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Synthesis, Characterization and Release Studies of Mutual Prodrugs Of Norfloxacin and Trimethoprim With Indomethacin For Colon-Specific Drug Delivery

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ABSTRACT : *Prodrug approach is one of the most conventional concepts used for the treatment of several intestinal bowel diseases (IBD) such as crohn's disease, ulcerative colitis, and colon cancer etc. The present study aims towards formation of mutual amide prodrugs of norfloxacin and trimethoprim with indomethacin (IN and IT) by coupling method for targeted drug delivery to the inflamed gut tissue in IBD. Their physico-chemical properties were determined and their structures were supported and analyzed by FTIR, ^{1}H NMR and Mass spectroscopy. Release study of the synthesized derivatives was done in different simulated gastro-intestinal fluids to identify the expected hydrolysis of these amide conjugates in gastrointestinal tract. They were found chemically stable in simulated gastric fluid and remarkable release was observed in simulated colonic fluid, 57.46 % from IN and 62.75 % from IT, although, simulated intestinal fluid reported negligible release of drugs. So, the purpose of colon-targeting delivery of the drugs for treating infection as well as inflammation may be achieved.*

KEYWORDS: *Amide conjugates, colon-targeting, IBD, indomethacin, Norfloxacin, Trimethoprim.*

I. INTRODUCTION

NSAIDs are primarily absorbed in the stomach and antibiotics / antibacterial are also absorbed in the jejunum or distal ileum. Thus, the treatment of intestinal bowel disease (IBD) had ever been a great problem due to non availability of NSAIDs and antibacterial in the distal intestinal region. Prodrug design is a very important concept to solve this particular problem and as per the Lipinski rule of five, the drugs having the molecular weight lesser than 500 can be absorbed from the gastrointestinal tract [1]. Furthermore, the drug will also be highly hydrophobic and will come under the fourth class of Biopharmaceutical classification system (BCS) [2]. Barring sulphasalazine the other prodrugs could not be successful as colon targeted in the treatment of IBD. However, earlier various approaches have been applied to synthesize several prodrugs [3, 4]. The problem with all those prodrugs had been the selection of the drug candidates for their formation. Most of the time 5-aminosalicylic acid has been generated which has lower potential as compare to other anti-inflammatory drugs and it is not adequate to be used as such because it is absorbed extensively and rapidly through the upper intestine before it reaches to colon [5]. To overcome this mesalazine, basalazine and olsalazine etc. have also been synthesized which protects 5-ASA from acidic pH of stomach and prevents its absorption from upper part of the gut [6, 7], but none of them have been reported to reach to the market and their used is limited up to the clinical trials only due to several side effects produced by the carriers such as hepatotoxicity, severe blood disorders etc. Dose of such prodrugs is also quite high. Their molecular weight was under 500 and therefore, such prodrugs could not prove worth for the treatment of IBD.

Therefore, in present study we have selected indomethacin which is a highly potent analgesic with a high molecular weight. It is also used in the form of suppositories to treat the rectal inflammation. Thus, we can say it will be pertinent to use it with the antibacterial in the form of mutual prodrugs. We have selected norfloxacin because of the fact that it is very much useful for the treatment of bacterial dysentery. Trimethoprim will be good to be used as a mutual prodrug along with sulfasalazine and other recently synthesized prodrugs such as of NSAIDs viz. ibuprofen, diclofenac, and flurbiprofen with sulfonamides like sulphamethoxazole and sulphanilamide via amide linkage using DCC coupling reaction [8] and mutual prodrug of aceclofenac with amino acids [9]. The present study describes the formation of mutual prodrugs of norfloxacin and trimethoprim (antibacterial drugs) with indomethacin (NSAIDs) by masking free carboxylic acid group of the indomethacin by norfloxacin and trimethoprim via amide linkage through simple coupling process in order to reduce local

irritation as well as to reduce infection. Their physico-chemical properties were determined and spectral characterizations were carried out. The hydrolysis behaviour of the synthesized prodrugs in different simulated fluids over 24 h was also studied.

II. MATERIALS AND METHOD

Indomethacin was purchased from Loba Chemie, Mumbai. Norfloxacin and Trimethoprim were gifted by Vivek Pharmachem India Ltd. Jaipur and all other reagents were procured from E. Merck (India) Ltd. and were of analytical grade. TLC of the synthesized derivatives was carried out on precoated plates of silica gel (Merck) with chloroform: methanol: glacial acetic acid (4:4:2) as solvent system using iodine vapours and UV light as detecting agent for visualization. Melting points of the intermediate and the conjugates were determined by the open capillary method and uncorrected. The λ_{max} of the synthesized prodrugs was determined in chloroform on a Shimadzu 1700 UV double beam spectrophotometer. The IR spectra were recorded on Shimadzu FTIR in KBr pellet (anhydrous) at the University of Rajasthan, Jaipur. The H^1 NMR spectrum of the synthesized compounds was recorded in DMSO-d_6 with TMS is used as internal standard and the chemical shifts are recorded in δ ppm, using Bruker Avance II 400 NMR spectrometer, SAIF, Panjab University, Chandigarh. The molecular weights of compounds were determined from their Mass spectrum recorded at Jeol SX-102(FAB) Mass spectrometer, SAIF, Panjab University, Chandigarh.

2.1 Synthesis of conjugates of indomethacin with Norfloxacin (IN) and Trimethoprim (IT) [10, 11, 12]

2.1.1Preparation of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetyl chloride (acyl chloride of indomethacin):

Indomethacin (0.01 mol, 3.57 g) was first dissolved in 100 ml of chloroform and freshly distilled thionyl chloride (0.01 mol+20% excess, 0.9 ml) was added slowly with 1-2 drop of DMF as catalyst. The reaction mixture was refluxed for 3 h at 70-80 º C, until the evolution of hydrogen chloride and sulphur dioxide ceased. The excess of thionyl chloride and benzene were distilled off under reduced pressure, to give white crystalline product, m.p. 100-102 °C, yield 79 %

2.1.2 Coupling of acyl chloride of indomethacin with norfloxacin:

The weigh amount of norfllloxacin (0.01 mol, 3.19 g) was taken in about 5% ice cooled solution of NaOH (5 ml) and 60 ml acetone was added to it and kept at magnetic stirrer for 1 hr to dissolve it completely. The above mixture was then placed in a round bottom flask containing about 10 ml of pyridine and acyl chloride of indomethacin (0.01 mol, 3.76 g). The mixture was refluxed for 1 h at 100º C on water bath. After cooling, it was kept aside. After 24 h the mixture was poured into crushed ice to give precipitate, which was filtered off and washed several times with water. The crude white to light yellow color solid was then recrystallized from rectified alcohol to give IN.

2.1.3 Coupling of acyl chloride of indomethacin with trimethoprim:

Weigh amount of trimethoprim (0.01 mol, 2.90 g) was taken in round bottom flask containing about 10 ml of pyridine and acyl chloride of indomethacin (0.01 mol, 3.76 g). The mixture was refluxed for 1 h at 100º C on water bath. After cooling, the mixture was kept aside for 24 h and then poured into crushed ice. The resultant prodrug (IT) was obtained as a precipitate, which was filtered and then washed with water and recrystallized from rectified alcohol.

2.2 Hydrolysis studies of the synthesized prodrugs

The drug release studies of the synthesized conjugates were carried out by diffusion method in different simulated fluids, viz, simulated gastric fluid (SGF, pH 1.2), simulated jejunal fluid (SJF, pH 4.5), simulated intestinal fluid (SIF, pH 7.4) and simulated colonic fluids (SCF, pH 7.0) using dissolution test apparatus (Type 1). Weigh amount (10 mg) each of the synthesized prodrugs were gently introduced over the surface of 900 ml of SGF taken in separate baskets and were kept thermostatically controlled at 37 ± 0.5^{o}C [13, 14, 15]. About 5 ml aliquots were withdrawn at various time intervals and were extracted with equal amount of ether in order to remove the interference by free drugs. The aliquots were now estimated on UV spectrophotometer for the amount of IN and IT remaining. Parent drugs which were supposed to be released from the synthesized conjugates did not interfere in the absorption range of IN and IT, as is obvious from the differences in the λ_{max} values of indomethacin (319 nm), norfloxacin (274 nm) and trimethoprim (287 nm) with their prodrugs IN (293 nm) and IT (307 nm). The study in SGF was carried for a period of 2 hours and further release studies were carried out in SJF, SIF and SCF similarly as described above, for two hours, two hours and 18 hours respectively, retaining solid contents and the amount of prodrug remaining in aliquots were estimated on UV spectrophotometer.

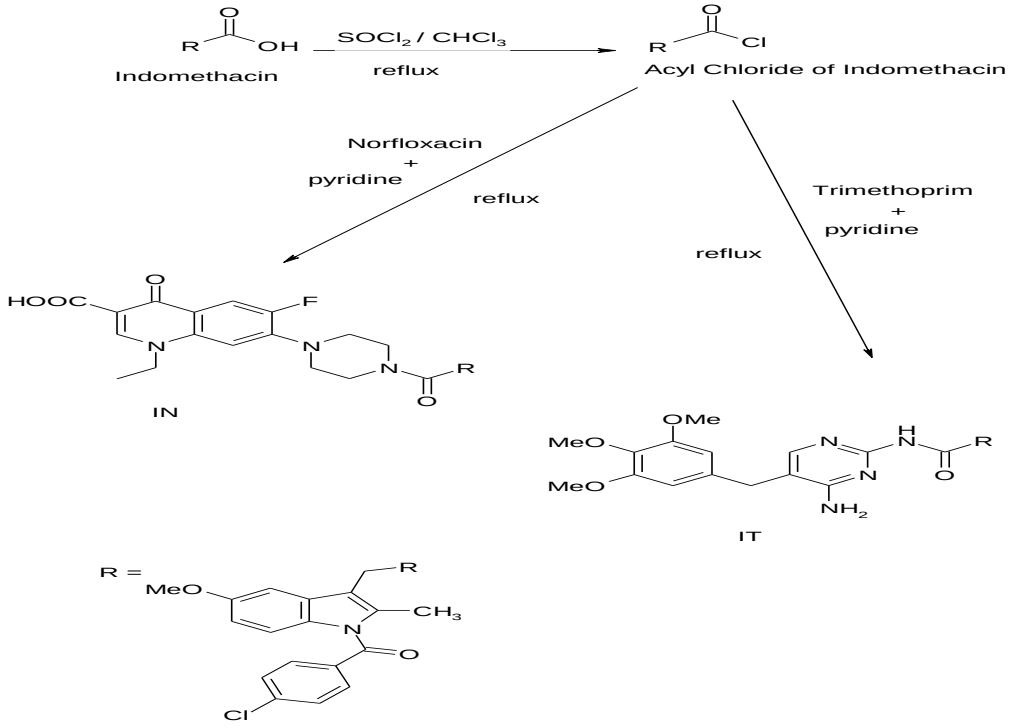

Figure 1: Scheme of synthesis of mutual amide prodrugs IN and IT

III. RESULTS AND DISCUSSION

Mutual amides prodrugs of indomethacin with norfloxacin and trimethoprim were synthesized by the scheme shown in Fig. 1 mentioned above. They were subjected to physico-chemical characterization, the data are shown in Table 1, and their structures were supported and confirmed by the FTIR, H^1 NMR and Mass spectroscopy as represented by Table 2. IR spectra of IN showed the characteristic absorption band for tertiary amide C=O stretching at 1643 cm^{-1} while IT showed –NH stretching at 3433 cm^{-1} and carboxyl stretching vibrations at 1672 cm^{-1}, thus confirmed the formation of amide bond in the synthesized prodrugs. The ^{1}H NMR spectra of the synthesized derivatives showed characteristic chemical shifts of the anticipated structures. The mass spectra showed the parent peak which confirmed the molecular weight of the synthesized prodrugs.

Table I: Physico-chemical properties of synthesized prodrugs

Code	*Chemical Formula*	*Molecular Weight*	*Elemental Analysis (%)*	*% Yield*	*Melting Point (°C)*	*R_f value*
IN	$C_{35}H_{32}ClFN_4O_6$	659.27	Calculated: C- 63.78; H, 4.89; Cl, 5.38; F, 2.88; N, 8.50; O, 14.56. Found: C, 63.13; H, 5.02; Cl, 5.27; F, 2.84; N, 8.38; O, 14.34	57	235-238 (melts with decomposition)	0.83
IT	$C_{33}H_{32}ClN_5O_6$	630.04	Calculated: C, 62.90; H, 5.12; Cl, 5.63; N, 11.11; O, 15.24. Found: C, 62.83; H, 5.06; Cl, 5.59; N, 10.98; O, 15.17	62	156-158	0.69

Table II: Spectral data of the synthesized mutual amide prodrugs

Compounds	*IR spectral data (cm^{-1})*	*^{1}H NMR spectral data (δ)*	*Mass spectral data $(M+H)^+$*
IN	3274 (carboxylic O-H str.), 3014 (aromatic C-H str.), 2997 (asym. C-H str. in methyl and methylene of ethyl and piperazine), 2842 (sym. C-H str. in methyl and methylene of ethyl and piperazine), 1720, 1687 (C=O str.), 1643 (C=O str. tert. amide), 1625 (C=O str. pyridone), 1473 (quinoline ring C-C and C-N str.), 1457 (O-CH_3 deformation of indole ring), 1241 (C-F and carboxylic C-O str.), 747 (C-Cl ?)	15.11 [S, 1H] OH- carboxylic acid, 8.22 [s, 1H] CH- pyridone, 7.32-7.66 [m, 4H] CH- (p-chlorobenzoyl), 7.10 [s, 1H], 5.83 [s, 1H] CH- benzene, 6.18-6.67 [m, 3H] CH- indole, 3.72 [s, 3H] –OCH_3, 3.86 [q, 2H] –N-CH_2-CH_3, 3.43-3.61 [m, 8H] -CH_2- piperazine ring, 3.28 [s, 2H] –CH_2-CO- , 2.21 [s, 3H] -CH_3 gp., 1.28 [t, 3H] –N-CH_2-CH_3	660
IT	3433 (N-H str. sec. amide), 3005 (aromatic C-H str.), 2966, 2935, 2837 (aliphatic C-H str.), 1672 (C=O str. sec. amide), 1507 (aromatic ring), 1477 (CH deformation), 1461 (O-CH_3 deformation of indole ring), 1239, 1138 (aromatic methoxy), 749 (C-Cl ?);	7.91 [s, 1H]-NH-CO-, 7.63-7.69 [m, 4H] CH- p-chlorobenzoyl, 7.49 [s, 1H] CH-pyrimidine, 6.56-7.10 [m, 3H] CH- indole, 6.15 [s, 2H] CH- benzene, 4.1 [b, 2H] –NH_2 gp., δ 3.75 [s, 3H]-OCH_3(C4), 3.73 [s, 6H] -OCH_3(C3, C5), 3.61 [s, 5H] –CH_2-, –OCH_3, 3.53 [s, 2H] –CH_2-CO- , 2.21 [s, 3H] -CH_3 gp.	631

The *in vitro* hydrolysis studies of the amide conjugates IN and IT indicates negligible release of parent drugs in SGF (pH 1.2) and SJF (pH 4.5), confirmed their stability in upper GIT which implying that they did not undergo hydrolysis and would be stable in the acidic pH of stomach. In simulated intestinal fluid, only 1.5 % and 4 % release of free drugs was observed for IN and IT, respectively. Thus, the objective of bypassing the upper GIT without any free drug release was achieved. The kinetics was further studied in SCF which indicates remarkable release of free drugs, 57.4 % from IN and 62.7 % from IT, as represented in Fig. 2

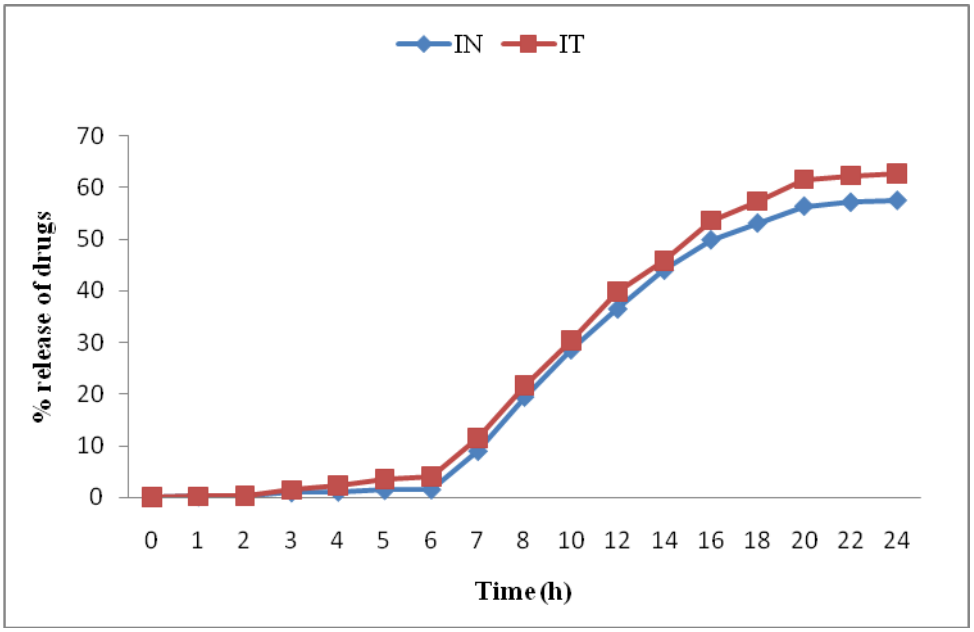

Figure 2: % release of drugs from IN and IT in different simulated fluids over 24 hrs

IV. CONCLUSION

The authors would like to conclude that the synthesized mutual prodrugs of indomethacin with norfloxacin and trimethoprim, through an amide linkage leads to a delivery system which is capable of releasing the drugs in colon without any appreciable release in upper GIT due to their higher molecular weight, however, they start releasing from prodrugs in distal intestinal region. As unwanted absorption of drugs from GIT is prevented, this will results in lowering of doses that will ultimately enhance the therapeutic utilization of drugs. The field is further open for the *in vivo* release studies of the synthesized prodrugs.

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