www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Synthesis and Evaluation of Azo Prodrugs of Mefenamic Acid as Enzyme Specific Drug Delivery system for Ulcerative Colitis.

Shweta Gogate[1*], Vishal Gupta[1]

1. **Mansarovar Global University, Bilkisganj, Sehore, M.P.**

Corresponding mail id: Shweta Gogate**,** Mansarovar Global University, Bilkisganj, Sehore, M.P.
gogateshweta@yahoo.com

Abstract:

Ordinary treatment of IBD requires frequent intake of anti-inflammatory drugs at higher doses. Most of these drugs are rapidly absorbed from small intestine with very small fraction actually reaching the site of action i.e. colon. Interaction with non-targeted sites leads to significant adverse effects. Therefore, out of the need to overcome this formidable barrier of GIT, colon-targeted delivery has evolved as an ideal drug delivery system for the topical treatment of local diseases of colon like inflammatory bowel disease. Minimizing drug-induced side effects and mortality are the main challenges during management of IBD. Prodrug approach is one of the important approaches for targeting drugs to colon. Prodrug design has paved a way to overcome the undesirable properties associated with the existing drug and successful site-specific drug delivery to varied organs and tissues. Colon-specific drug delivery through colon-specific prodrug activation may be accomplished by the utilization of high activity of certain enzymes at the target site relative to non-target tissues for prodrug to drug conversion. For the present studies mefenamic acid was selected because of being curative agents for most prevalent colon disease namely intestinal bowel disease due to any reason. At present there is no effective anti-inflammatory agent is available. Anti-inflammatory therapy, at present, involves use of corticosteroids, as all NSAIDs are absorbed in the stomach and they do not reach to colon. Most of the NSAIDs have free carboxylic acid groups, although, it is important for their activity but they can be targeted to colon via formation of mutual prodrugs (azo and amide). Hydrolytic enzymes of stomach to ileum do not hydrolyze such mutual prodrugs. Absorption of the NSAIDs primarily takes place in the stomach and followed with jejunum due to lipophilicity of the unionized form. Thus, they do not reach to the colon and also ulcerogenic which can also be avoided by formation of their mutual prodrugs.

Keywords: Mefenamic acid, Colon-specific drug delivery, Inflammatory bowel disease, Prodrugs, Ulcerative colitis, Amino acids.

www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Introduction:

Ulcerative colitis is a chronic gastrointestinal disorder that is limited to the large bowel (the colon). It does not affect all layers of the bowel, but only affects the top layers of the colon in an even and continuous distribution. The first symptom of ulcerative colitis is a progressive loosening of the stool. The stool is generally bloody and may be associated with cramping abdominal pain and severe urgency to have a bowel movement. In addition, there may be loss of weight, anemia, skin lesions, joint pain, eye inflammation, and liver disorders occur. Thus, an effective and safe therapy of these colonic disorders, using site-specific drug delivery systems is a challenging task to the pharmaceutical technologists in the field of drug development. One of the most important strategies used for this purpose is using prodrug approach to deliver the drug in colon. Thus, it is worthy to discuss both the concepts, viz, prodrug and colon targeting.[1,2]

IBD management often requires long-term treatment based on a combination of drugs to control the disease. Most people with mild or moderate UC are treated with corticosteroids like dexamethasone to reduce inflammation and relieve symptoms. Other drugs such as immunomodulators that reduce inflammation by affecting the immune system and aminosalicylates are available. 5- aminosalicylic acid (5-ASA) **and** corticosteroids are used as first-line therapy of IBD. Azathioprine, 6-mercaptopurine, methotrexate, calcineurin inhibitors and anti-TNF-α-antibodies have an important role in the treatment of severe disease stages.[3,4]

Prodrugs are pharmacologically inactive molecules of an active drug molecule that, prior to exerting a pharmacological effect, require an enzymatic and/or chemical transformation to release the active parent drug *in vivo.* Prodrugs can be used to bypass physicochemical, pharmaceutical, pharmacokinetic and pharmacodynamic barriers to drug formulation and delivery, such as poor aqueous solubility, chemical in stability, insufficient oral absorption, rapid presystemic metabolism, inadequate tissue penetration, toxicity and local irritation.[5]

Colon targeted drug delivery system:

The oral route is considered to be most convenient for administration of drugs to patients. Oral administration of conventional dosage forms normally dissolves in the stomach fluid or intestinal fluid and absorb from these regions of the GIT depends upon the physicochemical properties of the drug. It is a serious drawback in conditions where localized delivery of the drugs in the colon is required or in conditions where a drug needs to be protected from the hostile environment of upper

GIT. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, crohn's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. Also, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Apart from retarding and targeting dosage forms, a reliable colonic drug delivery could also be an important starting position for the colonic absorption of perorally applied, undigested, unchanged and fully active peptide drugs. The presence of colonic microflora (enterobacteria) that is responsible for specific enzymatic activity. The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substances such as carbohydrates and proteins that escape the digestion in the upper GI tract.There are many attributes of colon that can be explored and exploited for site-specific delivery of drugs such as: Less hostile environment., Near neutral pH, Less diversity and intensity of enzymatic activities than stomach and small intestine, Long colonic transit (20-30 h) for extended absorption window, Highly responsive to absorption enhancers, Unique microbial flora and enzymes, Minimized systemic exposure of drugs, Reduced risk of first-pass metabolism, More chances of drug being available in its effective concentration, Lower dosing and prevalence of systemic side effects abd attractive site for drugs which are hydrophilic or poorly absorbed from upper GIT . Colon-specific delivery system can be designed for drug candidates that are intended for the treatment of the local diseases of colon like IBD, IBS, colorectal cancer, diarrhea/constipation and intestinal infections (amoebiasis) with an aim of increasing the potency and decreasing their systemic side effects.[6,7]

Evaluation Techniques for CDDS

i) In vitro dissolution test:

Dissolution of controlled-release formulations employed for colon-specific drug delivery are mainly hard, and the dissolution techniques described in the USP cannot fully imitate in-vivo situation such as those relating to bacterial environment, pH and mixing forces. Dissolution tests describing to CDDS may be carried out using the conservative basket method. Parallel dissolution studies in diverse buffers may be undertaken to distinguish the behaviour of formulations at different pH levels. Dissolution tests of a colon- specific formulation in different media simulating pH circumstances and times likely to be stumble upon at different locations in the gastrointestinal tract have been studied. The media chosen were examined to simulate gastric fluid, pH 6.8 to

simulate the jejunal region of the small intestine, and Ph 7.2 to simulate the ileum segment. Enteric- coated capsules for CDDS have been examined in a gradient dissolution study in three buffers.[8,9]

ii) In vitro enzymatic tests:

Incubation of carrier drug system in fermenter holding appropriate medium for bacteria (B. ovatus and Strectococcus faccium).The quantity of drug produced at dissimilar time intervals are determined. Drug release study is completed in buffermedium containing enzymes (dextranase, ezypectinase), or rat or guinea pig or rabbit cecal contents. The quantity of drug produced in a particular time is done, which is directly proportional to rate of deprivation of polymer carrier.[10,11]

iii) In vivo evaluation:

A number of animals such as guinea pigs, rats, dogs, and pigs are used for screening the delivery of drug to colon because they look like the anatomic and physiological circumstances as well as the microflora of human GIT. While deciding a model for testing the CDDS, comparative model for the colonic diseases should also be measured. Guinea pigs are mainely used for experimental IBD model. The distribution of azoreductase and glucouronidase potential in the GIT of rat and rabbit is fairly equivalent to that in the human.[12]

Methodology:

Melting Point Determination:

The melting points of the drug and the synthesized conjugates were determined by open capillary tube using Toshniwal Melting Point Apparatus and errors are uncorrected.[13,14]

Thin Layer Chromatography:

The purity of the synthesized derivatives was ensured by subjecting to thin layer chromatography. It was carried out on silica gel precoated plates of Merck with acetone: chloroform: acetic acid (3:2:1). as solvent system used for prodrugs and iodine vapours and UV light were used as detecting agent for visualization.[15]

Spectroscopic Methods for Characterization of Synthesized Derivatives

The synthesized compounds were analyzed and their structures were supported and corroborated by spectroscopic analyses viz., FTIR and NMR.[16]

Partition coefficient determination:

Partition coefficient was determined in octanol/ phosphate buffer (pH 7.4) at 37± 1ºC . n-Octanol and water were mutually saturated with each other prior to use. A prodrug (10 mg) was dissolved in n-octanol (10 mL) and 10 mL distilled water was slowly added to it and the octanol-

water mixture was shaken for 24 h on a wrist shaker to reach distribution equilibrium. The two layers were separated by separating funnel and aqueous layer was estimated by JascoV-530, UV- Visible double beam spectrophotometer at pre-determined λmax.[17]

Synthesis of azo conjugates of NSAIDs with amino acids:

Esterification of amino acids.

Synthesis of diazonium salt of amino acids.

Coupling of diazotised salt of amino acids with mefenamic acid.

Procedure Synthesis of methyl ester hydrochlorides of amino acids:

Freshly distilled (0.05 M, 6 ml) of thionyl chloride was slowly added to methanol (100 ml) with cooling and amino acid (0.1 M) was added to it. The mixture was refluxed for 6-8 h at 60-70ºC with continuous stirring on magnetic stirrer. Excess thionyl chloride and solvent was removed under reduced pressure giving crude amino acid methyl ester hydrochloride. It was treated with 20 ml portion of cold ether at 0ºC until the excess of dimethyl sulphate was removed. The resulting solid product was collected and dried under vacuum.It was recrystallized from hot methanol by slow addition of 15-20 ml ether followed by cooling at 0ºC. The crystals were collected on next day and washed twice with ether- methanol mixture (5:1) followed by pure ether and dried under vacuum.[18,19]

Diazotization of amino acid :

Amino acid ester (0.01 mol) was dissolved in a suitable volume of water containing 2.5-3 equivalents of hydrochloric acid (0.02 mol; 1.7 mL of 35% HCl), by the application of heat (if necessary) and then solution was cooled in ice. The temperature was maintained at 0-50C on a cryostatic bath and an aqueous solution of sodium nitrite (2 mol, 1.4 g in 10 mL) was added (portion wise), through syringe with complete assurance that the tip of the syringe was always dipped completely in the solution. The addition of sodium nitrite solution was continued till the solution gave an immediate positive test for excess of nitrous acid with an external indicator i.e. moist potassium iodide-starch paper. The precipitated amino acid, if any, got dissolved during the diazotization to give a clear solution of the highly soluble diazonium salt. To stabilize the diazonium salt and to minimize secondary reactions (proper condition of acidity was maintained throughout) by adding excess of acid (0.5-1 equivalents). The reaction mixture was kept in cryostatic bath at 0-50C during the course of reaction (which is exothermic in nature), in order to avoid the hydrolysis of diazonium salt.[20,21]

Coupling of diazotised salt of amino acid with mefenamic acid:

Mefenamic acid (0.01 mol) was completely dissolved in sodium hydroxide solution (2 mol; 0.08g/mL). The solution was then cooled at below 50C. Then slowly diazotised salt of Amino acid was added with continuous stirring, through syringe. Alkaline condition was constantly maintained. After completing the reaction, water was evaporated and crude product was recovered. It was recrystallized by dissolving in methanol and cooling at 00C. Purified product was dried under vacuum. The reaction was monitored by TLC .[22]

In vitro release studies of synthesized mutual prodrugs

Release studies of drug in SGF at pH 1.2

The mutual prodrugs were *in vitro* studied for the release of drugs in SGF using dissolution test apparatus I described in U.S.P. XXIV. Accurately weigh amount of mutual prodrug (10 mg) was kindly spread over the surface of 900 ml of SGF taken in basket and the contents were rotated at 100 rpm and were kept thermostatically controlled at 37 ± 0.5oC as specified in the I.P. Perfect sink condition was maintained during the dissolution of drug. 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 aliquots were now estimated spectrophotometrically. The study in SGF was carried for a period of 2 hours. Similar procedure is followed for in vitro release study in intestinal and colonic fluids.[23]

Pharmacological screening of drugs and prodrugs:

Drugs as well as the synthesized prodrugs were evaluated for anti inflammatory activity, analgesic activity, ulcerogenicity and histopathology. A comparative study between pharmacological activities of parent drug and their prodrugs were performed.[24]

Anti Inflammatory Activity:

In the present study, the anti-inflammatory activity of drugs and prodrugs were determined by hind paw oedema method using carrageenan (0.1 ml, 1 % w/v) as phlogistic agent. Wistar albino rats (150-200 g) were divided into groups, each comprising of six animals, including a control and a standard group. The initial volume of right hind paw of rat was measured by plethysmometer without administration of drug. The 1 % sodium carboxy methyl cellulose (CMC) suspension containing drug (100 mg) was prepared and a volume of this suspension containing an equivalent dose (Mefenamic acid-50 mg/kg/body wt) was administered orally to the standard groups. Similarly equivalent quantity of each prodrug was administered to the test groups. After 30 min

of administration of the drug or prodrugs, carrageenan solution in normal saline was injected into the planter surface of right hind paw of each animal. The volume of swelling of right hind paw of each rat was measured after 0.5, 1, 2, 4 and 6 h. The mean increase in the volume of the right hind paw of rats was compared with control and standard. The percent inhibition of paw oedema was calculated.[25,26]

Analgesic Activity:

The analgesic activity of the drugs and prodrugs was determined by thermal stimulus using tail flick method. Analgesiometer was used for the determination of pain threshold of albino rat. Cold water was circulated through the water jackets of the instrument to avoid heating of the area around the hot wire. Rats were divided into groups, each comprising six animals, including a control and standard group. The mice were placed in a holder through which the tail of the animal protruded out. The normal reaction time, i.e., the time taken to flick the tail was noted at 0.5, 1, 2, 3 and 4 h after the treatment and cut-off time was 9 s. The animals, which showed significant (above 9 s) delayed response, were rejected. 1 % sodium carboxy methyl cellulose (CMC) suspension containing drug (100 mg) was prepared and a volume of this suspension containing an equivalent dose (Mefenamic acid-50 mg/kg/body wt) was administered orally to the standard groups. Similarly equivalent quantity of prodrugs was administered to the test groups. Percentage analgesia was calculated.[27]

Ulcerogenic Activity:

Gastrointestinal toxicity of the drugs and prodrugs was measured and compared with the parent drug by measuring mean ulcer index. Wistar albino rats were divided into groups, each comprising six animals, including a control and standard group. The control group was administered orally by 2 % acacia suspension. Test compounds and standard were administered orally (at 10 times higher dose) as a suspension with 2 % acacia daily for 5 days. The rats were fasted after the administration of last dose, thereafter they were sacrificed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. Ulcers greater than 0.5 mm were recorded. The mean ulcer index (UI) was calculated by severity of gastric mucosal lesions which are graded as grade 1: less than1 mm erosions, grade 2: 1-2 mm erosions and grade 3: more than 2 mm erosions. The UI was calculated.[28]

TNBS induced experimental colitis model:

www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Induction of Colitis:

Rats were fasted for 24 h before experimentation. Rats were lightly anesthetized with ketamine and xylazine (20mg/kg and 5mg/kg, i.m.). A polyethylene catheter with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm. For ulcerative colitis induction, TNBS dose was 150 mg/kg of body weight of TNBS in ethanol, 50% solution) was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec. The catheter was left in place for few seconds then gently removed. For 3 days the rats were housed without treatment to maintain the development of a full inflammatory bowel disease model with full access of food and water *ad libitum*. The animals of standard and test groups received orally sulfasalazine and prodrugs respectively, once daily for five continuous days. The normal control and colitis control groups received only 1% carboxy methylcellulose instead of free drug or prodrug.[29,30]

Assessment of colonic damage by clinical activity score:

The animals of all groups were examined for weight loss, stool consistency and rectal bleeding throughout the 11 days study. Colitis activity was quantified with a clinical activity score assessing these parameters as previously applied. The clinical activity score was determined by calculating the average of the above three parameters for each day, for each group and was ranging from 0 (healthy) to 4 (maximal activity of colitis). They were sacrificed 24 h after the last drug administration for study.[31]

Result and discussion:

Characterization of azo prodrugs:

Table1: Physicochemical characterization prodrugs

Prodrug	Colour	Melting point (ºC)	Yield (%)	Rf value	Partition coefficient
MAZ1	Off white	158-159	74	0.56	0.76
MAZ2	Creamy white	168-169	75	0.64	0.74
MAZ3	Off white	172-175	68	0.58	0.75

MAZ4	Yellowish white	179-182	79	0.67	0.67

Spectral data of azo prodrugs of mefenamic acid :

i) Spectral data of azo prodrug of mefenamic acid-Isoleucine (MAZ1)

IR (KBr, cm-1): 3476 (NH str.), 2981 and 2862 (CH str.), 3021(CH str. of aromatic ring), 1705 (CO str. of ester), 1607, 1573, 1455 and 1410(C=C of aromatic ring), 1490 (N=N str.). 1248 (OCH3), 753 (1,2-ortho disubstituted); Single spot TLC data of synthesized compound along with NMR values assured the purity of synthesized prodrug.

^{1}H NMR (DMSO- *d*6, 400 MHz, δ) 8.28-8.22 (m, 4H, aromatic ring), 7.39-7.19 (d, 3H, CH in ring), 4.8 (s, 1H, NH), 3.62 (s, 3H of OCH_3), 3.58(s, 1H of CH) 2.47(s, 3H, of CH_3), 1.37 (d, 2H of CH_2).

ii) Spectral data of azo prodrug of mefenamic acid-Cysteine (MAZ2)

IR (KBr, cm-1): 3437 (NH str.), 2921 and 2810 (CH str.), 3012(CH str. of aromatic ring), 2551(SH str.), 1697 (CO str. of ester), 1612, 1586, 1490 and 1412(C=C of aromatic ring), 1494 (N=N str.), 1267 (OCH3), 746 (1,2-ortho, disubstituted);

1H NMR (δ, ppm) (DMSO): 8.52-7.26(m, 4H, aromatic ring), 7.25-7.13 (t, 3H, CH in ring), 4.80 (1H, NH in ring), 3.61 (t,1H, SH), 2.47 (s, 3H of OCH_3), 1.83(s, 1H of CH) 1.81(s, 3H, of CH_3), 1.37 (d, 2H of CH_2).

iii) Spectral data of azo prodrug of mefenamic acid-Glutamic acid (MAZ3)

IR (KBr, cm-1): 3412 (NH str.), 3011(CH str. of aromatic ring), 2919 and 2850 (CH str.), 1711 (CO str. of ester), 1612, 1586, 1460 and 1412(C=C of aromatic ring), 1505 (N=N str.), 1265 (OCH_3), 753 (1,2-ortho , disubstituted); Single spot TLC data of synthesized compound along with NMR values assured the purity of synthesized prodrug.

1H NMR (δ, ppm) (DMSO): 8.13-8.03(m, 4H, aromatic ring), 7.99-7.11 (t, 3H, CH in ring), 4.70 (1H, NH in ring), 3.59 (s, 3H of OCH3), 2.39(s, 1H of CH) 1.76(s, 3H, of CH_3), 1.36 (d, 2H of CH_2).

iv) Spectral data of azo prodrug of mefenamic acid-Aspartic acid (MAZ4)

IR (KBr, cm-1): 3413 (NH str.), 3018(CH str. of aromatic ring), 2949 and 2880 (CH str.), 1708

www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

(CO str. of ester), 1612, 1576, 1490 and 1452(C=C of aromatic ring), 1510 (N=N str.), 1262 (OCH_3), 758 (1,2-ortho , disubstituted); Single spot TLC data of synthesized compound along with NMR values assured the purity of synthesized prodrug.

1H NMR (δ, ppm) (DMSO): 8.16-8.03(m, 4H, aromatic ring), 7.99-7.23 (t, 3H, CH in ring), 4.80 (1H, NH in ring), 3.58 (s, 3H of OCH_3), 2.39(s, 1H of CH) 1.764(s, 3H, of CH_3), 1.37 (d, 2H of CH2).

Hydrolysis studies of mefenamic acid prodrugs:

Table2 : Percentage release of drug on hydrolysis in SIF

Time(h)	Azo Prodrug Hydrolyzed in SIF (%)			
	MAZ1	MAZ2	MAZ3	MAZ4
15	0.00	0.00	0.00	0.00
30	1.11	1.12	1.14	1.03
45	1.54	1.50	1.50	1.42
60	2.17	2.19	2.23	2.41
75	3.23	3.29	3.39	3.22
90	3.37	3.79	3.53	3.81
105	4.03	4.52	4.43	4.65
120	4.61	4.74	4.78	4.81
240	5.13	5.24	5.17	4.94
360	5.17	5.33	5.36	5.02

Table 3: Percentage release of drug on hydrolysis in rat fecal matter

Time (min)	% Drug Released			
	MAZ1	MAZ2	MAZ3	MAZ4
15	0.00	0.00	0.00	0.00
30	12.7	15.2	13.2	16.9
45	23.4	23.9	24.9	28.6
60	37.8	38.8	38.7	41.3
75	44.9	45.3	45.8	49.8
90	53.2	54.2	52.7	59.2
105	66.9	67.1	69.2	69.7
120	71.8	71.9	72.7	76.4
240	81.5	82.5	78.2	86.6
360	96.6	96.8	97.5	99.4

www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Pharmacological study of mefenamic acid prodrugs:

i) Anti-Inflammatory Activity

Table4: Anti-inflammatory activity of azo prodrugs

Group	Treatment	Percentage anti-inflammatory activity				
		0.5 hr	1 hr	2 hr	4 hr	6 hr
I	Normal % CMC	nil	nil	nil	nil	nil
II	Mefenamic acid	48.0 ± 1.1	62.0 ± 1.2	60.6 ± 2.1	56.1 ± 1.2	42.3 ± 1.5
III	Sulfasalazine	42.0 ± 1.2	50.1 ± 1.0	59.0 ± 1.3	68.1 ± 2.3	72.4 ± 1.2
IV	MAZ1	58.3 ±1.6	60.1 ±1.3	62.22 ±1.5	68.1 ±1.3	69.4 ±1.2
V	MAZ2	55.1 ±1.0	56.4 ±2.0	57.77 ±1.3	59.7 ±1.1	72.4 ±1.7
VI	MAZ3	61.4 ±1.1	63.0 ±1.3	64.44 ±1.1	72.9 ±1.2	74.0 ±1.2
VII	MAZ4	62.0 ±1.4	64.0 ±2.0	71.0 ±1.8	78.1 ±1.5	81.2 ±1.3

Analgesic Activity:

Table 5: Analgesic activity of azo prodrugs

Group	Prodrug	Analgesic activity (%)				
		0.5 h	1 h	2 h	3 h	4 h
I	Normal Control	-	-	-	-	-
II	MA	52.0 ± 1.4	70.1 ±1.1	82.2 ±1.3	72.1 ±1.1	71.0 ±1.3
III	MAZ1	32.1 ±1.0	38.3 ±1.0	46.2 ±1.1	53.0 ±1.0	77.4 ±1.0
IV	MAZ2	31.2 ±1.1	35.0 ±2.9	43.2 ±2.5	67.1 ±1.7	74.7 ±1.0
V	MAZ3	33.0 ±1.3	36.2 ±2.0	45.3 ±2.0	54.8 ±1.0	76.6 ±1.4
VI	MAZ4	30.3 ±1.0	31.4 ±2.4	38.1 ±2.5	46.7 ±1.6	86.7 ±1.2

Ulcerogenic Activity:

The ulcer index of the prodrugs was recorded to observe the extent of gastrointestinal side

www.ijbar.org
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

effects and the mean ulcer index was determined

Table 6 : Results of ulcerogenic activity:

Compound	Ulcer index ± S.D.
Normal Control	0.6 ± 0.12
Diseases Control	28.4 ± 1.6
Standard (Sulfasalazine)	5.4 ± 0.15
Mefenamic acid	45.6 ± 1.8
MAZ1	5.9 ± 0.14
MAZ2	5..6 ± 0.4
MAZ3	4.7 ±0.87
MAZ4	4.5 ± 0.12

Table 7: Clinical activity score rate

GROUPS	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
HC	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00
DC	0±00	0.7±1.3	1±1.73	1.6±1.5	1.6±1.5	1.8±1.7	3.1±1.0	3.2±1.0	3.3±1.1	3.3±1.1	3.33±1.15
Mefenamic acid	0±00	0.6±1.1	1.0±1.7	1.6±1.5	2±1.73	2.7±1.3	3.0±1.0	2.3±0.5	1.9±0.6	1.3±1.1	0.99±1.1
SLZ	0±00	0.3±0.6	0.8±0.9	1.8±0.8	2.7±0.6	2.8±0.8	2.4±0.5	1.6±1.13	1.1±1.0	0.7±0.75	0.38±0.6
MAZ1	0±00	0.7±0.1	1.2±0.3	1.7±0.1	2.3±0.2	2.5±0.3	2.8±0.1	2.4±0.3	1.9±0.2	1.3±0.3	0.6±0.2
MAZ2	0±00	0.6±0.1	1.2±0.3	1.6±0.5	2.2±0.5	2.6±0.3	2.6±0.5	2.5±0.3	2.1±0.5	1.5±0.4	0.5±0.17
MAZ3	0±00	0.7±1.8	1.2±1.5	1.7±0.8	2.7±0.6	2.7±1.1	2.3±0.9	2.0±0.86	1.6±0.6	1.3±0.75	0.4±1.2
MAZ4	0±00	0.6±1.2	1.3±0.8	2.0±0.9	2.6±0.6	2.7±0.8	2.4±1	2.0±0.6	1.4±0.8	0.8±0.5	0.3±1.1

The mean ulcer index of standard drug Mefenamic acid was found to be more than prodrugs. The minimized side effect obtained in the prodrugs might be due to the inhibition of direct contact of carboxylic acid group of the drug to the gastric mucosa which is mainly responsible for the damage.

TNBS-induced experimental colitis model:

i) Assessment of colonic damage by clinical activity score rate

The animals of all groups were examined for three quantifying parameters weight loss, stool consistency and rectal bleeding daily at a specified time throughout the 11 days study. Depending on severity of inflammation, a score was assigned to each parameter on each day The clinical activity score rate was determined by calculating the average of the above three parameters for each day, for each group and was ranging from 0 (healthy) to 4 (maximal activity of colitis)**.**

Conclusion:

Prodrug design concept is a part of drug discovery process which was initiated for improving drug therapy, in which a unique substance is created to have desirable pharmacokinetic characters in order to optimize pharmacologically potent structures which ultimately lead to the design of better drugs. As most of the drugs are absorbed in the upper gastro-intestinal tract like NSAIDs which are primarily absorbed in the stomach ,the treatment of IBD had ever been a great problem due to non availability of these drugs in the distal intestinal region. In the present research, it was envisaged to synthesize mutual prodrugs of NSAIDs with amino acids to deliver them effectively to colon without their absorption at upper part of GIT. This concept will not only target the drugs to colon but also avoid gastric irritation and will maximize the therapeutic availability that will ultimately result in lowering of the doses. The therapeutic efficacy of the synthesized prodrugs was studied on pre-existing TNBS- induced experimental colitis model in Wistar rats. The anti-colitic activity of prodrugs was compared with standards SLZ and mefenamic acid . The results of pharmacological screening revealed that the ulcer indices of prodrugs is profoundly lower than mefenamic acid. And also maximum concentration of drug is reached to colon This indicates that the synthesized prodrugs have a very low potential of causing ulcers with high efficacy.

References

1. Ajayi B. O., Adedara I. A., Farombi E. O. (2015). *Phytotherapy Research*, 29(4), 566–572.
2. Philip A. K., Philip B. (2010). *Oman Medical Journal*, 25(2), 70–77.
3. Ashford M., Fell J. T., Attwood D., Sharma H., Woodhead P. (1994). *Journal of Controlled Release*, 30, 225–232.

4. Azad K., Truelove S. C., Aronseq J. K. (1982). *British Journal of Clinical Pharmacology*, 13, 523–528.
5. Azad Khan A. K., Truelove S. C., Aronseq J. K. (1977). *British Journal of Clinical Pharmacology*, 13, 523–528.
6. Badamaranahalli S. S., Kopparam M., Bhagawati S. T., Durg S. (2015). *European Journal of Pharmaceutical Sciences*, 76, 73–82.
7. Batta A. K., Tint G. S., Xu G., Shefer S., Salen G. (1998). *Journal of Lipid Research*, 39, 1641–1646.
8. Bayat A., Larijani B., Ahmadian S., Junginger H., Rafiee-Tehrani M. (2008). *[Journal details missing]*.
9. Chan R. P., Pope D. J., Gilbert A. P., Baron J. H., Lennard-Jones J. P. (1983). *Digestive Diseases and Sciences*, 28, 609–615.
10. Chavan M. S., Sant V. P., Nagarsenker M. S. (2001). *Journal of Pharmacy and Pharmacology*, 53, 895–900.
11. Devereux J. E., Newton J. M., Short M. B. (1990). *Journal of Pharmacy and Pharmacology*, 42, 500–508.
12. Duan L., Zheng Q., Li X., Quan D., Ge J. (2011). *Journal of Controlled Release*, 152(Suppl. 1), e18–e20.
13. Fedorak R. N., Haeberlin B., Empey L. R. (1995). *Gastroenterology*, 108, 1688–1699.
14. Flourie B., Molis C., Achour L., Dupas H., Hatat C., Rambaud J. C. (1993). *Journal of Nutrition*, 123, 676–681.
15. Friend D. R., Chang G. W. A. (1984). *Journal of Medicinal Chemistry*, 27, 261–266.
16. Galvez L. M., Del Carmen R. I. M., Gálvez J., García D. R. (2013). *Molecular Diversity*, 17(3), 573–593.
17. Garjani A., Davaran S., Rashidi M., Maleki M. (2004). *DARU Journal of Pharmaceutical Sciences*, 12, 24–30.
18. Garreto M., Ridell R. H., Wurans C. S. (1981). *Gastroenterology*, 84, 1162–1167.
19. Gliko-Kabir I., Yagen B., Baluom M., Rubinstein A. (2000). *Journal of Controlled Release*, 63(1), 129–134.
20. Gugulothu D., Kulkarni A., Patravale V., Dandekar P. (2014). *Journal of Pharmaceutical Sciences*, 103(2), 687–696.

21. Haeberlin B., Rubas W., Nolen H. W. II, Friend D. R. (1994). *Pharmaceutical Research*, 10, 1553–1562.
22. Karunaratne D. N., Farmer S., Hancock R. E. W. (1993). *Bioconjugate Chemistry*, 4, 434–440.
23. Khan M. Z., Prebeg Z., Kurjakovic N. (1999). *Journal of Controlled Release*, 58, 215–222.
24. Kim C. K., Shin H. J., Yang S. G., Kim J. H., Oh Y. (2001). *Pharmaceutical Research*, 18, 454–459.
25. Kubba R. M., Kadhim M. M., Khadom A. A. (2021). *Results in Chemistry*, 3, 100212.
26. Laali M. (2020). *Digestive Diseases and Sciences*, 32, 598–602.
27. Lamprecht A., Torres H. R., Schafer U., Lehr C. M. (2000). *Journal of Controlled Release*, 69, 445–454.
28. Loftsson T., Brewster M. E., Derendorf H., Bodor N. (1991). *Pharmazeutische Zeitung Wissenschaft*, 4, 5–10.
29. Khalaf M., Jordan P. M. (2023). *International Journal of Frontiers in Chemistry and Pharmacy Research*, 3(1), 32–41.
30. Mahdi M. F., Alsaad H. N. (2012). *Pharmaceuticals*, 5, 1080–1091.
31. Makhija D. T., Somani K. R., Chavan A. U. (2013). *Indian Journal of Pharmaceutical Sciences*, 75(3), 353–357.
32. Nagpal D., Singh R., Gairola N., Bodhankar S. L., Dhaneshwar S. S. (2006). *Indian Journal of Pharmaceutical Sciences*, 68(2), 171–178.
33. Nakamura J., Asai K., Nishida K., Sasaki H. A. (1992). *Chemical and Pharmaceutical Bulletin*, 40, 2164–2168.
34. Ojha M., Madhav N. V. S., Singh A. (2011). *International Current Pharmaceutical Journal*, 1, 209–212.
35. Paliwal V. J., Rajewski R. A. (2017). *Pharmaceutical Research*, 14, 556–567.
36. Pellicciari R., Garzon-Aburbeh A., Natalini B. (1993). *Journal of Medicinal Chemistry*, 36, 4201–4209.
37. Raffi R., Franklin W., Cerniglia C. E. (1990). *Applied and Environmental Microbiology*, 56, 2146–2150.
38. Rajput A. P., Gore R. P. (2013). *Der Pharma Chemica*, 3(3), 409–421.
39. Rasheed A., Aishwariya K., Basha B. N., Reddy B. S., Swetha A. (2009). *Der Pharma Chemica*, 1(2), 124–132.