig. 7: - Salicylic Acid-Ornidazole (SA-2) conjugate

IR (KBr cm^{-1}): 3369 (OH*str*, Aro), 3100 (C-H*str* ,Aro.), 2860 (CH*str*, Aliph.), 1740 (C=O*str*, Aro. ester), 1578 (C-C*str*Aro.), 1535 (N-O*str*), 1275 (C-O*str*Aro ester), 763 (C-Cl*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.46 (s, 3H, -CN=N-), 3.67 (p, 2H, -CH_2Cl, -CH_2N), 4.56 (d, 2H, -CHO, -CH_2Cl), 6.95 (p, 1H, Ar-H), 8.02 (s, 1H, -CNO_2, CN/ imidazole). MS (70 eV) *m/z*: m/z 339, $(M+H)^+$.

2.5.3 Analytical Data of Synthesized Salicylic Acid-Ciprofloxacin (SA-CF) Conjugates (SA3)

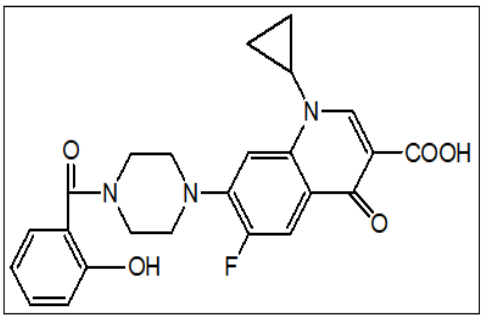


Fig. 8: - Salicylic Acid-Ciprofloxacin (SA-3) conjugates

IR (KBr cm^{-1}): 3383 (N-*str,* amide), 3225 (OH*str,* Aro), 3130 (C-H*str*, Aro.), 2850 (CH*str*, Aliph.), 2575 (OH*str*, Carb. acid), 1655 (C=O*str,* amide), 1299 (C-F*str*), 1611 (C-C*str*Aro.), 1242 (CN*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 1.18 (d, 2H, -CHN-), 1.33 (t, 1H, $-CH_2-CH_2$), 3.33 (t, 2H, $-CH_2$-N/piperazine), 3.56 (t, 2H, $-CH_2$N/piperazine), 6.95 (q, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.80 (d, 1H, =CH-COOH). MS (70 eV) *m/z*: m/z 451, $(M+H)^+$.

2.5.4 Analytical Data of Synthesized Salicylic Acid-Norfloxacin (SA-NF) Conjugates (SA4)

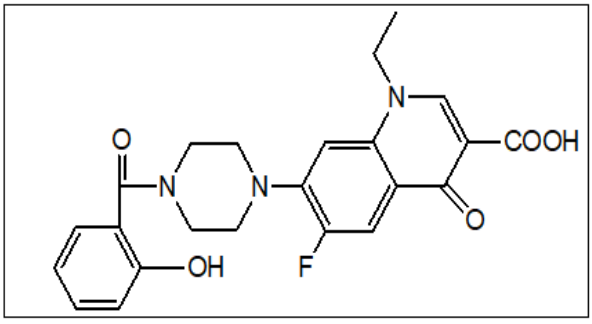


Fig. 9: - Salicylic Acid-Norfloxacin (SA-4) conjugates

IR (KBr cm^{-1}): 3343 (N-*str,* amide), 3203 (OH*str,* Aro), 3120 (C-H*str*, Aro.), 2893 (CH*str*, Aliph.), 2465 (OH*str*, Carb. acid), 1648 (C=O*str,* amide), 1300 (C-F*str*), 1625 (C-C*str*Aro.), 1243 (CN*str*). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 1.39 (t, 3H, $-CH_2$N-), 2.50 (q, 2H, $-CH_3$), 3.37 (t, 2H, $-CH_2$N/ piperazine), 3.57 (t, 2H, $-CH_2$N/ piperazine), 6.80 (q, 1H, Ar-H), 7.20 (d, IH, Ar-H), 7.36 (t, 1H, Ar-H), 7.7 (d, 1H, Ar-H), 7.86 (d, 1H, =C-COOH). MS (70 eV) *m/z*: m/z 439, $(M+H)^+$.

2.5.5 Analytical Data of Synthesized Salicylic Acid-Sulfamethoxazole (SA-SM) Conjugates (SA-5)

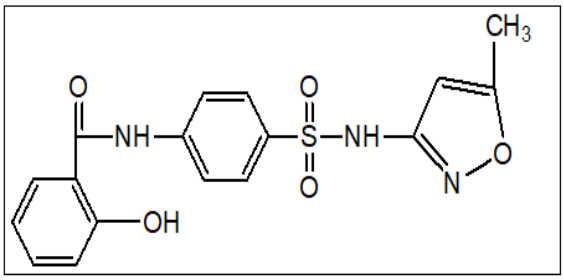


Fig. 10: - Salicylic Acid-Sulfamethoxazole (SA-5) conjugates

IR (KBr cm^{-1}): 3378 (N-*str,* amide), 3200 (OH*str,* Aro), 3140 (C-H*str*, Aro.), 2869 (CH*str*, Aliph.), 1657 (C=O*str,* amide), 1598 (C-C*str*Aro.), 1330 (S=O*str,* Sulfo.). ^{1}HNMR (500 MHz, DMSOd6) δ ppm: 2.28 (s, 3H, -CO=C-), 6.62 (d, 1H, Ar-H), 6.96 (t, 1H, Ar-H) 7.48 (t, 1H, Ar-H), 7.81 (s, 1H, Ar-H). MS (70 eV) *m/z*: m/z 373, $(M+H)^+$.

2.6 Release Studies

2.6.1 In-Vitro Release Studies

Colon-targeted prodrugs must remain intact as they move through the stomach and small intestine to reach the colon, where enzymes in the colon microflora must break them down. Because the current study seeks to transfer antimicrobial drugs (MTZ, OZ, CF, NF, and SM) to the colon through their azo prodrugs, their stability in the stomach and small intestine must be evaluated. [39] Following the synthesis of the azo prodrugs and confirmation of their structure by physicochemical and spectral characterization, in-vitro stability tests in hydrochloric acid buffer (pH 1.2, SGF), phosphate

buffer (pH 7.4, SIF), and rat fecal matter (RFM) were carried out. [40]

2.6.1.1 Release Studies in Simulated Gastric Fluid (SGF, Ph 1.2)

Each azo-prodrug (S1-S5, 10 mg) was gently placed on the surface of 900 ml of HCl buffer (simulated gastric fluid, SGF) in separate baskets that revolved at 100 rpm and maintained a temperature of 37±10°C. [41] During drug dissolution, perfect sink conditions were maintained. At 30-minute intervals, the solutions were agitated, and 5 ml aliquots were taken from the dissolving vessel. The study involved six hours in completing. The removed samples were evaluated using a UV spectrophotometer at λ_{max} 295 nm, 336 nm, 285 nm, 302 nm, and 282 nm, respectively.

2.6.1.2 Release Studies in Simulated Intestinal Fluid (SIF, Ph 7.4)

Drug release tests in phosphate buffer (simulated intestinal fluid, SIF, pH 7.4) were carried out in the same manner as described above, except that simulated intestinal fluid was used instead of HCl buffer (simulated gastric fluid, SGF, pH 1.2). [42]

2.6.1.3 Release Studies in Rat Fecal Matter (RFM)

For the release tests in rat feces, the sensitivity of the azo bond to reduction by bacterial azoreductase was assessed by diffusion method. [43] Each of the prodrugs (S1-S5) was dissolved in phosphate buffer (pH 7.4) to achieve a final concentration of 250 μg/ml. Fresh rat (Wistar rat) feces (about 1 g) were weighed and deposited in various test tubes. In the tube, 1 ml of the prodrug solution was added and diluted to 5 ml with phosphate buffer. The suspensions were incubated at 37°C for 30 minutes at a time. The experiment was completed in 6 hours; aliquots were taken, filtered, and monitored using a UV spectrophotometer (Shimadzu UV 1700) at λmax 295 nm, 336 nm, 285 nm, 302 nm, and 282 nm, respectively. It was found that the λmax of any free drugs that were assumed that were released by the produced azo prodrugs did not overlap with the λmax of the prodrugs because their λmax was significantly different from that of the prodrug. [44]

2.6.2 In-Vivo Release Studies (Pharmacological Evaluation)

2.6.2.1 Animals

For this investigation, male Wistar rats (180-200 g, 12-15 weeks) were employed. The rats were housed in sterile metal cages (a maximum of five rats per cage) and kept at room temperature with unrestricted access to food and water. The rats were held at 25°C on a 12-hour light/dark cycle. The rats fasted for 24 hours before colitis induction, although they had constant access to tap water. The rats were divided into eight groups of five rats each for the studies. All experimental procedures were carried out during the light cycle and followed the guidelines for the care and use of laboratory animals established by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC). The Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Chemistry, Acharya Narendra Dev College of Pharmacy, Babhnan, Gonda UP-271313, India, reviewed and approved the experimental protocol, and its animal facility was approved by CPCSEA: 1585/PO/E/5/11/CPCSEA, Registration date: 23/12/2011, and Author permission IAEC approval Reference No: IAEC/AND/CP/3/2021.

2.6.2.2 Induction of Colitis and Treatment

For the in-vivo effectiveness research, a rat colitis model was established with minor modifications, employing TNBS as the colitis-inducing drug. The rats fasted for 24 hours while having unlimited access to water. Under mild anesthesia, TNBS was administered into the rat colon using ketamine and xylazine (20 mg/kg and 5 mg/kg, i.m.). A 2 mm polyethylene catheter with a tip 8 cm proximal to the anus was inserted rectally into the colon. After instilling 0.5ml of 5% w/v TNBS (150mg/kg body weight) in 50% ethanol into the intestinal lumen of all rats (except the normal control group), the rats were kept upright for 45 seconds before being returned to their cages. [45] The rats were observed without treatment for three days to generate a suitable model for inflammatory bowel disease with unlimited access to food and water *ad libitum*. Group 1 (normal control) and Group 2 (disease control/TNBS-induced ulcerative colitis group) received 1-carboxymethylcellulose (CMC) after three days. Group 3 (standard control) got sulfasalazine (500 mg/kg), whereas Groups 4, 5, 6, 7, and 8 (treatment control) received azo prodrugs of S1, S2, S3, S4, and S5 (500 mg/kg). All treatment regimens were followed for a total of eight days. The drug and azo prodrugs were suspended in CMC and given by oral gavage once a day.[46]

2.6.2.3 Assessment of Colonic Damage by Disease Activity Index Score

During the 11-day research, all groups of rats measured changes in body weight, fecal consistency, and fecal occult blood (Table 04). In addition, colitis activity was assessed using a disease activity index score (DAI score), as previously published by Hartmann et al. 2000 and Lamprecht et al., 2001. [47-48] The disease activity index was calculated by averaging the three previous characteristics for each day for each group, and it varied from 0 (healthy) to 3 (unhealthy) (maximum colitis activity). [49] The rats were euthanized under isoflurane anesthesia 24 hours after the last drug dose, and an 8 cm long colon section was removed. The colon weight/length ratio was calculated to measure the inflammation.

2.6.2.4 Histopathological Studies

For histological examinations, tissue samples (3 cm long segments) were removed from each colon and preserved in 10% formalin. Then, histopathological exams of the colon were done, and microscopic color pictures of colon sections were captured using a RADICAL RXL-4T trinocular microscope coupled to an IS500 5.0 camera S/N KC 500305157.

2.7 Statistical Analysis

One-way ANOVA with Tukey's test was used to compare the stability and release results (*in-vitro*) of azo prodrugs in SGF, SIF, and RFM. In contrast, one-way ANOVA with the Newman-Keuls test was used to analyze the *in-vivo* release studies. Mean ±, and standard deviation represents all text and graphs data. The data were analyzed using GraphPad Prism (5.01 version) statistical software. P values of < 0.05 were regarded as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Physicochemical and Analytical Characterization

All azo prodrugs (S1-S5) and intermediates (SA1-SA5) were synthesized according to the scheme, which includes the formation of diazonium salts of Sulfamethoxazole and, subsequently, a coupling reaction between diazonium salts of Sulfamethoxazole and salicylic acid derivatives (SA1-SA5). The physicochemical constants of the synthesized azo prodrugs (S1-S5) and salicylic acid derivatives (SA1-SA5) are presented in Tables 1 and 2. FTIR, ^{1}HNMR, and mass spectral data confirmed by the structures of the intermediates (SA1-SA5), whereas Infrared spectroscopy (FTIR), Nuclear Magnetic Resonance (^{1}H and ^{13}C), Mass spectroscopy, and Elemental analysis assigned the structures of the final synthesized azo prodrugs (S1-S5).

Table 1: Physical constants and physicochemical characteristics of Azo Prodrugs (S1-S5)

Compounds Code	Molecular Formula	Mol. Weight	Melting Point (0^0C)	Yield (%)	Rf Value
S-1	$C_{23}H_{21}N_7O_8S$	555	272-276	65	0.66
S-2	$C_{24}H_{22}ClN_7O_8S$	603	264-268	67	0.69
S-3	$C_{34}H_{30}FN_7O_8S$	715	272-276	68	0.71
S-4	$C_{33}H_{30}FN_7O_8S$	703	268-272	69	0.67
S-5	C27H23N7O8S2	637	266-270	72	0.71

Mobile Phase: Chloroform: methanol (9:1) and Chloroform: ethyl acetate: methanol (2:2:1)

Table 2: Physical constants and physicochemical characteristics of Salicylic acid derivatives (SA1-SA5)

Compounds Code	Molecular Formula	Mol. Weight	Melting Point (0^0C)	Yield (%)	Rf Value
SA-1	$C_{13}H_{13}N_3O_5$	291	188-192	66	0.65
SA-2	$C_{14}H_{14}ClN_3O_5$	339	202-206	68	0.64
SA-3	$C_{24}H_{22}FN_3O_5$	451	220-224	72	0.72
SA-4	$C_{23}H_{22}FN_3O_5$	439	218-222	71	0.69
SA-5	$C_{17}H_{15}N_3O_5S$	373	212-216	70	0.68

Mobile Phase: chloroform:methanol (4:1) and ethyl acetate:chloroform:ammonia (25:25:1)

Infra-Red spectrum of synthesized azo prodrugs (S1-S5) and intermediates (SA1-SA5) shows the characteristics absorption bands at 1735-1750 cm^{-1} due to carbonyl (-C=O) ester group, 1630-1690 cm^{-1} due to carbonyl amide group, 1335-1370 strong S=O stretching in sulphonamide, and 1400-1600cm^{-1} due to (–N=N– str. unsymmetrical p-substituted azobenzene) azo group (S1-S5). [50]^{1}HNMR of the synthesized azo prodrugs and intermediates showed the chemical shift value at δ 2.29-2.70 (s, 3H, Ar-C-CH_3) ppm for the methyl group (CH_3) attached to the oxazole (SM), imidazole (MTZ and OZ) ring, δ 4.22-4.79 (t, 2H, N-CH_2, MTZ, OZ) and δ 4.56-4.57 (d, 2H, CH_2Cl, OZ) ppm for the methylene group (CH_2). Chemical shift values at δ 3.35-3.59 (m, 2H, piperazine-H) ppm were observed for the piperazine ring (S-3, S-4). The chemical shift value at δ 6.0-8.5 (d, 1H) ppm was also observed for the aromatic ring (Ar-H). In ^{13}CNMR, the characteristics of chemical shifts were observed for the synthesized azo prodrugs (S1-S5), showed a value of δ ppm at 0-35 for CH_3, 50-90 CH_2O, 125-150 Ar-C-, 160-185 C=O ester and 160-175 C=O for amide. The aromatic ring carbon bonded to the COOH group resonated at δ 169.81-172.17 ppm, while the aromatic ring carbon atoms bonded to the azo bond resonated at δ 153.40-158.88 ppm, confirming the formation of an azo bond. The carbon atom of the carbonyl group (C=O) showed a chemical shift value at δ 165.41-171.88 for ester (S1, S2) and 167.96-169.85 for amide. [51] The mass spectra of synthesized azo prodrugs (S1-S5) showed parent ion/ molecular peak (m/z) at 555, 603, 715, 703, and 637, and for intermediates (SA1-SA5) were found at 291, 339, 451, 439 and 373 respectively, which confirms the molecular weight of the synthesized compounds. [52] The elemental analysis also confirms the synthesis of azo prodrugs. The detailed spectral data are shown in the characterization data of synthesized conjugates.

3.2 In-Vitro Release Studies

In vitro release experiments of the synthesized azo prodrugs S1, S2, S3, S4, and S5 revealed that they were stable, with the low release of the parent drug in SGF (pH 1.2) (Fig. 11), meaning they did not undergo hydrolysis and could be stable in acidic gastric pH and SIF (pH 7.4), with about 10% release was observed (Fig. 11). This avoided the GIT without releasing drugs. To confirm the colonic release of the synthesized prodrugs, the kinetics were investigated in rat fecal matter (RFM), which reveals a considerable release of free drugs from the prodrug, with a percent release of 81.72 for MTZ (S1), 80.49 for OZ (S2), 80.08 for CF (S3), 80.24 for NF (S4), and 84.55 for SM (S5) (Fig. 12). [53-55] There was a significant difference between in-vitro release in SGF, SIF, and RFM, with more drugs released in RFM than in SGF and SIF. The table and graphs (Table-3, Fig. 12) illustrate the maximum amount of drug released from azo prodrugs in rat fecal matter.

Table-3: % Drugs released from azo prodrug (S1-S5) in rat fecal matter					
Time (Hrs)	% Release of drug from azo prodrugs (S1-S5) in rat fecal matter (RFM)				
	S-1	S-2	S-3	S-4	S-5
0.0	00.00±0.00	00.00±0.00	00.00±0.00	00.00.±0.00	00.00.±0.00
0.5	09.24±0.27	08.90±0.05	10.17±0.37	08.72±0.09	09.37±0.08
1.0	16.92±0.26	19.13±0.24	17.08±0.09	18.76±0.14	19.96±0.14
1.5	28.44±0.12	26.59±0.14	29.90±0.22	26.05±0.05	28.75±0.30
2.0	35.64±0.06	36.51±0.13	34.83±0.68	36.66±0.04	39.96±0.72
2.5	47.04±0.03	43.57±0.37	45.68±0.13	44.67±0.05	47.28±0.30
3.0	54.72±0.54	54.41±0.18	54.31±0.31	55.23±0.09	57.76±0.28
4.0	62.52±0.51	62.39±0.21	64.42±0.48	63.66±0.08	65.81±0.91
5.0	71.04±0.52	72.72±0.24	71.57±0.34	71.34±0.11	76.61±0.27
6.0	81.72±0.06	80.49±0.28	80.08±0.37	80.24±0.17	84.55±0.26

Results are expressed as mean ± S.D, an average of three readings.

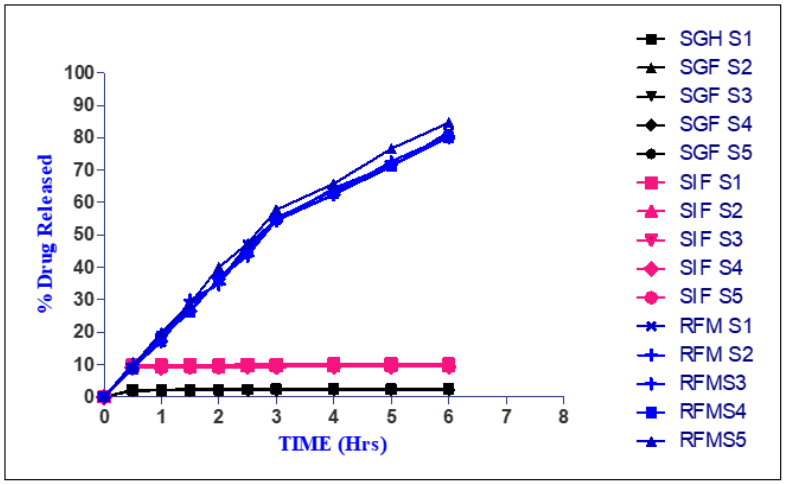


Fig. 11: - The maximum amount of drug released from synthesized azo prodrugs (S1-S5) in SGF (pH 1.2), SIF (pH 7.4), and RFM (rat fecal matter).

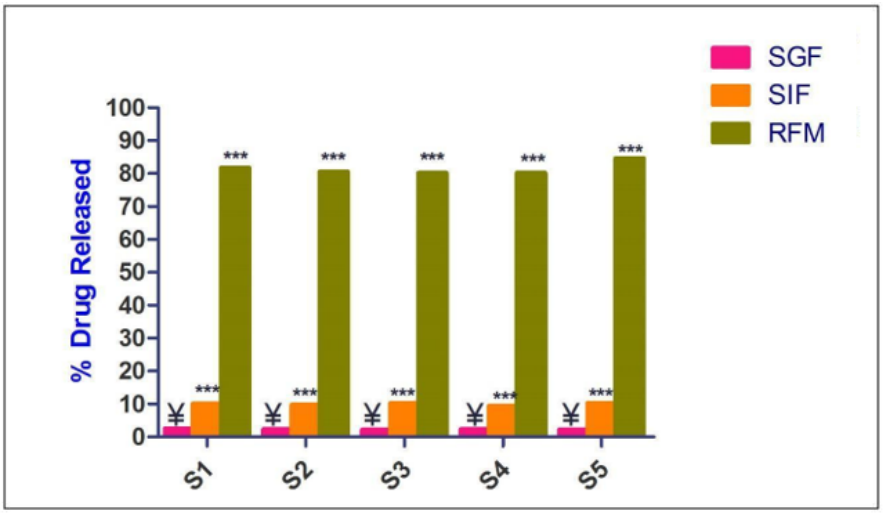


Fig. 12: - The maximum amount of drug released from synthesized azo prodrugs (S1-S5) in SGF (pH 1.2), SIF (pH 7.4), and RFM (rat fecal matter). The distinction was statistically significant (* p<0.05 SGF vs. SIF and *** p<0.05 SGF vs. RFM).**

3.3 In-Vivo Release Studies

Animal models of intestinal inflammation are essential for understanding CD and UC, the two primary human IBDs. [56] In rats, the TNBS model causes intestinal inflammation with various clinical and histological characteristics similar to human IBDs. [57]. In *In-vivo* experiments, after experimental colitis was induced, all groups' DAI scores raised immediately and persistently over 3 days. All groups that received azo prodrugs demonstrated a decrease in infection and inflammation within 48 hours, which became significant on day 6. Standard control groups (Sulfasalazine, prodrug of 5-ASA) and treatment

control groups (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5) decreased DAI score during treatment (Table-5, Fig. 13A). There was no significant difference between sulfasalazine (STD) and the synthesized prodrugs (treatment groups), but significant difference observed between DC (disease control) and sulfasalazine (STD) and treatment groups. (**p<0.05 disease control (DC) vs. Standard (STD), TRTS1 and TRTS2, *p<0.05 disease control (DC) vs. Treatment (TRTS3, TRTS4, and TRTS5) (Table-5, Fig. 13B). On day 11 (24 h post drug administration), rats were euthanized, and colon weight/colon length ratio was measured to evaluate inflammation. Standard and prodrug-treated groups had lower colon weight/ colon length ratio than the colitis control group (Table-6, Fig. 14). [58] There was a significant difference between normal control group (NC) and sulfasalazine (STD) treated group and azo prodrugs treated groups (treatment groups). Sulfasalazine treated group shows more reduction in colon-to-colon length ratio than treatment groups (**p<0.05 Normal control (NC) vs. Standard (STD), ***p<0.05 Normal control (NC) vs.TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5). Histopathological results validated the anti-infective and anti-inflammatory properties of mutual azo prodrugs.

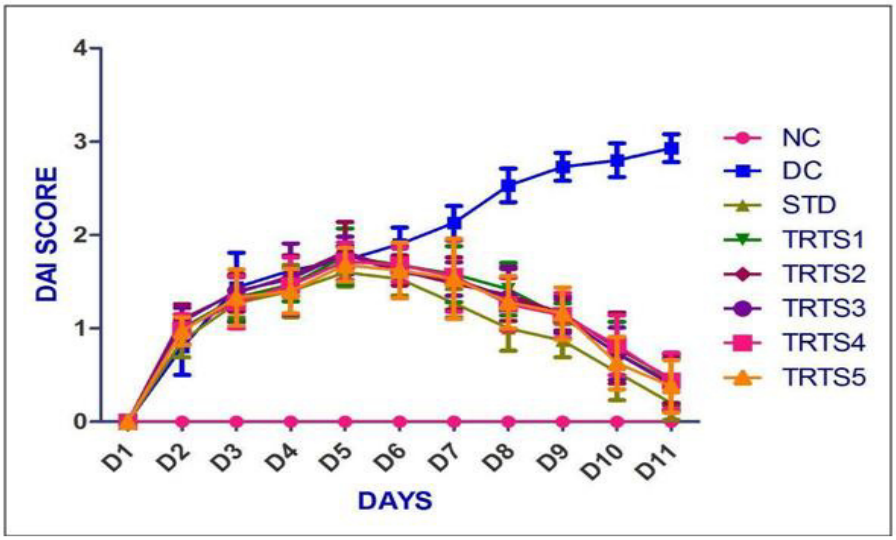
Table- 4: Scoring rate of Disease Activity Index

S.No	Activity	Descriptions			
1	% Change in Body wt	Gained body wt/remained the same	Lost 1-3 % of original body wt	Lost 3-5 % of initial body wt	Lost > 5% of initial body wt
2	Fecal occult blood	Occult blood absent	Occult blood detected	Bloodstains in feces	Significant bleeding in feces
3	Fecal consistency	Normal feces	Soft feces	Mild diarrhea	Severe diarrhea
4	Score Rate	0	1	2	3

Table- 5: Disease Activity Index* Score

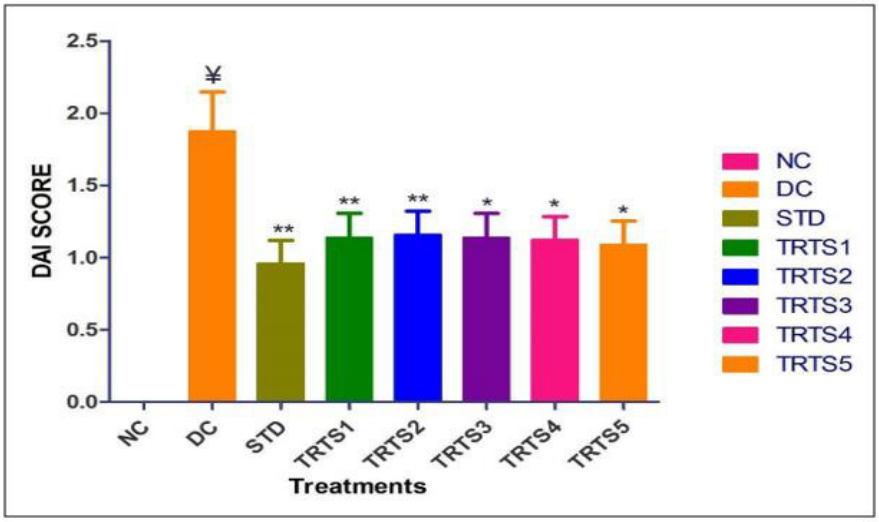
Days	No. of Animals/ rats (n)	Groups							
		NC	DC	STD	TRTS1	TRTS2	TRTS3	TRTS4	TRTS5
D1	5	0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
D2	5	0.00	0.80±0.30	0.87±0.18	1.00±0.00	1.10±0.16	1.07±0.15	0.99±0.16	0.97±0.15
D3	5	0.00	1.44±0.37	1.27±0.15	1.33±0.24	1.38±0.20	1.40±0.15	1.28±0.28	1.33±0.30
D4	5	0.00	1.62±0.00	1.40±0.28	1.47±0.18	1.56±0.22	1.53±.038	1.46±0.30	1.40±0.24
D5	5	0.00	1.73±0.15	1.60±0.15	1.77±0.30	1.82±0.32	1.80±0.18	1.72±0.20	1.68±0.18
D6	5	0.00	1.90±0.18	1.53±0.18	1.68±0.18	1.62±0.16	1.60±0.28	1.68±0.18	1.62±0.30
D7	5	0.00	2.13±0.18	1.27±0.15	1.58±0.30	1.48±0.28	1.53±0.18	1.56±0.38	1.53±0.43
D8	5	0.00	2.53±0.18	1.00±0.24	1.42±0.28	1.36±0.28	1.33±0.33	1.26±0.28	1.28±0.28
D9	5	0.00	2.73±0.15	0.87±0.18	1.12±0.15	1.16±0.18	1.13±0.18	1.14±0.24	1.16±0.28
D10	5	0.00	2.80±0.18	0.53±0.30	0.74±0.33	0.79±0.38	0.73±0.28	0.82±.032	0.63±0.28
D11	5	0.00	2.93±0.15	0.20±0.18	0.42±0.24	0.46±0.26	0.40±0.28	0.44±0.30	0.38±0.28

Results are expressed as mean±S.D, *average of five readings.



DAI scores were decreased by administration of Sulfasalazine and azo prodrugs (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5). The results are expressed as the mean±SD average of five rats.

Fig. 13A: - Effect of Sulfasalazine and azo prodrugs (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5) on the DAI score in rats with TNBS-induced colitis.



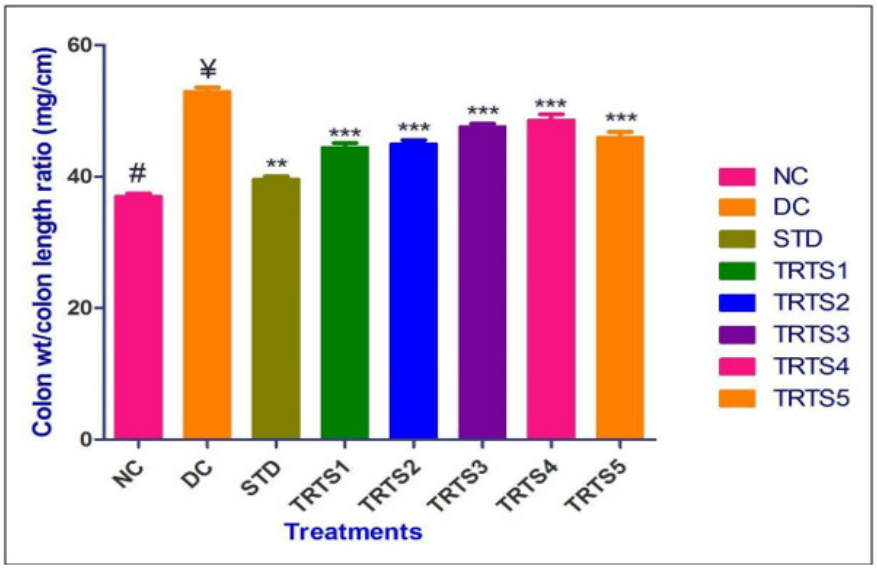
*DAI scores were decreased by administration of Sulfasalazine and azo prodrugs (S1, S2, S3, S4, and S5). The results are expressed as mean±SEM of five rats. **p<0.05 disease control (DC) vs. Standard (STD), TRTS1 and TRTS2, *p<0.05 disease control (DC) vs. Treatment (TRTS3, TRTS4, and TRTS5).*

Fig. 13B: -Effect of Sulfasalazine and azo prodrugs (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5) on the DAI score in rats with TNBS-induced colitis.

TABLE- 6: Colon Weight/Colon Length Ratio*

Colon weight/colon length ratio (w/w) ± SD	GROUPS							
	NC	DC	STD	TRTS1	TRTS2	TRTS3	TRTS4	TRTS5
	37.02±0.93	53.38±1.30	39.62±1.47	44.46±1.49	45.28±1.28	47.61±1.36	48.62±1.96	46.71±1.86

*Results are expressed as mean±S.D, *average of five rats.*



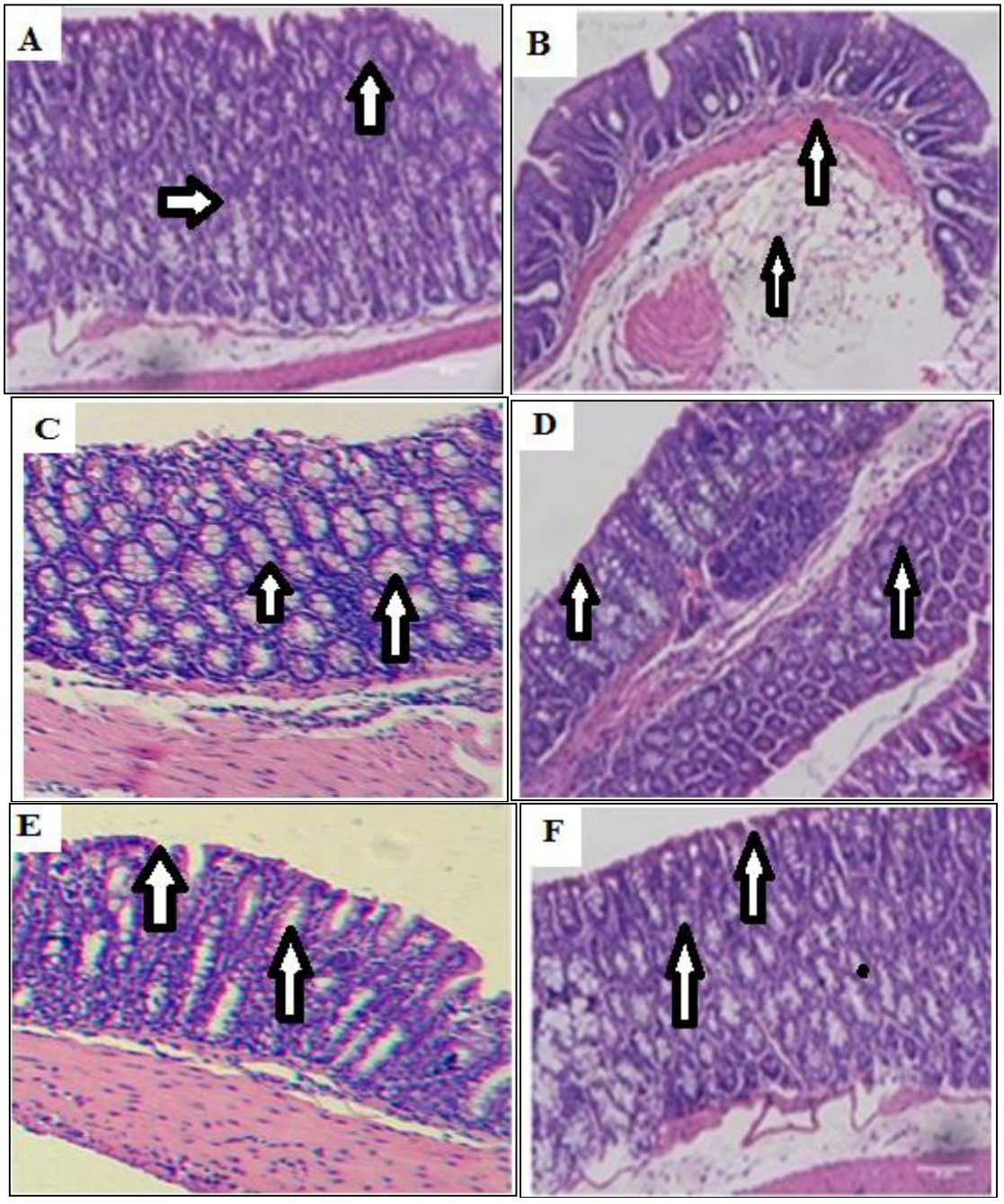
*Colon weight to colon length ratios was decreased by administration of Sulfasalazine and azo prodrugs (S1, S2, S3, S4, and S5). The results are expressed as the mean±SD of five rats. **p<0.05 Normal control (NC) vs. Standard (STD), ***p<0.05 Normal control (NC) vs. TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5.*

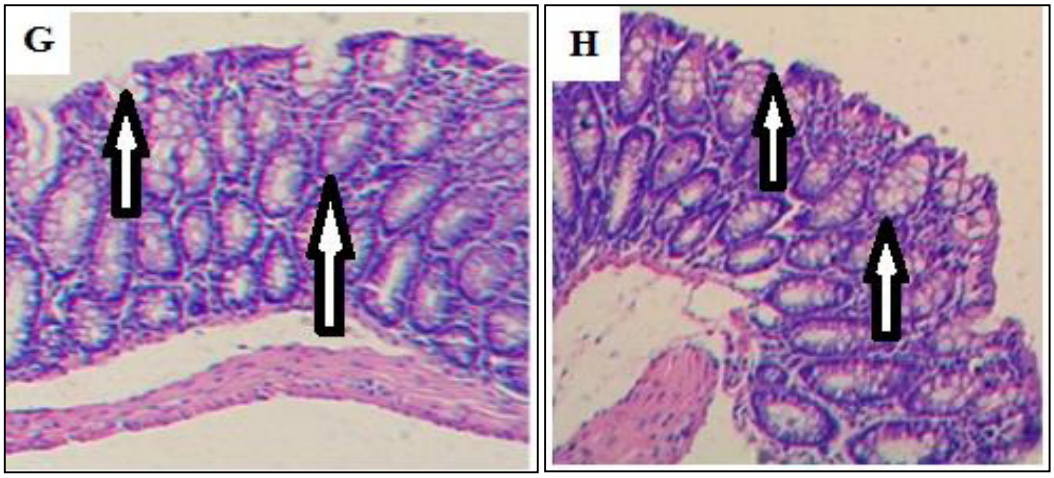
Fig.14: - Effect of Sulfasalazine, azo prodrugs (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5) on the colon weight to colon length ratio in rats with TNBS-induced colitis.

3.4 Histopathological Studies

The NC (Normal control), DC (Disease control), STD (Standard control), and Treatment control groups (TRTS1, TRTS2, TRTS3, TRTS4, and TRTS5) were histologically examined. All the samples were collected from rats sacrificed on the 11th day after drug and prodrug treatment. Normal control groups OR normal colon structure exhibited healthy mucosa with enterocytes and goblet cells and connective tissue (lamina propria, Fig. 15A). In contrast, untreated/disease control rats showed necrosis, extensive epithelial damage, inflammatory cell infiltration, crypt abscess, loss of goblet cells, and crypt architecture distortion (Fig. 15B). [45, 59] Standard/sulfasalazine-treated rats exhibited normal mucosa,

mucosal thinning, and gradual restoration of crypt architecture, goblet cells, and epithelium, as well as a reduction in inflammatory cell infiltration (Fig. 15C). The severity and extent of colonic injury were significantly reduced after treatment of infected rats with synthesized azo prodrugs. The treated groups exhibited normal mucosal structure with slight inflammation in the muscular propria (TRTS1, Fig. 15D), progressive restoration of crypt architecture, goblet cells, and epithelium (TRTS2, Fig. 15E), normal mucosal structure and restoration of crypt architecture (TRTS3, Fig. 15F), normal mucosa, presence of goblet cells (TRTS4, Fig. 15G), and normal mucosa, presence of goblet (TRTS5, Fig. 15H). [60] Histological analyses demonstrate the utility of mutual azo-prodrugs in the treatment of inflammatory bowel disease (ulcerative colitis).





A- Normal Control (NC) arrow shows normal mucosa and goblet cells. B- Disease Control/Colitis control (DC), arrow shows the presence of mucus, necrosis, and distortion of crypt architecture; C- Standard control (STD)/ sulfasalazine treated group, arrow shows the presence of goblet cells, and restoration of crypt architecture, D- TRTS1 arrow shows the presence of united goblet cells and inflammation, E.-TRTS2 arrow shows the presence of goblet cells and restoration of crypt architecture, F-TRTS3 arrow shows the presence of united goblet cells and inflammation, G-TRTS4 arrow shows the presence of goblet cells and H- TRTS5 arrow shows the presence of goblet cells and restoration of crypt architecture. The original magnification was 10x X 10x.

Fig. 15: - Histopathological studies of TNBS- induced colitis

4. LIST OF ABBREVIATIONS

Abbreviations	Full Name
IBD	Inflammatory bowel diseases
13C-NMR	Carbon Nuclear Magnetic Resonance
1H-NMR	Proton Nuclear Magnetic Resonance
CD	Crohn's disease
CF	Ciprofloxacin
DAI	Disease Activity Index
DC	Disease Control
DMSO	Dimethyl Sulfoxide
EDAC	1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimide
FTIR	Fourier Transform Infrared spectroscopy
IP	Indian Pharmacopoeia
KBr	Potassium Bromide
NC	Normal Control
m/z	Mass/ Charge Ratio
MS	Mass Spectroscopy
MTZ	Metronidazole
NF	Norfloxacin
OZ	Ornidazole
S1	Azo prodrugs of Sulfamethoxazole and Salicylic acid derivative of Metronidazole
S2	Azo prodrugs of Sulfamethoxazole and Salicylic acid derivative of Ornidazole
S3	Azo prodrugs of Sulfamethoxazole and Salicylic acid derivative of Ciprofloxacin
S4	Azo prodrugs of Sulfamethoxazole and Salicylic acid derivative of Norfloxacin
S5	Azo prodrugs of Sulfamethoxazole and Salicylic acid derivative of Sulfamethoxazole
SA-1	Salicylic acid derivative of Metronidazole
SA-2	Salicylic acid derivative of Ornidazole
SA-3	Salicylic acid derivative of Ciprofloxacin
SA-4	Salicylic acid derivative of Norfloxacin
SA-5	Salicylic acid derivative of Sulfamethoxazole
SCF	Simulated Colonic Fluid
SD	Standard Deviation
SEM	Systematic Error Mean
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SM	Sulfamethoxazole

STD	Standard Control
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
TRTS1	Treatment group of S1 azo prodrugs
TRTS2	Treatment group of S2 azo prodrugs
TRTS3	Treatment group of S3 azo prodrugs
TRTS4	Treatment group of S4 azo prodrugs
TRTS5	Treatment group of S5 azo prodrugs
UC	Ulcerative colitis
WHO	World Health Organisation

5. CONCLUSION

In conclusion, we have successfully synthesized specific mutual azo prodrugs of sulfamethoxazole and salicylic acid derivatives (S1-S5). According to the findings of this research, the synthesis of mutual azo-prodrugs of Sulfamethoxazole and salicylic acid derivatives (S1-S5) improved drug delivery to the colon. Due to the considerable molecular weight of the synthesized azo prodrugs (>500) and according to the Lipinski rule of five, "compounds having molecular weight more than 500 will not be absorbed in upper GIT" therefore, all the azo prodrug was transported to the colon, where bacterial azoreductase divided it to release drugs (antimicrobial drugs). According to release results, drug release from prodrugs begins in the distal colon. There is a considerable release in the colon, implying that synthesized azo-prodrugs show promise and are effective for treating IBDs (UC).

6. ACKNOWLEDGEMENTS

We are grateful to the late Dr. Anil Kumar Shrivastava, sir, for his excellent guidance and enthusiastic support throughout this research. We also thank GLA University Mathura, INDIA, for IR spectra and SAIF Punjab University, Chandigarh, PUNJAB, INDIA, for providing NMR (^{1}H and ^{13}C), Mass spectra, and Elemental analysis data.

7. ETHICAL STATEMENT

The release studies on which this study is based employed animals. The Department of Pharmaceutical Chemistry, Acharya Narendra Dev College of Pharmacy, Babhnan, Gonda UP-271313, India, conducted release studies on the synthesized conjugates, and CPCSEA approved their animal facility AND THE STUDY : 1585/PO/E/ 5/11/CPCSEA, Registration date: 23/12/2011, and Author permission IAEC approval Reference No: IAEC/ANDCP/3/2021. The Institutional Animal Ethics Committee has approved the experimental protocols for these studies.

8. AUTHORS CONTRIBUTION STATEMENT

Dr. Ashutosh Mishra conceptualized the study, and Mr. Anoop Kumar designed and prepared the final draft. Mr. Anoop Kumar gathered the data, reviewed the approach and methodology with Mishra Sir, and ran the analysis. The analysis of results, interpretation of data, and final article were all made by Mr. Anoop Kumar after discussing with Dr. Ashutosh Mishra. All authors have read and accepted the final version of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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