SYNTHESIS, CHARACTERIZATION AND RELEASE STUDIES OF MUTUAL PRODRUGS OF NORFLOXACIN AND TRIMETHOPRIM WITH ASPIRIN FOR COLON RELEASE

Arshi Hussain*, Pradeep Parashar¹, Anil Kumar Shrivastava²

*Department of Pharmaceutical Chemistry, School of Science, Suresh Gyan Vihar University, Jaipur-302001, Rajasthan, India

¹Department of chemistry, Govt. L.B.S P.G. College, Kotputli, Jaipur, Rajasthan, India

²Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda-271303, Uttar Pradesh, India.

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) and Antibiotics are commonly used for the treatment of Intestinal bowel diseases (IBD) such as ulcerative colitis, crohn’s disease etc. Prodrug designing is one of the several approach used to treat such colonic diseases. However, mutual prodrug concept also can be exploited for this purpose. In view of this context, mutual amide prodrugs of norfloxacin with aspirin (AN) and trimethoprim with aspirin (AT) were synthesized by coupling method. Their physico-chemical characterizations were carried out by analytical and spectral methods. In vitro release study of the synthesized derivatives was done in different simulated fluids to identify the expected hydrolysis of these mutual prodrugs in gastrointestinal tract. It was revealed that these compounds were chemically stable in simulated gastric fluid and an appreciable release of drugs was observed in simulated colonic fluid with 74.29 % from AN and 79.21 % from AT, although, simulated intestinal fluid reported a little release of drugs. So, the purpose of targeting the drugs to colon for treating infection as well as inflammation may be achieved.

Keywords: IBD, Mutual prodrugs, Norfloxacin, Trimethoprim, Aspirin, colon-targeting.
INTRODUCTION

Inflammatory bowel disease (IBD) involves chronic inflammation of all parts of digestive tract. IBD primarily includes ulcerative colitis and Crohn's disease.\[1, 2\] It can be painful and debilitating, and sometimes leads to life-threatening complications. This chronic condition is without a medical cure and commonly requires a lifetime of care. Each year in the United States, IBD accounts for more than 700,000 physician visits, 100,000 hospitalizations, and disability in 119,000 patients. Over the long term, up to 75% of patients with Crohn’s disease and 25% of those with ulcerative colitis will require surgery.

The exact cause of the IBD is not clearly understood yet, but it is believed that IBD is induced by the bacteria present in the colon and an infection always leads to inflammation.\[3, 4, 5\] To treat infection and inflammation, antibacterial / antibiotics and NSAIDs are used respectively, but both of them are primarily absorbed in the upper gastrointestinal tract. Thus, treatment of IBD had ever been a big problem due to non availability of drugs in colon. So, the main object of the hypothesis is to get control over bacterial population present in colon as well as the treatment of inflamed gut tissue which can be best accomplished by mutual prodrug concept, by the fact that although administered orally, the drugs are not absorbed in the gut, so the majority of the dose is delivered to the distal bowel and in addition, it undergoes reductive metabolism by colon bacteria, converting the prodrug into its active components.\[6, 7\] Therefore, antibacterial drugs can be coupled with NSAIDs as they may also be useful to overcome some side effects of NSAIDs particularly gastrointestinal irritation.\[8, 9\]

In the view of this background, the present study was conducted to design and synthesize mutual prodrugs of aspirin with antibacterial drugs namely norfloxacin and trimethoprim by masking free carboxylic acid group of the NSAIDs in order to reduce local irritation as well as to reduce infection by antibacterials via amide linkage through simple coupling process. Their chemical and spectral analysis was carried out. Further, the study of release behaviour of the prodrugs in different simulated fluids was also performed.

MATERIALS AND METHOD

Norfloxacin and Trimethoprim were gifted by Vivek Pharmachem India Ltd. Jaipur. Aspirin was purchased from Loba Chemie, Mumbai and all other reagents were procured from E. Merck (India) Ltd. and were of analytical grade. The $\lambda_{\text{max}}$ of the 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 H¹ NMR spectrum of the synthesized compounds was recorded in DMSO-d₆, 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.

**General procedure for preparation of mutual amide prodrugs:**¹⁰,¹¹,¹²

Synthesis of amide conjugates of aspirin with norfloxacin and trimethoprim was carried out by the formation of its acyl chloride followed by coupling with the given antibacterial drugs. The scheme of synthesis is depicted in Fig. 1 given below.

![Synthesis Scheme](image)

**Fig. 1: Scheme of synthesis of AN and AT**

(I is Aspirin, II is Acyl chloride of aspirin, III is Norfloxacin, IV is Trimethoprim)

**(a) Synthesis of mutual prodrug of Aspirin with Norfloxacin (AN)**

*Preparation of acyl chloride of aspirin (2-acetoxybenzoyl Chloride):* Aspirin (0.01 mol, 1.8 g) was first dissolved in 20 ml of sodium-dried benzene and freshly distilled thionyl chloride (0.01 mol+20% excess, 0.9 ml) was added slowly with a drop of DMF as catalyst. The reaction mixture was refluxed for 3 h at 70-80 °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.

*Coupling of 2-acetoxybenzoyl Chloride with norfloxacin:*¹³

Norfloxacin (0.01 mol, 3.19 g) was dissolved in about 60 ml acetone and then placed in a
round bottom flask containing about 10 ml of pyridine and 2-acetoxybenzoyl Chloride (0.01 mol, 1.99g). The mixture was refluxed for 1 h at 100 °C on water bath. After cooling, it was kept aside. After 24 h the mixture was poured into crushed ice and the resultant compound (AN) was obtained as a precipitate, which was filtered, washed with water and drained well. The crude white to light yellow conjugate was then recrystallized from rectified alcohol.

(b) **Synthesis of mutual prodrug of Aspirin with Trimethoprim (AT)**

Preparation of 2-acetoxybenzoyl chloride was done by the same procedure as described above. In next step, trimethoprim (0.01 mol, 2.90 g) was placed directly in round bottom flask containing about 10 ml of pyridine and 2-acetoxybenzoyl chloride (0.01 mol, 1.99g). The mixture was refluxed for 1 h at 100º C on water bath. After cooling the mixture, it was kept aside. After 24 h it was poured into crushed ice and the resultant prodrug was obtained as a precipitate, which was filtered and then washed with water. The product (AT) was purified by recrystallization from rectified alcohol.

**Release studies**

The ability of the prodrug to release the drugs in colon is tested in vitro by incubating it under conditions mimicking mouth-to-colon transit using dissolution test apparatus (Type 1).\(^{[14]}\)

The release study of the synthesized compounds was carried out by diffusion method in different 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). Simulated gastric fluid and Simulated Intestinal fluid were prepared as described in USP (2004). Simulated colonic fluid was prepared by using the formula as was used by Gliko-Kabir et al.\(^{[15]}\). About 10 mg each of the synthesized prodrugs were gently spread over the surface of 900 ml of SGF taken in two different baskets rotated at 100 rpm and were kept thermostatically controlled at 37 ± 0.5°C.\(^{[16, 17, 18]}\) Perfect sink condition was prevailed during the drug dissolution. The samples were withdrawn at intervals of 30 minutes, while first sample was withdrawn after an hour from the dissolution vessel and replaced with equal volume of SGF. The study was carried for a period of 2 hours. The withdrawn samples were assayed spectrophotometrically at 254 nm and 261 nm for the amount of AN and AT remaining, respectively. Free drugs which were supposed to be released by the synthesized prodrugs did not interfere with absorption of AN and AT because their λ\(_{max}\) were found to be substantially different from prodrugs.
The studies for release of drugs in SJF, SIF and SCF were carried out by similar method as described above for two hours, two hours and 18 hours respectively, retaining solid contents. The amounts of prodrug remaining in aliquots were estimated directly on UV spectrophotometer.

RESULTS AND DISCUSSION

The synthesis of amides conjugates of aspirin with norfloxacin and trimethoprim was achieved successfully. Characterization of the synthesized prodrugs were carried out, their physical properties are shown in Table 1. The formation of the prodrugs was confirmed by the FTIR, H$^1$ NMR and Mass spectroscopy. Infra-red spectroscopy of AN showed the characteristic absorption peaks at 3268 cm$^{-1}$ for carboxylic O-H stretching, 3054 cm$^{-1}$ for aromatic C-H stretching, 2994 cm$^{-1}$ for asym. C-H stretching in methyl and methylene of ethyl and piperazine, 2852 cm$^{-1}$ for sym. C-H stretching in methyl and methylene of ethyl and piperazine, 1649 cm$^{-1}$ for C=O stretching in tertiary amide, 1723 cm$^{-1}$ for C=O stretching in ester gp., 1629 cm$^{-1}$ for pyridone C=O stretching , 1479 cm$^{-1}$ for quinoline ring C-C and C-N str., 1241 cm$^{-1}$ for C-F and carboxylic C-O stretching. AT also showed absorption bands at 3469 cm$^{-1}$ for asym. N-H str. in primary amino gp., 3318 cm$^{-1}$ for sec. amide N-H stretching, 3123 cm$^{-1}$ for N-H str. amino gp.), 3012 cm$^{-1}$ for aromatic C-H stretching, 1506 cm$^{-1}$ for aromatic ring, 1235 cm$^{-1}$ and 1126 cm$^{-1}$ for aromatic methoxy.

The $^1$H NMR spectra of AN showed chemical shifts for protons at $\delta$ 15.1 [ s, 1H] OH gp. carboxylic, $\delta$ 8.8 [s, 1H] CH- pyridone, $\delta$ 8.10 [ s, 1H], $\delta$ 7.87 [ s, 1H], $\delta$ 7.27 [s, 2H] CH- O-acetoxybenzoyl, $\delta$ 7.11 [d, 1H], $\delta$ 6.83 [ d, 1H] CH- benzene, $\delta$4.59 [q, 2H]–N-CH$_2$-CH$_3$, $\delta$ 3.36-3.66 [m, 8H] CH$_2$- piperazine ring, $\delta$ 2.08 [s, 3H] –O-CO-CH$_3$, $\delta$ 1.42 [t, 3H] –N-CH$_2$-CH$_3$. $^1$H NMR spectra of AT showed chemical shift peaks for protons at $\delta$ 7.82 [s, 1H] of -NH-CO-, $\delta$ 7.53 [s, 1H] $\delta$ 6.56 [s, 1H], $\delta$ 6.14 [s, 2H] of O-acetoxybenzoyl, $\delta$ 6.56 [s, 1H] of CH- pyrimidine, $\delta$ 5.7 [s, 2H] of CH- benzene, $\delta$ 3.53 [s, 2H] of –NH$_2$ gp., $\delta$ 3.40 [s, 2H] of –CH$_3$- gp., $\delta$ 3.72 [s, 6H] of –OCH$_3$ gp., $\delta$ 3.62 [s, 3H] of –OCH$_3$ gp., $\delta$ 2.08 [s, 3H]- of -O-CO-CH$_3$. The mass spectra for AN and AT showed parent peak (m/z) at 481 and 452 respectively, thus confirming the molecular weight of the synthesized compounds.
Table 1: Physical properties of synthesized mutual prodrugs

<table>
<thead>
<tr>
<th>Mutual Prodrugs</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>% Yield</th>
<th>Description</th>
<th>Melting Point (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;F</td>
<td>481.47</td>
<td>59%</td>
<td>Wight-brown Crystals</td>
<td>310-312 (melts with decomposition)</td>
</tr>
<tr>
<td>AT</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>452.46</td>
<td>64%</td>
<td>White crystals</td>
<td>211-213</td>
</tr>
</tbody>
</table>

The hydrolysis studies of the synthesized amide conjugates AN and AT confirmed that they were stable and indicates negligible release of parent drugs in SGF (1.2 pH) and SJF (4.5 pH), implying that they did not undergo hydrolysis and would be stable in the acidic pH of stomach and in SIF (7.4 pH), about 7.63 % and 10.32 % release was observed, respectively. Thus, the objective of bypassing the upper GIT without any free drug release was achieved. To confirm the colonic hydrolysis of the synthesized prodrugs, the kinetics was further studied in SCF (7.0 pH) which indicates appreciable release of free drugs from the prodrug, with % release of 74.29 from AN and 79.21% from AT as shown in Fig. 2 due to enzymatic hydrolysis of prodrugs in SCF.

**CONCLUSION**

From the present studies, it can be concluded that the formation of mutual prodrugs of aspirin and antibacterial drugs certainly improves the drug delivery in the intestinal region. Release studies suggest that drugs start releasing from prodrug in the distal intestinal region and an appreciable release was observed in colon. Thus, anti-inflammatory action of aspirin can be capitalized and the antibacterial drug is also not absorbed due to its higher molecular weight.
from GIT. As a result the prodrug and released drugs remain in the GIT only. Abolition of unwanted absorption of drugs will lower the doses, thus increasing in the therapeutic utilization of drugs. However, further in vivo studies are required, which will be considered only after the permission of IAEC, Suresh Gyan Vihar University.

ACKNOWLEDGEMENT
The authors are thankful to Suresh Gyan Vihar University, Jaipur for providing the facilities to carry out this research work. The authors wish to thank Vivek Pharmachem India Ltd., Jaipur for providing the gift samples of the required drugs; The Head, Department of Chemistry, University of Rajasthan, Jaipur for carrying out IR studies; The Director, SAIF, Panjab University, Chandigarh, for H\textsuperscript{1} NMR, C\textsuperscript{13} NMR and mass spectral data.

REFERENCES


