ORIGINAL RESEARCH ARTICLES

SYNTHESIS AND EVALUATION OF NAPROXEN ESTER PRODRUGS

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ABSTRACT

The present works deals with simple and efficient method of improving therapeutic efficacy of naproxen by retarding gastrointestinal side effects through masking of carboxylic group chemically. This is achieved by synthesis of ester prodrugs of naproxen with various naturally available antioxidants; menthol, thymol, eugenol, guiacol, vanillin and sesamol by the dicyclohexyl carbodiimide (DCC) coupling method. The title compounds are purified and characterized by spectral data. Further, their partition coefficients have been determined and hydrolytic studies have been performed. The synthesized compounds are more lipophilic compared to the parent moieties and are stable in acidic environment, which is a prerequisite for their oral absorption. Under gastric as well as intestinal pH conditions, these prodrugs showed variable susceptibility towards hydrolysis. The title compounds when evaluated for anti-inflammatory and analgesic activities showed improvement over the parent drug. Prodrugs were also found to be significantly less ulcerogenic then parent drugs.

Keywords: Naproxen, anti-inflammatory, analgesic, ulcerogenicity.

INTRODUCTION

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are the most frequently used drugs today for the treatment of peripheral pain and inflammation. They act by the inhibition of an enzyme cyclooxygenase (COX). It is well established that two isoforms, cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) exist[1]. The potentially harmful effects of NSAIDs on gastrointestinal tract are due to inhibition of cytoprotective COX-I and a direct irritant action on the GIT[2,3] and thus, their long term use leads to ulcers, bleeding and other gastric side effects[4-6]. Due to this, some selective COX-II inhibitors devoid of the carboxylic acid functionality e.g., celecoxib, rofecoxib, etoricoxib and valdecoxib, were introduced in the market. However, serious cardiovascular side effects arising on their long term use has resulted in the withdrawal of some of these drugs from market.[7] It is now well known that localized production of reactive oxygen species (ROS) play a vital role in the gastric ulcerations associated with the NSAID treatment[8,9]. Thus, antioxidants can play a key role in the prevention of gastric ulcer formation. Several naturally occurring antioxidant compounds are considered promising in the treatment of free radical mediated diseases.[10] Naturally occurring antioxidants like thymol, menthol, eugenol and sesamol can be considered as suitable promoieties for mutual prodrugs as, besides masking the irritant carboxylic acid function, they provide the additional antioxidant effect[11-13]. Based on this rationale, prodrugs of diclofenac[14], biphenyl acetic acid[15], ibuprofen[16,17], mefenamic acid[18,19], aceclofenac[20] and ketoprofen[21] with natural phenolic and alcoholic antioxidant compounds have been reported. Naproxen, (*R,S*) 6-methoxy-α-methyl-2-naphthalene acetic acid, is one of the most widely used NSAIDs for relieving arthritic pain. Free carboxylic group of naproxen has severe gastrointestinal side effects on oral administration that restricts its use[22].

There are several reports on the synthesis and evaluation of prodrugs of naproxen with promoieties like glycerides[23], stigmasterol and estrone[24], glucosyl thiamine[25] and glycolamide[26]. Several polymeric prodrugs of naproxen with vinyl ether[27], polyoxyethylene[28] and hydroxymethyl methacrylate[29] are also reported. Mutual prodrugs of naproxen with nicotinic acid[30], paracetamol[31] and propyphenazone[32] have been synthesized and

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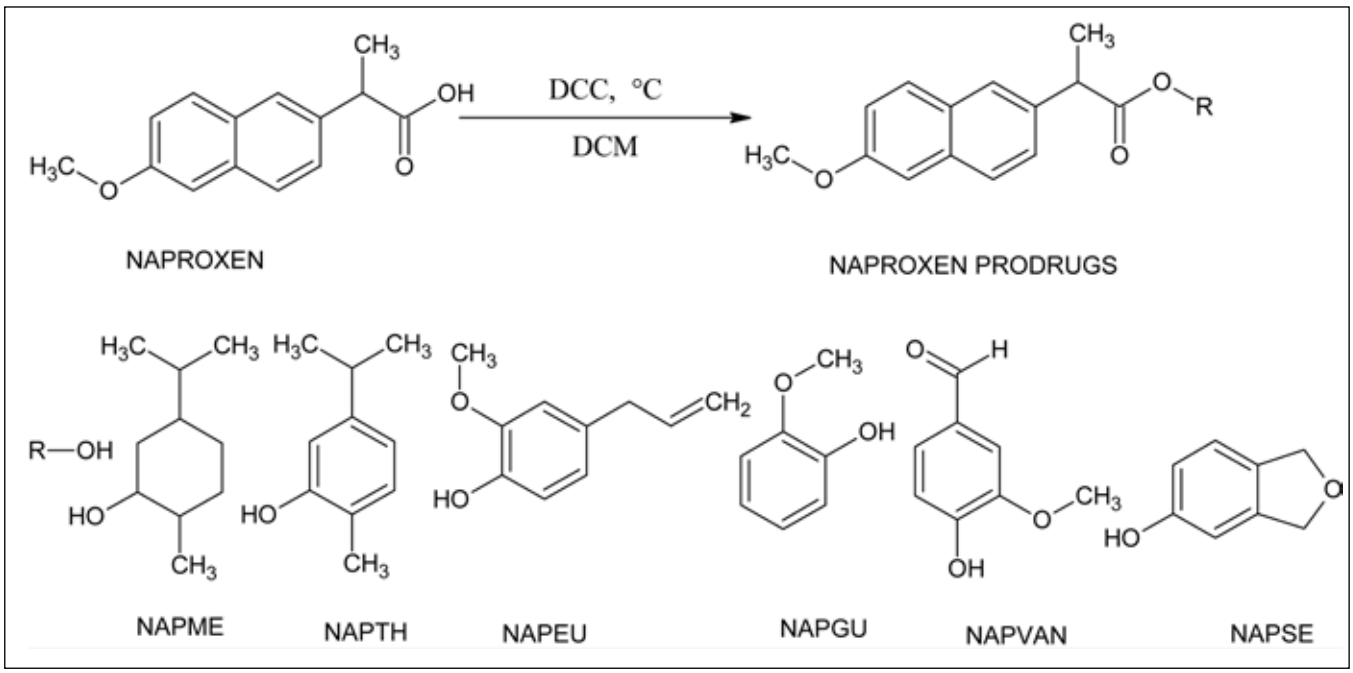

Fig. 1: Synthesis of naproxen prodrugs. DCM- dichloromethane, DCC- *N, N'*- dicyclohexylcarbodimide, DMAP- dimethylaminopyridine

evaluated. The earliest conjugation of naturally occurring phenolic/alcoholic compounds with naproxen is hitherto unreported.

In the present research, naproxen has been conjugated with thymol, menthol, eugenol, guiacol, vanillin and sesamol to obtain its ester prodrugs. The synthesized prodrugs have been characterized by spectral analysis. Further, they have been studied for physicochemical properties like solubility, partition coefficient and hydrolytic stability. These title prodrugs have also been evaluated for anti-inflammatory, analgesic activity and ulcerogenic potential.

MATERIALS AND METHODS

Materials

Naproxen was obtained from Ozone Laboratories Ltd (Mumbai, India). Thymol, menthol, eugenol, guiacol, vanillin, sesamol, dimethylaminopyridine and *N*, *N'*-dicyclohexyl carbodimide, as well as all other reagents and solvents were commercially procured from Loba Chemicals Pvt. Ltd. (Mumbai, India). IR spectra were recorded on a Bruker FT-IR spectrometer (Model –Alpha). The ^{1}H NMR spectra were recorded on Bruker AVANCE III HD 500 MHz spectrometer. The mass spectra were recorded on JEOL GCMATE II GC-MS system. The HPLC system used for determining the partition coefficient and hydrolysis studies of the compounds was Jasco PU-2089 plus Quaternary model with a UV/Vis detector and a C-18 column (Finepak SIL, 250 x 4.6 mm, 5 µm). The HPLC software used was Jasco-ChromNAV (1.19.1 Version). Digital plethysmometer (UGO-BASILE-7140 Barcelona, Italy was used for anti-inflammatory studies.

Methods

Synthesis of naproxen ester prodrugs: General Procedure

To a well stirred and cooled solution of naproxen (0.575 g, 2.5 mmol) in dichloromethane (50 mL), the appropriate alcohol/phenol (2.5 mmol) was added at 0°C, followed by *N*, *N'*-dicyclohexyl carbodimide (0.515 g, 2.5 mmol) and dimethylaminopyridine (0.012 g, 0.1 mmol) over 30 min. The reaction mixture was thereafter allowed to stir at 0°C for 1 hour and at room temperature for next 12 hours. The reaction mixture was filtered thereafter, to separate the precipitated *N*, *N'*- dicyclohexyl urea. The filtrate was washed with aq. 5% $NaHCO_3$ solution (25mL x 2). The aqueous layer was separated and organic layer washed aq. 5% NaOH solution (5mL x 2). The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4) and the solvent distilled out under reduced pressure. The crude product was purified by column chromatography using hexane-ethyl acetate as eluent.

5-Isopropyl-2-methylphenyl 2-(6-methoxynapthalen-2yl) propanoate (NAPTH)

UV (λ_{max}): (MeOH) 234 nm, IR (KBr) cm^{-1}: 2943.37 and 2865.51 (C-H str.), 1750.12 (C=O str. ester), 1238.70 (C-O str. ester), 1628.11 and 1603.03 (C=C str. aromatic), 815.29 (C-C), 1023.83 (C-O-CH_3), 1325.78 (C-H str, -CH_3). ^{1}H NMR (500 MHz, DMSO): δ 0.9-1.0 (m, 2H, 2-CH-), δ 0.93 (d, 6H, -CH-(CH_3)$_2$), δ 1.1 (d, 1H, -CH-), δ 1.61 (d, 3H, -CH-CH_3), δ 2.28 (s, 3H, Ar-CH_3), δ 2.52 (m, 1H, -CH-(CH_3)$_2$, δ 3.92 (s, 3H, Ar-OCH_3), δ 4.32 (q, 1H, -CH-CH_3), δ 6.71- 7.19 (m, 3H, Ar-H, Thymol), δ 7.19 - 8.08 (m, 6H Ar-H, NAP). Mass: (70 eV) m/z 363.52.

5-Isopropyl-2-methylcyclohexyl 2-(6-methoxy napthalen-2yl) propanoate (NAPME)

UV (λ_{max}): (MeOH) 233.5 nm, IR (KBr) cm^{-1}: 2927.00 and 2868.09 (C-H str.), 1716.24 (C=O str. ester), 1264.78 (C-O str. ester), 1629.80 and 1604.87 (C=C str. aromatic), 809.89 (C-C), 1033.93 (C-O-CH_3), 1372.77 (C-H str, -CH_3). ^{1}H NMR (500 MHz, DMSO): δ 0.91 (d, 6H, -CH-(CH_3)$_2$), δ 1.0-1.1 (m, 1H, -CH-), δ 1.2-1.3 (s, 6H, 3-CH_2), δ 1.50 (d, 3H, -CH-CH_3), δ 1.09- 2.0 (s, 3H, Cycloohexyl-CH_3, Menthol), δ 2.51 (m, 1H, -CH-(CH_3)$_2$, δ 3.90 (s, 3H, Ar-OCH_3), δ 4.60 (q, 1H, -CH-CH_3), δ 7.01 - 8.10 (m, 6H Ar-H, NAP). Mass: (70 eV) m/z 368.44.

5-Allyl-2-methoxyphenyl 2-(6- methoxy napthalen-2yl) propanoate (NAPEU)

UV (λ_{max}): (MeOH) 233 nm, IR (KBr) cm^{-1}: 2973.84 and 2936.28 (C-H str.), 1750.11 (C=O str. ester), 1263.19 (C-O str. ester), 1633.19 and 1604.41 (C=C str. aromatic), 816.65 (C-C), 1030.77 (C-O-CH_3), 1326.89 (C-H str, -CH_3). ^{1}H NMR (500 MHz, DMSO): δ 1.63 (d, 3H, -CH-CH_3), δ 3.51 (m, 2H, Ar-CH_2-), δ 3.60 (s, 3H, Ar-OCH_3, Eugenol), δ 3.82 (s, 3H, Ar-OCH_3, NAP), δ 4.23 (q, 1H, -CH-CH_3), δ 5.13 (t, 2H, =CH_2), δ 5.92 (m, 1H, -CH=), δ 6.50- 7.12 (m, 3H, Ar-H, Eugenol), δ 7.12 - 8.03 (m, 6H Ar-H, NAP). Mass: (70 eV) m/z 377.52.

2-Methoxyphenyl 2-(6-methoxy napthalen-2yl) propanoate (NAPGU)

UV (λ_{max}): (MeOH) 233 nm, IR (KBr) cm^{-1}: 2931.30 and 2852.09 (C-H str.), 1747.42 (C=O str. ester), 1263.42 (C-O str. ester), 1629.67 and 1607.09 (C=C str. aromatic), 811.19 (C-C), 1023.97 (C-O-CH_3), 1322.44 (C-H str, -CH_3). ^{1}H NMR (500 MHz, DMSO): δ 1.59 (d, 3H, -CH-CH_3), δ 3.80 (s, 3H, Ar-OCH_3, Guiacol), δ 3.89 (s, 3H, Ar-OCH_3, NAP), δ 4.20 (q, 1H, -CH-CH_3), δ 6.80- 7.20 (m, 4H, Ar-H, Guiacol), δ 7.20 - 8.00 (m, 6H Ar-H, NAP). Mass: (70 eV) m/z 337.54

4-Formyl-2-methoxyphenyl 2-(6-methoxy napthalen-2yl) propanoate (NAPVAN)

UV (λ_{max}): (MeOH) 235 nm, IR (KBr) cm^{-1}: 2930.81 and 2848.66 (C-H str.), 1763.76 (C=O str. ester), 1266.57 (C-O str. ester), 1628.51 and 1603.15 (C=C str. aromatic), 812.29 (C-C), 1024.67 (C-O-CH_3), 1328.07 (C-H str, -CH_3), 1701.19 (-C=O, -CHO). ^{1}H NMR (500 MHz, DMSO): δ 1.68 (d, 3H, -CH-CH_3), δ 3.60 (s, 3H, Ar-OCH_3 Vaniliin), δ 3.81 (s, 3H, Ar-OCH_3, NAP), δ 4.25 (q, 1H, -CH-CH_3), δ 7.00- 8.00 (m, 3H, Ar-H, Vanillin), δ 7.00 - 8.00 (m, 6H Ar-H, NAP), δ 10.02 (s, 1H, -CHO). Mass: (70 eV) m/z 365.38.

1,3-Dihydroisobenzofuran-5-yl 2-(6-methoxy napthalen-2yl) propanoate ester (NAPSE)

UV (λ_{max}): (MeOH) 232.5 nm, IR (KBr) cm^{-1}: 2934.45 and 2854.67 (C-H str.), 1754.17 (C=O str. ester), 1247.14 (C-O str. ester), 1633.19 and 1606.60 (C=C str. aromatic), 817.43 (C-C), 1037.77 (C-O-CH_3), 1327.03 (C-H str, -CH_3). ^{1}H NMR (500 MHz, DMSO): δ 1.81 (d, 3H, -CH-CH_3), δ 3.80 (s, 3H, Ar-OCH_3, NAP), δ 4.20 (q, 1H, -CH-CH_3), δ 6.02 (s, 2H, -CH_2-), δ 6.20- 7.20 (m, 3H, Ar-H, Sesamol), δ 7.00 - 8.00 (m, 6H Ar-H, NAP). Mass: (70 eV) m/z 348.48.

Solubility and partition coefficient determination:

10 mg of the synthesized prodrug was tested for solubility in 0.5 mL of each of the solvents, *viz.,* ethanol, methanol, chloroform and dichloromethane in separate test tubes. After gentle shaking solubility was observed. Further 0.5 mL of solvent was added if required to completely dissolve the compound. The partition coefficients of synthesized prodrugs were determined in *n*-octanol-phosphate buffer, pH 7.4 (1:1) as follows. The prodrug, 10 mg, was added to 10 mL of aqueous phase followed by addition of 10 mL of n-octanol. The contents were thoroughly shaken for 2 hrs at room temperature and left for 1 hr. The concentrations in the aqueous and organic phase was determined using acetonitrile: buffer (90:10 V/V) as mobile phase for determination and flow rate of 1.0 mL/min with UV detection at 232 nm by using HPLC[33] and partition coefficient computed.

***In vitro* hydrolysis**

The hydrolysis kinetics of prodrugs was studied in aqueous buffer solutions at pH 1.2 and pH 7.4 at 37 °C using hydrochloric acid-buffer and phosphate buffer, respectively. Solutions of 10 mg of the prodrug prepared in 90 mL of hydrochloric acid- buffer (pH 1.2) or phosphate buffer (pH 7.4) were kept in screw capped tubes maintained at 37 ± 0.5 °C. At definite time intervals (15, 30, 60,

120, 240 min), aliquots were withdrawn from tubes and analyzed by HPLC for the amount of drug released after the hydrolysis of the prodrug. Pseudo first order rate constants (K_{obs}) and half-life ($t_{1/2}$) were calculated[34].

Pharmacological evaluations

The title prodrugs were evaluated for analgesic, anti-inflammatory and ulcerogenic potential. Wistar rats (albino rats) of either sex weighing 100 to 200 g were divided into eight groups of 6 animals each for the evaluation of anti-inflammatory activity and ulcerogenic potential and albino mice were used for the evaluation of analgesic activity. The animals were housed in standard polypropylene cages in an air-conditioned room at 22 ±3 °C with 55 ±5% humidity and provided with standard laboratory diet and water *ad libitum.* The study protocol was approved by Institutional Animal Ethics Committee (448/01/c/CPCSEA/PRCOP/14-15/12).

Anti-inflammatory activity

The anti-inflammatory activity of naproxen ester prodrugs was determined by using carrageenan-induced rat paw edema model.[35,36] Group I served as the control and received only vehicle (0.5% w/V aq.CMC suspension). Group II received naproxen (10 mg/kg) while, the groups III to VIII received prodrugs in the doses molecularly equivalent to naproxen, *p.o*. After 30 min of compound administration, 0.1 mL of 1% w/V carrageenan in normal saline was injected into the sub planter region of left hind paw and the edema volume was measured before injection and at the several intervals up to 12 h. The initial volume of right hind paw was measured using a digital plethysmometer without administration of drug/ prodrug.

Analgesic activity

Analgesic activity was carried out by the acetic acid induced writhing method[37] using the Swiss albino mice model. A 1% V/V aqueous solution of acetic acid was used to induce writhings. The animals of either sex were used and were divided in eight groups of 6 animals each. Group I served as a control group, group II received standard drug, naproxen (10 mg/kg) and all other six groups received prodrugs in molecularly equivalent doses, orally in a 1 % w/V aqueous suspension of sodium carboxymethylcellulose. Acetic acid was administered intraperitoneally at 1 mL/100g body weight of the animal. Test compounds were administered orally 3 h prior to acetic acid injection. The number of writhings in 10 min within the control group and the standard and test compounds groups, were counted and compared. Analgesic activity was measured as percentage decrease in writhing as compared to the control.

Ulcerogenic potential

Gastrointestinal toxicity of the synthesized prodrugs was compared with that of the parent drug, naproxen by measuring the ulcer index. For this albino Wistar rats of either sex, weighing around 100-150 g each, were divided in eight groups of six animals each and fasted for 24 h prior to administration of drug/prodrug. The naproxen (standard, 100 mg/kg) and prodrugs (dose of prodrugs molecularly equivalent to naproxen) were administered orally as aqueous suspension in 0.5 % w/V acacia. The control group was administered as 0.5 % w/V acacia aqueous suspension only. Animals were sacrificed 12 h after the treatment. The stomach was removed, opened along the curvature, rinsed with 5 mL saline and examined by means of a magnifier. The ulcer index was calculated as mean for all animals in the group[38].

Statistical Analysis

Statistical analysis was carried out for pharmacological evaluation data using analysis of variance (ANOVA) test, followed by Dunnet's Test for determining level of significance. P values < 0.05 were considered statistically significant.

RESULTS

Chemistry

The naproxen ester prodrugs were synthesized by the DCC coupling method using menthol, thymol, eugenol, guiacol, vanillin and sesamol as promoieties. Purity of the synthesized prodrugs was ascertained by melting point and thin layer chromatography (TLC). The products were obtained in reasonable yields (63-75%). The title compounds were confirmed by FTIR, ^{1}H NMR and Mass spectroscopic data. The IR spectra of these compounds show characteristics C=O stretching bands around 1716-1763 cm^{-1} and C-O stretching bands around 1238-1266 cm^{-1} for the esters which confirms the formation of esters. The ^{1}H NMR spectra of synthesized compounds showed characteristic chemical shifts, which anticipated their structures. Presence of parent peak in mass spectra further confirms the molecular weight.

Solubility and partition coefficient

The synthesized naproxen ester prodrugs were subjected to solubility studies. It was observed that naproxen was highly soluble in 0.1 N sodium hydroxide solution. Prodrugs were found to be sparingly soluble in

0.1 N NaOH. All the prodrugs showed higher solubility than parent drug in organic solvents such as methanol, ethanol, chloroform and dichloromethane indicating their lipophilic nature. The partition coefficients of naproxen and ester prodrugs were determined in n-octanol-aqueous buffer (pH 7.4) system (Table I).

Table I: Percentage yield, melting point, Retention factor and partition coefficients of Naproxen prodrugs

Prodrug	Yield (%)	Melting point (^{0}C)a	Rf Value b	Log P
NAP	-	152-154	0.80	3.29
NAPTH	74.9	62-65	0.84	5.49
NAPME	69.5	120	0.86	6.34
NAPEU	72.3	105-110	0.88	5.14
NAPGU	63.4	100-101	0.94	4.18
NAPVAN	69.5	100-102	0.91	5.04
NAPSE	72.8	110-112	0.89	6.08
a Uncorrected; **b** TLC (Ethyl acetate: n-hexane, 1:4)				

Chemical Stability

The hydrolysis kinetics of naproxen ester prodrugs was studied in aqueous buffer solution at pH 1.2 and pH 7.4 to confirm the extent of release of parent drug. The decrease in concentration of ester prodrugs was monitored by HPLC. The result showed longer half-life of prodrugs in acidic pH 1.2 as compared to pH 7.4, which implies it may pass unhydrolyzed through stomach and possess enough stability to be absorbed from intestine. The values of the rate parameters K_{obs} for hydrolysis of prodrugs at different pH and 37^{0}C are listed in Table II along with their half-lives ($t_{1/2}$). The chemical degradation of ester prodrugs of naproxen followed first order kinetics and are quantitatively converted to parent drug as revealed by HPLC analysis. In the acidic buffer solution of pH 1.2 all prodrugs showed high chemical stability which implied that the compounds passed unhydrolyzed through the stomach on oral administration, while at neutral pH 7.4 their $t_{1/2}$ ranging from 79 min to 270 min.

Table II: Values of the rate parameters K_{obs} for hydrolysis of prodrugs at different pH and 37^{0}C

pH	1.2		7.4	
Prodrug	K_{obs}	$t_{1/2}$ (h)	K_{obs}	$t_{1/2}$ (h)
NAPME	1.569 × 10-3	7.36	2.566 × 10-3	4.51
NAPTH	1.899 × 10-3	6.08	3.600 × 10-3	3.20
NAPEU	2.960 × 10-3	3.89	8.750 × 10-3	1.32
NAPGU	2.310 × 10-3	5.01	3.824 × 10-3	3.02
NAPVAN	2.366 × 10-3	4.88	6.170 × 10-3	1.87
NAPSE	1.669× 10-3	6.93	2.953 × 10-3	3.91

Pharmacological evaluation

Synthesized prodrugs were evaluated for anti-inflammatory, analgesic and ulcerogenic potential. The prodrugs (in molecularly equivalent dose) showed comparable inhibition of carrageenan induced inflammation. Naproxen-eugenol and naproxen-vanillin prodrugs showed higher anti-inflammatory activity than parent drug, which may be due to synergistic activity of eugenol. The prodrugs show retention of

Table III: Anti-inflammatory, analgesic and ulcerogenic activity of naproxen prodrugs

Group	% Inhibition of Inflammation				Analgesic activity [a](%)	Ulcer Index (± SEM)[b]
	2 h	4 h	6 h	12 h		
Vehicle	-	-	-	-	-	*0.080 ± 0.097*
NAP	*32.50*	*52.04*	*63.82*	*78.57*	*68.81*	*3.672 ± 0.374*
NAPME	*30.72*	*50.00*	*65.43*	*79.89*	*74.72*	*1.463 ± 0.387*
NAPTH	*29.75*	*53.86*	*66.89*	*82.14*	*74.33*	*1.088 ± 0.197*
NAPEU	*34.39*	*56.19*	*68.22*	*86.96*	*81.63*	*0.879 ± 0.136*
NAPGU	*33.80*	*53.71*	*66.51*	*80.71*	*55.39*	*2.894 ± 0.358*
NAPVAN	*33.85*	*54.73*	*67.43*	*86.33*	*60.78*	*1.968 ± 0.140*
NAPSE	*30.18*	*48.61*	*63.51*	*77.41*	*60.48*	*2.486 ± 0.396*

Data represented as mean ± SEM, n=6.
Statistical analysis was performed with ANOVA followed by dunnett test
[a]P < 0.05 with respect to control , [b]P < 0.1 with respect to control

anti-inflammatory activity with percentage inhibition of 77%-86% as compared to 78 % when studied up to 12 hour (Table III). For analgesic activity, the decrease in number of writhings was expressed as a percentage protection from pain analgesic activity by test compounds with reference to control. The title compounds showed considerable retention of analgesic activity (55 %-81 % as compared to 68 % for naproxen). Naproxen-eugenol prodrug showed highest analgesic activity amongst all the prodrugs (Table III). All the synthesized prodrugs showed lower ulcer index value as compared to drug, naproxen, thus indicating decrease in gastrointestinal side effects through successful masking of free carboxylic group of the drug. The data represents that the risk of gastric ulceration is reduced by 2 to 5 times in prodrugs. Eugenol prodrug caused lowest ulceration of GI tract.

DISCUSSION

Natural phytophenols and alcohols are good free radical scavengers/ antioxidants and also exhibit NSAID type activites. In the present study six such compounds were employed as promoieties to prepare the title ester prodrugs of naproxen. The title compounds were synthesized by dicyclohexylcarbodimide coupling and evaluated for analgesic, antinflammatory activites and ulcrogenic potential, as well as hydrolysis kinetics. The aim and rationale behind the present study was of achieving synergistic effect and reducing gastrointestinal side effects associated with naproxen. The synthesized prodrugs showed improved solubility in organic solvents which implies their lipophilic character. They were chemically stable, biolabile and showed comparable analgesic and anti-inflammatory activities to the parent drug, with reduced ulcerogenicity. Retention of activity along with reduction in ulcerogenicity may be due to analgesic properties of some phytophenols and prevention of direct contact of carboxylic group with gastric mucosa. The study shows that mutual prodrug approach can be successfully used in improving therapeutic effectiveness of NSAID's.

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