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RESEARCH ARTICLE

Synthesis and Evaluation of Amino Acid Prodrug of Naproxen

Muzumdar N[1]*, Garg G[1], Mishra K[1], Singh A[2]

[1]Faculty of Pharmacy VNS Institute, Neelbud Bhopal (MP) – 462042.

[2]Centre for Research & Development, Ipca Laboratories Ltd, Sejavta, Ratlam (MP) – 457002.

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ABSTRACT

Prodrug approach is very effective and helpful in decreasing the problem related with solubility, absorption, distribution, site specificity, instability, toxicity, formulation and bioavailability problem. Literature reveals that many efforts had made to synthesis prodrugs via masking carboxylic acid group by forming ethyl ester, methyl ester, glycolamide ester and amide prodrug using various amino acids. Also attempts were made to develop amide prodrugs of different NSAIDs using amino acid. The advantages of using amino acids for this purpose are owing to their characteristics like normal dietary constituent, non-toxic in moderate doses, healing effect on gastric toxicity, marked anti-inflammatory activity and site specificity. In this background, the present research aims to synthesize the amide prodrug of Naproxen with various amino acids like Glycine, Glutamic acid, Aniline, pralines and Leucine and a study on their various physicochemical characters, anti-inflammatory activity and ulcer index as prodrugs. The main side effects of Naproxen include GIT disturbance, peptic ulceration and gastric bleeding. These gastroenteropathies are generally believed to be resulted from the direct contact effect, which can be attributed to the combination of local irritation produced by the free carboxylic group in the molecular structure and by local blockage of prostaglandin biosynthesis in the GI tract. Therefore, the development of new NSAIDs without these side effects has long been awaited. The use of prodrugs to provisionally hide the acidic group of NSAIDs has been proposed as an approach to reduce or suppress the GI toxicity due to the direct contact effect.

KEYWORDS

Naproxen, Non-steroidal Anti-inflammatory drug, Prodrug, Amino acid

INTRODUCTION

Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs or NAIDs, but also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIAs) or non-steroidal Anti-inflammatory medicines (NSAIMs), are drugs with analgesic and antipyretic (fever-reducing) effects and which have, in higher doses, anti-inflammatory effects.

***Address for Correspondence:**
Neha Muzumdar
Department of Pharmaceutical Chemistry,
VNS Institute of Pharmacy, VNS Campus, Vidhya Vihar,
Berkheda Nathu, Neelbud, Bhopal (M.P.) India.
E-Mail Id: muzumdar.neha@gmail.com

The term "non-steroidal" is used to distinguish these drugs from steroids, which, among a broad range of other effects, have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group of drugs are aspirin, ibuprofen, and naproxen, all of which are available over the counter in many areas[1]. NSAIDs are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. The widespread use of NSAIDs has meant that the adverse effects of these drugs have become increasingly prevalent. The two

main adverse drug reactions (ADRs) associated with NSAIDs relate to gastrointestinal (GI) effects and renal effects of the agents. These effects are dose-dependent, and in many cases severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, and death, limiting the use of NSAID therapy[2].

The main adverse drug reactions (ADRs) associated with use of NSAIDs relate to direct and indirect irritation of the gastrointestinal (GI) tract. NSAIDs cause a dual assault on the GI tract: the acidic molecules directly irritate the gastric mucosa, and inhibition of COX-1 and COX-2 reduces the levels of protective prostaglandins. Inhibition of prostaglandin synthesis in the GI tract causes increased gastric acid secretion, diminished bicarbonate secretion, diminished mucus secretion and diminished trophic effects on epithelial mucosa. Common gastrointestinal ADRs include: Nausea/Vomiting, Dyspepsia, Gastric ulceration/bleeding, Diarrhea[3].
The term prodrug refers to a pharmacologically inactive compound that is converted to an active drug by a metabolic biotransformation which may occur prior, during and after absorption or at specific target sites within the body. According to IUPAC (International Union of pure and applied chemistry): Prodrug is defined as any compound that undergoes biotransformation before exhibiting its pharmacological effects. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of chronic inflammatory diseases. Prolonged administration of these drugs exhibit several undesired side effects; the most important are gastro-intestinal irritation and ulceration which represent still an unsolved therapeutic problem. The development of a gastrointestinal tract (GIT)-safe anti-inflammatory therapy for the treatment of disease of joints presents a unique challenge. Literature reveals that many efforts had been made to synthesize amino acid ester, glycolamide ester, and amide prodrugs using various amines but few attempts were made to develop amide prodrugs using amino acids[4]. The salient features of the usefulness of conjugation of amino acids with NSAIDs are as follows: (i) Amino acids are normal dietary constituent and they are non-toxic in moderate doses as compared to other promoities; (ii) Amino acids have healing effect on gastric lesions produced by NSAIDs; (iii) A drug with free carboxyl group can be derivatized into corresponding esters or amide of amino acids, so as to alter the physical properties of a parent drug with one or more of the hydrolase enzymes serving as the *in-vivo* reconversion site(s); (iv) Being a nutritional substance, the use of amino acids as a derivatizing group might also permit more specific targeting site for enzymes involved in the terminal phase of digestion; (v) Many amino acids possess marked anti-inflammatory activity against gelatin induced hind paw oedema in rats; (vi) By using different types of amino acids like non-polar, polar, acidic and basic, the drug molecule can be made more or less polar, or more or less soluble in given solvent[5-7]. In this background, the present research aims to synthesize the amide prodrug of Naproxen with various amino acids like Glycine, Glutamic acid, Aniline, proline and Leucine and a study on their various physicochemical characters, anti-inflammatory activity and ulcer index as prodrugs.

MATERIAL AND METHOD

All of the chemicals used in these experiments were of reagent grade and Naproxen was obtained as a gift sample from Ranbaxy, Gurgaon. The synthetic route used for the synthesis of amino acid linked amide Prodrugs of naproxen is shown in scheme 1. They were prepared in three steps.

RESULTS AND DISCUSSION

All the synthesized derivatives showed anti-inflammatory activity less but near to the parent drug Naproxen, which was used as a standard. The %inhibition of the standard drug naproxen was calculated as 66.81% and that of NM1, NM2, NM3, NM4 and NM5 was found to be 62.09%, 46.52%, 50.53%, 63.13% and 59.44% respectively. Also the ulcer causing side effect of the drug was quite much reduced, as depicted by the visibly reduced ulcer index of the

synthesized compound as shown in figure 5.2. The order of the effectiveness of the drug against the ulcer causing side effect is:

NM1 > NM3, NM5 > NM2, NM4 > Naproxen

This indicates the effectiveness of the synthesized amino acid conjugates in preventing GI side effects of Naproxen as was expected.

Synthesis of Prodrug of Naproxen

Naproxen 2-(6-methoxynaphthalen-2-yl) propanoic acid and the synthesis of prodrugs was carried out by Schotten Baumann technique.

Step 1: Synthesis of Naproxen Chloride

Naproxen (0.05 M) was dissolved in minimum amount of chloroform and freshly distilled thionyl chloride (0.05 M) was added slowly to it. The mixture was refluxed for 15 h at 60-70ºC with continuous stirring on magnetic stirrer. The viscous liquid was immediately poured on petridish and was vacuum dried to give yellow colour crude Naproxen chloride.

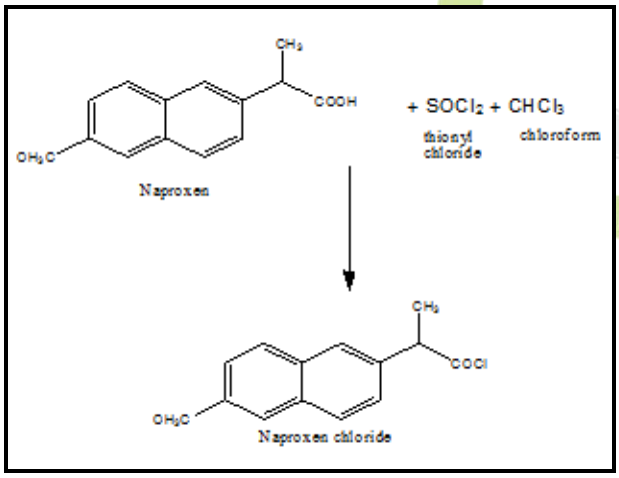

Figure 1: Synthesis of Naproxen chloride

Step 2: Synthesis of Methyl Ester Hydrochlorides of Different Amino Acids

Freshly distilled thionyl chloride (0.05 M) was slowly added to methanol (100 ml) with cooling and amino acid (Glycin) (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 titrated 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 re-crystallized 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 to give pure amino acid methyl ester hydrochloride.

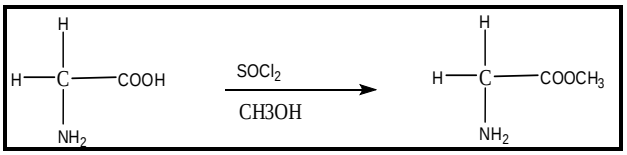

Figure 2: Synthesis of methyl ester of glycin

Step 3: Synthesis of Prodrugs of Naproxen with Methyl Esters of Different Amino Acid

Ice cold, aqueous sodium hydroxide solution (5%) was taken in 250 ml beaker and methyl ester of Glycin (0.05 M) was added to it. The reaction mixture was mechanically stirred for 30 min at room temperature, after which the beaker was transferred to an ice bath kept on mechanical stirrer, maintaining the temperature at 10ºC. Naproxen chloride (0.01 M) was added in small portions with continuous stirring for 7-8hrs. The solid that separated out was filtered using vacuum pump and dried. The crude prodrug was recrystallized from methanol.

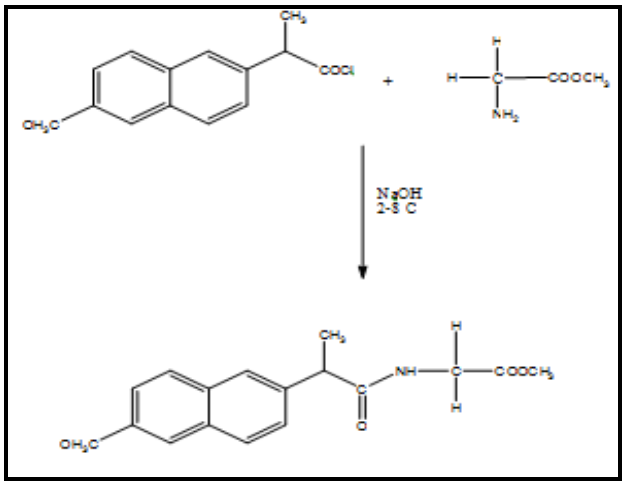

Figure 3: Synthesis of NM1

(Scheme 1: Common root of synthesis)

(The same procedure was followed to synthesize naproxen prodrugs with methyl ester hydrochlorides of other amino acids like glutamic acid, Alanine, Leucine and Proline methyl ester)

NM1: [2-(6-methoxy-naphthalen-2-yl)-propionylamino]-acetic acid methyl ester (naproxen glycin conjugate)

$C_{17}H_{19}NO_4$ - M.P.: 282-284 ^{0}C; Yield (%): 62%; UV (λ_{max}): 344;IR(cm-^{1}): 3400.9(NH str. of amide), 2959.2(CH str.), 1542.8-1604.3(C=O), 1455.8-1479.8 (C-C), 1213.8 (C-N), 814.8-855.13 (C-H aromatic); 1H NMR (DMSO, δ(ppm)): 6.98-7.57 (CH, 2-napthelene), 3.89 (CH, methine), 4.16 (CH_2, methylene), 8.0 (NH, secondary amide).

NM2: 2-[2-(6-methoxy-naphthalen-2-yl)-propionylamino]- pentanedioic acid dimethyl ester (naproxen glutamate conjugate)

$C_{21}H_{25}NO_6$- M.P.:322 ^{0}C; Yield (%): 56%; UV (λ_{max}): 342;IR (cm^{-1}): 3418.4 (N-H stretching), 2955.9(C-H stretching), 1543.8-1604.2 (C=O), 1457.1-1479.9(C-C), 1213.8(C-N), 814.8-855.13 (C-H aromatic);1H NMR (DMSO, δ(ppm)): 6.98-7.57 (CH, 2-napthelene), 3.89-4.42(CH, methine), 2.25-2.29 (CH_2, methylene, S), 3.67-3.73(CH3, Methyl).

NM3: 2-[2-(6-methoxy-naphthalen-2-yl)-propionylamino]- propionic acid methyl ester (naproxen alanine conjugate)

$C_{18}H_{21}NO_4$ - M.P.:340-342 ^{0}C; Yield (%): 58%; UV (λ_{max}): 340; IR (cm^{-1}): 3418.4 (N-H stretching), 2955.9(C-H stretching), 1543.8-1604.2 (C=O), 1457.1-1479.9(C-C), 1213.8(C-N), 814.8-855.13 (C-H aromatic); 1H NMR (DMSO, δ(ppm): 6.98-7.57 (CH, 2-napthelene), 1.48-3.73(CH_3, methyl), 3.89-4.50(CH, methine), 8.0 (NH, secondary amine).

NM4: 2-[2-(6-methoxy-naphthalen-2-yl)-propionylamino]- 5-methyl-hexanoic acid methyl ester (naproxen leucine conjugate)

$C_{22}H_{29}NO_4$ - M.P.:350 ^{0}C; Yield (%): 63%; UV (λ_{max}): 370; IR **(**cm^{-1}**):** 3400.9(N-H stretching), 2959.2(C-H stretching), 1542.8-1604.3(C=O), 1455.8-1479.8(C-C), 1213.8(C-N), 814.8-855.13 (C-H aromatic); 1H NMR (DMSO, δ(ppm): 6.89-7.57(CH), 1.83-3.89(CH, methine), 1.09-1.25(CH_2, methylene, S), 1.01-3.73(CH_3, methyl).

NM5: 1-[2-(6-methoxy-naphthalen-2-yl)-propionyl]- 2,5-dihydro-1H-pyrrole-2-carboxylic acid methyl ester (naproxen proline conjugate)

$C_{20}H_{21}NO_4$ - M.P.: 358-360 ^{0}C; Yield (%): 58%; UV (λ_{max}): 332; IR (cm^{-1}): 2959.2(C-H stretching), 1542.8-1604.3(C=O), 1455.8-1479.8(C-C), 1213.8(C-N), 814.8-855.13(C-H aromatic); 1H NMR (DMSO, δ(ppm): 6.98-7.57(CH, 2-napthelene), 1.58-3.73(CH_3, methyl), 3.87(CH_2, methylene), 5.11(CH, Methine, S), 1.58-3.73(CH_3, methyl), 5.75 (H, 1 ethylene).

***In-vivo* Study**

Anti-inflammatory Activity

The anti-inflammatory activity was evaluated using formaldehyde-induced edema of rat paw. Albino rats (100- 200g) were divided into seven groups of six animals each. Group 1 served as control group, group II received Naproxen 20 mg/kg, group III to group VII received prodrug NM1 to NM5 respectively, where the dose was molecularly equivalent to the free drug. The initial volume of right hind paw of albino rat was measured by plethysmometer without administration of drug. The drug was administered orally in 1% suspension of sodium CMC. After 30 min of drug administration, formaldehyde (0.2 ml, 2% w/v solution in normal saline) was injected into the planter surface of right hind paw of each animal as phlogistic agent. The volume of right hind paw of albino rats was measured after 2 and 4 hrs. The mean difference in the volume of the right hind paw of rats was compared with control. Percent anti-inflammatory activity is calculated by

% inhibition = (1-Vt/Vc) *100

Where Vc – mean relative change in paw edema volume in control group and Vt - mean relative change in paw edema volume in test group. All

Table 1: Anti-inflammatory Activity of amide derivatives (NM1-NM5) of Naproxen

Compound	Dose (mg/kg)	% Increase in paw volume mean ± SEM		% Inhibition
		2 hrs	4 hrs	
control	20	42.85 ± 1.32	66.99 ± 1.88	0
Naproxen	20	17.81 ± 0.71	25.82 ± 0.68	66.81
NM1	20	16.81 ± 0.83	25.96 ± 1.01	62.09
NM2	20	22.61 ± 0.67	35.52 ± 0.71	46.52
NM3	20	23.67 ± 0.04	35.61 ± 0.55	50.53
NM4	20	22.61 ± 0.67	31.51 ± 0.71	63.13
NM5	20	21.02 ± 1.23	30.81 ± 0.85	59.44

Table 2: Anti-Ulcerogenic activity of amide derivatives (NM1-NM5) of Naproxen

S no.	Test compound	Oral dose(mg/kg)	Ulcer index
1	Control	80	0
2	Standard	80	2.55
3	NM1	80	0.38
4	NM2	80	1.16
5	NM3	80	0.77
6	NM4	80	1.16
7	NM5	80	0.77

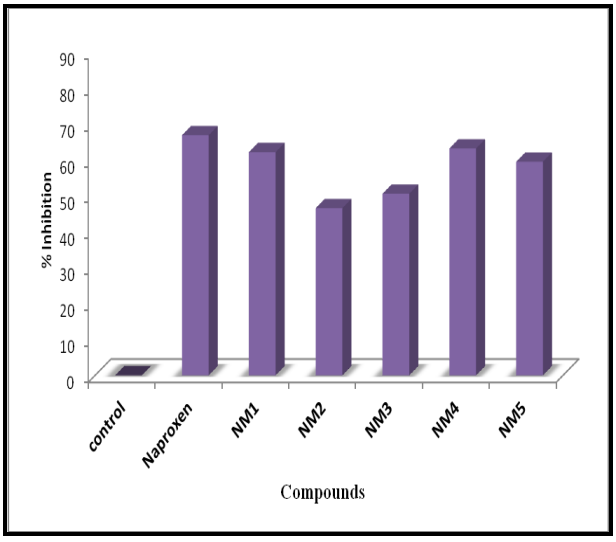

Figure 4: Graph representing % inhibition of the synthesized compounds

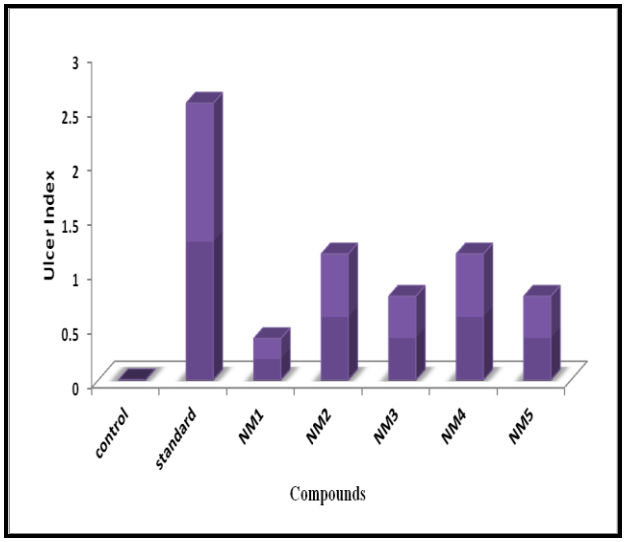

Figure 5: Graph representing Ulcer index of the synthesized compounds

the results were expressed as mean ± SEM. Statistical evaluation was performed using analysis of variance followed by the T Dunnet test for sub group comparison. (Anti-inflammatory Activity of amide derivatives (NM1-NM5) of Naproxen were shown in table 1 and Graph representing % inhibition of the synthesized compounds were shown in Figure 4).

Ulcerogenic Activity

Gastrointestinal toxicity of the synthesized prodrugs was measured and compared with the drug by measuring ulcer index. For the purpose, male albino rats were selected, weighing between 100-200 g, the rats were divided into seven groups each comprising of six rats, including a control and a standard group. The prodrug was suspended in 10 ml of 2% w/v suspension of acacia. Measured volume of the suspension containing dose equivalent to 20 mg/kg of body weight of Naproxen was administered orally to the test group 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 ulcer index was calculated by severity of gastric mucosal lesions which are graded as grade1 = less than1mm erosions, grade 2 = 1- 2mm erosions and grade 3 = More than 2mm erosions.

The UI was calculated as

UI= [1× (number of lesions of grade 1) + 2× (number of lesions of grade 2) + 3× (number of lesions of grade 3)]/10.

(Anti Ulcerogenic activity of amide derivatives (NM1-NM5) of Naproxen were shown in table 2 and Graph representing Ulcer index of the synthesized compounds were shown in Figure 5)

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