Journal of Pharmaceutical Research Vol. 10, No. 4, October 2011 : 149-153.

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF ESTER PRODRUGS OF NAPROXEN

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Received on : 30.03.2011 Revised : 12.10.2011 Accepted : 19.10.2011

ABSTRACT

Naproxen suffers from the general side effects of NSAIDs, owing to presence of free carboxylic acid group. The study aimed to retard the adverse effects of gastrointestinal origin. Ten prodrugs of Naproxen were synthesized by amidation with N-Substituted-2-chloroacetamide. Purified synthesized prodrugs were characterized by their M.P., TLC, IR, ^{1}H NMR, MS and elemental analysis. The purity of the synthesized prodrugs was monitored by HPLC. Synthesized prodrugs were subjected to acute oral toxicity studies, anti-inflammatory activity and reduced ulcerogenic activity. Marked reduction of ulcerogenic activity and anti-inflammatory activities were obtained in all cases as compared to Naproxen. Among synthesized prodrugs, viz. A-5, A-6 and A-8 showed significant anti-inflammatory activity and A-2 and A-5 showed reduced ulcerogenic activity compared to Naproxen. In acute oral toxicity studies, all synthesized prodrugs were found to be non toxic at dose of 2000 mg/kg.

Keywords: *Naproxen; Prodrugs; Glycolamide ester conjugates; Characterization; Pharmacological activity.*

INTRODUCTION

Naproxen, one of non-steroidal anti-Inflammatory Drugs (NSAIDs), could not be used up to its potential, because of its adverse reactions offered due to presence of free carboxylic acid group. The NSAIDs are widely used for indications extending from inflammation and pain to cardiovascular and genitourinary diseases. In the recent years, a number of NSAIDs have been introduced into clinical practice. The research is on to relieve pain and inflammation with freedom of undesirable effects. Gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs and often these reactions lead to GIT ulceration and hemorrhage. GI mucosal injury produced by NSAIDs is generally believed to be caused by two different mechanisms[1]. The first mechanism involves a local action composed of a direct contact while the other has indirect effect on the GI mucosa. The direct contact effect can be attributed to a combination of a local irritation produced by acidic group of NSAIDs and local inhibition of prostaglandin synthesis in the GI tract. The indirect effect can be attributed to combination of an ion trapping mechanism of NSAIDs from the lumen into the mucosa. The second mechanism is based on a generalized systemic action occurring after absorption, which can be demonstrated following intravenous dosing. Recently, considerable attention has been focused in the development of bio-reversible derivatives by temporarily masking the acidic group of NSAIDs, as a promising mean of reducing or abolishing the GI toxicity.

Prodrug has been the concept of retro metabolic drug design that incorporates targeting, metabolism and the duration of action consideration into the design process. The carboxylic group of NSAIDs can be temporarily masked and its direct effect on gastric mucosa can be minimized.

In the present study well-recognized NSAID, viz. naproxen was selected, which produces gastrointestinal side effects. Literature review revealed that many efforts had been made to synthesize prodrug via masking carboxylic acid group such as acyloxyalkyl ester of ketoprofen and naproxen prodrug have been synthesized to improve the dermal delivery[2]. Glucosamine conjugate prodrug of flurbiprofen was synthesized to reduce their GIT irritation[3]. Alkyl ester prodrug of Ibuprofen had been synthesized to reduce gastrointestinal side effect associated with long term oral administration[4]. Aminocarbonyloxy/methyl ester prodrug of naproxen and flufenamic acid have been synthesized to improve its transdermal delivery[5]. Ester prodrugs of naproxen have been synthesized using N-hydroxy methyl succinimide and N-hydroxy methyl isatin as promoieties to reduce their GIT irritation and improve bioavailability[6]. Polymeric prodrugs of naproxen have also been synthesized to improve the

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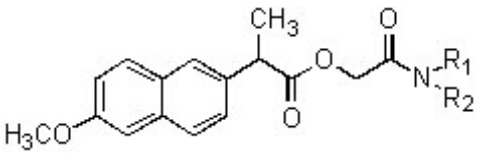
potency and duration of action[7]. A putative prodrug of ibuprofen and glyceryl-3-nitrate, glyceryl-1,2-diibuprofenate-3-nitrate showed less gastric irritation[8]. An increase in permeability of Niflumic acid to brain by developing its triglyceride prodrugs has been observed[9]. Prodrugs of several NSAIDs, such as diclofenac, ibuprofen, ketoprofen, etc. have been synthesized using 1,4-dihydro-1- methylpyridine-3-carboxylate as a carrier to brain to treat Alzheimer's disease[10].

Thus present work aims to synthesize ester prodrugs of Naproxen using Glycolamide ester conjugates to get nontoxic prodrugs with minimized GIT disturbances while maintaining the therapeutic activity. The list of synthesized prodrugs along with product codes, chemical names, possible trivial names and molecular structures is given in Table 1.

Table 1: *List of synthesized prodrugs of Naproxen*



Prodrug Code	Chemical name	R_1	R_2
A-1	2-oxo-2-(phenylamino) ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_6H_5
A-2	2-(4-chlorophenylamino) -2-oxoethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_6H_4Cl
A-3	2-(p-tolylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_7H_7
A-4	2-(4-nitrophenylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	$C_6H_4NO_2$
A-5	2-(4-fluorophenylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_6H_4F
A-6	2-(cyclohexylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_6H_{11}
A-7	2-(dimethylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	CH_3	CH_3
A-8	2-(diethylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	C_2H_5	C_2H_5
A-9	2-(butylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	H	C_4H_9
A-10	2-(dibutylamino) -2-oxo ethyl-2-(6-methoxynaphthalene-2-yl) Propanoate	C_4H_9	C_4H_9

MATERIALS AND METHODS

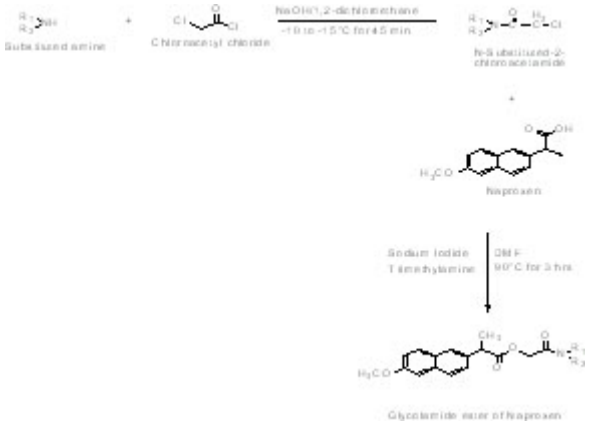
Materials

Substituted amine, chloroacetyl chloride, sodium hydroxide, 1,2-dichloroethane, trimethylamine, sodium iodide were procured from M/S Hi-Media Ltd., Mumbai. Drug Naproxen was obtained as gift sample from M/S Knoll, Mumbai. Other reagents and solvents used were of analytical/spectroscopic/HPLC grade as the case desired.

Synthesis of N-substituted-2-chloroacetamide

A mixture of 29.3 g of appropriate amine, 500 mL of 20% sodium hydroxide solution and 150 mL of 1,2-dichloroethane was taken and the mixture was added to 56.3 g of chloroacetyl chloride at temperature of -10°C to -15°C over a period of 45 minutes. The temperature was then raised to 10°C. After completion of the reaction (monitored by TLC), 200 mL water was added and extracted with dichloromethane (250 mL). The combined organic layer was washed with 5% sodium bicarbonate solution (200 mL) followed by brine (200 mL). The organic layer was then dried over anhydrous sodium sulphate and concentrated under vacuum to obtain a crude solid of N-substituted-2-chloroacetamide. Schematic representation of the reaction is given in Scheme 1.

Scheme 1: Chemical reaction adopted for the synthesis of ester prodrugs of Naproxen



Synthesis of glycolamide ester of Naproxen

A mixture of naproxen (0.01 M), appropriate N-substituted-2-chloroacetamide (0.011 M), sodium iodide (0.001 M), trimethylamine (0.011 M) in DMF (10 mL) was stirred at 90°C for 3 h. After completion of reaction (monitored by TLC), 200 mL water was added and extracted with dichloromethane (250 mL). The combined organic layer was washed with 5% sodium bicarbonate solution (200 mL) followed by brine (200mL). The organic layer was then dried over anhydrous sodium sulphate and concentrated under vacuum to obtain a crude solid, which was recrystallized from ethyl acetate-hexane mixture to get the corresponding glycolamide esters (A-1 to A-10). Schematic representation of the reaction is given in Scheme 1.

Characterization of synthesized prodrugs

The synthesized prodrugs were subjected to thin layer chromatography to check the completion of reactions. The prepared plates of silica gel G were dried and activated. The solvent system chloroform: methanol:ammonia 75:3:0.5 was used for naproxen.

Iodine vapor was used for the detection of spot. All the synthesized prodrugs were produced as single spot. The melting points of the synthesized prodrugs was determined by open capillary tube using Toshniwal melting point apparatus and are uncorrected.

The IR spectra of the prodrugs were obtained on IR spectrophotometer (ABB Boomem FTIR) in KBr pellets. The PMR spectral analyses of the synthesized prodrugs were done on NMR spectrophotometer (JOEL GSX400) using $CDCl_3$ as solvent .The mass was determined on QP5000 Shimadzu mass spectrometer. The elemental analysis of synthesized prodrugs was performed on FLASH 2000 HT Elemental analyzer (Thermo scientific). The purity of the synthesized prodrugs were done on HPLC analyser (Agilent) using YMC hydrosphere C_{18} (4.6 X 150)mm, 3.0 µ column and 0.05% TFA in water and acetonitrile as mobile phase.

Biological evaluations

All the synthesized prodrugs along with naproxen were evaluated for acute oral toxicity studies, anti-inflammatory and ulcerogenic activities. The prodrugs were compared with naproxen for these activities. The methods employed for this purpose are as follows.

Acute oral toxicity studies

Acute oral toxicity study of synthesized prodrugs was carried out in swiss albino rats of either sex (120-165 g) according to OECD guidelines no. 423. Synthesized prodrugs at different doses upto 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, toxic reaction and mortality upto 48 h[11].

Anti-inflammatory activity

The anti-inflammatory activity of synthesized prodrugs was determined by carrageenan-induced rat hind paw oedema method[12] utilizing carrageenan as phlogistic agent (0.1 mL of 1% w/v solution). The animals used were Wistar rats (albino rats). Rats (100-200 g) were divided into twelve groups, each comprising of six rats, including a control and standard group. The Group I was treated with carrageenan 0.1 mL/kg. This group served as control. The Group II was treated with naproxen 100 mL/kg, i.p. This group served as standard. The Group III to XII was treated with synthesized prodrugs 100 mL/kg, i.p. This group served as test. The initial volume of left hind paw of albino rats was measured by plethysmograph, without administration of the drug/prodrugs. After one hour of administration of prodrugs, 0.1 ml of carrageenan was injected into the lateral malleolus of the sub-plantar region of the left hind paw. Inflammation was determined for all the animals by using plethysmograph and after the administration of carrageenan at 1, 2, 3 and 4 h respectively.

Ulcerogenic activity

Albino rats weighing 150-200 g were starved for overnight having access to drinking water. During this time they were housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Six animals were used for synthesized prodrugs and control group. Under ether anaesthesia, a midline abdominal incision was made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. Grasping the stomach with instrument is to be meticulously avoided; else ulceration will invariably develop at such points. The abdominal wall was closed by suturing it. The synthesized prodrugs were given orally by gavage after the animals recovered from anesthesia.

The animals were deprived of food and water post operatively and the animals were sacrified after 4 h of pyloric ligation. The stomach was dissected out along the greater curvature and examined for lesions and the contents were drained in the centrifuge tubes and subjected to the analysis for pH, gastric volume, free acidity and total acidity. The stomach was observed and subjected to histopathological studies and ulcer/ lesions were counted. Ulcer index was calculated[13].

The gastric content was centrifuged at 1000 rpm for 10 min, the supernatant liquids were transferred to the measuring cylinder and the volume was measured. 1 mL of supernatant was diluted with 9 mL of distilled water and was titrated with 0.1N sodium hydroxide run from a microburette using 3-4 drops of Topfers reagent as indicator until canary yellow colour was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink color was restored. This gives total acidity. Free acidity and total acidity was expressed in terms of mL of 0.1N HCl per 100 g of gastric contents. This is the same as mEq/Lit/ 100g. To obtain this figure multiply the burette reading obtained from titration by 10.

Each stomach was examined grossly and the ulcers were graded according to the method suggested by J. Kunchandy[14]. Mean ulcer score for each animal is expressed as ulcer index.

Overall there are seven groups of animals consisting six rats, normal control group is not ligated, while positive control is ligated but not protected against ulceration. Third control group is previously protected with standard drug Ranitidine 20 mg/kg body weight. Remaining five groups were treated with synthesized prodrugs with doses 100 mg/kg.

Results obtained from the above mentioned ulcerogenic activity parameters were subjected for

intergroup difference; each parameter was analyzed separately using Graph pad prism software and one way analysis of variance (ANOVA) was carried out. Dunnets test was used for individual comparisons[15].

RESULTS AND DISCUSSIONS

Naproxen is a propionic acid derivative of NSAID drug. It has the side effects of GI toxicity, CVS, CNS, etc., due to the direct local effect caused by its free carboxylic acid. In order to reduce the GI toxicity, the glycolamide esters of naproxen prodrugs were synthesized. The glycolamide ester of Naproxen prodrugs (A-1 to A-10) were prepared by the reaction of appropriate N-substituted-2-chloroacetamide with naproxen. The purity of all the synthesized compounds was confirmed by HPLC and melting point and the results are shown in Table 2. The synthesized prodrugs were characterized by IR, ^{1}H NMR, Mass spectroscopy and Elemental analysis. All the synthesized prodrugs showed characteristic absorption in IR, ^{1}H NMR. Expected molecular (M^+) fragments were observed for all the synthesized prodrugs in the mass spectra. IR, ^{1}H NMR, mass spectral and HPLC data of the synthesized prodrugs are listed in Table 3 and elemental analysis data of the synthesized prodrugs are listed in Table 4. These data are in conformity with the structure.

Table 2: *Physicochemical properties of the synthesized prodrugs*

Prodrug Code	Molecular formula	M/W Calculated	(%) Yield	R_f value	Melting point (°C)[a]
A-1	$C_{22}H_{21}NO_4$	363	63	0.73	160-163
A-2	$C_{22}H_{20}ClNO_4$	397	65	0.77	135-138
A-3	$C_{23}H_{23}NO_4$	377	69	0.68	128-130
A-4	$C_{23}H_{24}N_2O_4$	408	61	0.72	118-121
A-5	$C_{22}H_{20}FNO_4$	381	67	0.70	175-178
A-6	$C_{23}H_{23}NO_4$	369	65	0.74	120-123
A-7	$C_{18}H_{21}NO_4$	315	62	0.69	58-61
A-8	$C_{20}H_{25}NO_4$	343	69	0.68	112-114
A-9	$C_{20}H_{25}NO_4$	343	63	0.70	91-94
A-10.	$C_{24}H_{25}NO_4$	399	66	0.71	106-108

[a]*Melting point are uncorrected* [b] *Solvent system – Chloroform: methanol:ammonia 75:3:0.5*

The results of acute oral toxicity studies indicate that none of the prodrugs studied showed any behavioral changes, toxic reaction and mortality even after 48 h. The synthesized prodrugs were found to be safe at the dose of 2000 mg/kg.

Anti-inflammatory activity of the synthesized prodrugs was carried out by carrageenan induced hind paw oedema method in rats. The activity of synthesized prodrugs was compared with the Naproxen at dose of 100 mg/kg. The prodrugs A-5, A-6 and A-8 exhibited significant anti-inflammatory activity. The prodrugs A-3, A-7, A-9 and A-10 showed moderate significant activity. The prodrugs A-1, A-2 and A-4 showed mild anti-inflammatory activity as shown in Table 5. This activity might be due to the various substituents on secondary and tertiary amides in naproxen prodrugs.

Table 3. *IR, ^{1}H NMR, mass spectral and HPLC characterization of synthesized prodrugs of Naproxen*

Prodrug Code	Characteristic peak of IR spectra	Characteristic peak of ^{1}H NMR spectra and mass spectra	HPLC
A-1	3008 (aromatic CH str.), 1652 (C=O str. of ester), 3281 (CONHR str., d 2° amide), 1091 (aromatic CO-CH- str.), 804 (aromatic C-C str.), 1597 (aromatic C=C str.)	δ 7.07.5 (m, 6H, naphthalene H), 7.0-7.6 (m, 5H, aromatic H), 3.5 (s, 1H, aromatic CH), 1.5 (d, 3H, RCH-CH-), 3.8 (s, 3H, aromatic O-CH-), 5.0 (d, 2H, -COOCH-), 8.0 (s, 1H, -CO-NH) and m/z 363.	97.5 %
A-2	3144 (aromatic CH str.), 1701 (C=O str. of ester), 3389 (CONHR str. of 2° amide), 1077 (aromatic CO-CH- str.), 853 (aromatic C-C str.), 1603 (aromatic C=C str.), 818 (aromatic C-Cl str.)	δ 7.0-7.6 (m, 6H, naphthalene H), 7.25-7.5 (m, 4H, aromatic H benzene), 1.5 (d, 3H, RCH-CH-), 3.75 (s, 1H, aromatic CH), 1.5 (s, 3H, aromatic O-CH-), 5.1 (d, 2H, -COOCH-), 8.1 (s, 1H, -CO-NH) and m/z 397.	98.2 %
A-3	3083 (aromatic CH str.), 1730 (C=O str. of ester), 3393 (CONHR str. of 2° amide), 1056 (aromatic CO-CH- str.), 825 (aromatic C-C str.), 1606 (aromatic C=C str.), 2939 (aromatic C-CH-).	δ 6.25-7.5 (m, 6H, naphthalene H), 7.0-7. 1(m, 4H, aromatic H benzene), 1.5 (s, 3H, aromatic CH-), 3.5 (s, 1H, aromatic CH) 2.0-2.5 (d, 3H, RCH-CH-), 3.7 (s, 3H, aromatic O-CH-), 5.0 (d, 2H, -COOCH-), 8.0 (s, 1H, -CO-NH) and m/z 377.	97.2 %
A-4	3056 (aromatic CH str.), 1771 (C=O str. of ester), 3273 (CONHR str. of 2° amide), 1079 (aromatic CO-CH- str.), 962 (aromatic C-C str.), 1602 (aromatic C=C str.), 1498 (aromatic C-NO-).	δ 6.57.2 (m, 6H, naphthalene H), 7.5-7.8 (m, 4H, aromatic H benzene), 3.25 (s, 1H, aromatic CH), 1.5 (d, 3H, RCH-CH-), 3.53.7 (s, 3H, aromatic O-CH-), 5.1 (d, 2H, -COOCH-), 8.2 (s, 1H, -CO-NH) and m/z 408.	95.6 %
A-5	3105 (aromatic CH str.), 1686 (C=O str. of ester), 3482 (CONHR str. of 2° amide), 1112 (aromatic CO-CH- str.), 851 (aromatic C-C str.), 1598 (aromatic C=C str.), 1340 (aromatic C-F str.).	δ 6.27.8 (m, 6H, naphthalene H), 6.2-7.8 (m, 4H, aromatic H benzene), 3.5 (s, 1H, aromatic CH), 1.8 (d 3H, RCH-CH-), 3.2-3.8 (s, 3H, aromatic O-CH-), 5.2 (d, 2H, -COOCH-), 8.0 (s, 1H, -CO-NH) and m/z 381.	98.3 %
A-6	3003 (aromatic CH str.), 1728 (C=O str. of ester), 3192 (CONHR str. of 2° amide), 1050 (aromatic CO-CH- str.), 896 (aromatic C-C str.), 1604 (aromatic C=C str.).	δ 6.57.2 (m, 6H, naphthalene H), 3.5 (s, 1H, CH cyclohexane), 3.4 (s, 1H, aromatic CH), 1.5 (d, 3H, RCH-CH-), 3.7 (s, 3H, aromatic O-CH-), 4.8 (d, 2H, -COOCH-), 1.4-1.8 (m, 10H, -CH- cyclohexane), 8.2 (s, 1H, aromatic NH) and m/z 389.	97.8 %
A-7	3099 (aromatic CH str.), 1716 (C=O str. of ester), 3192 (CONR, str. of 3° amide), 1074 (aromatic CO-CH- str.), 859 (aromatic C-C str.), 1599 (aromatic C=C str.), 1380 (N(CH-)- str. of amide)	δ 6.47.5 (m, 6H, naphthalene H), 3.5 (s, 1H, aromatic CH), 1.4 (d, 3H, RCH-CH-), 2. 53.0 (dd,6H, N(CH-)-), 3.6 (s, 3H, aromatic O-CH-), 4.8 (d, 2H, -COOCH-) and m/z 315.	98.2 %
A-8	3103 (aromatic CH str.), 1717 (C=O str. of ester), 3197 (CONR, str. of 3° amide), 1074 (aromatic CO-CH- str.), 800 (aromatic C-C str.), 1591 (aromatic C=C str.).	δ 6.57.5 (m, 6H, naphthalene H), 3.4 (s, 1H, aromatic CH), 1.5 (d, 3H, RCH-CH-), 1.01.4 (dd, 10H, N(C-H-)-), 3.6 (s, 3H, aromatic O-CH-), 5.0 (d, 2H, -COOCH-) and m/z 343.	97.8 %
A-9	3109 (aromatic CH str.), 1721 (C=O str. of ester), 3444 (CONHR str. of 2° amide), 1074 (aromatic CO-CH- str.), 955 (aromatic C-C str.), 1599 (aromatic C=C str.).	δ 6.37.3 (m, 6H, naphthalene H), 3.6 (s, 1H, aromatic CH), 1.43 (d, 3H, RCH-CH-), 3.7 (s, 3H, aromatic O-CH-), 4.8 (d, 2H, -COOCH-), 2.2-3.2 (m, 6H, -NCH-CH-CH-), 0.9-1.0 (s, 3H, -CH-), 8.0 (s, 1H, NH) and m/z 343.	98.7 %
A-10	3108 (aromatic CH str.), 1716 (C=O str. of ester), 3192 (CONR, str. of 3°amide), 1045 (aromatic CO-CH- str.), 955 (aromatic C-C str.), 1621 (aromatic C=C str.).	δ 6.47.5 (m, 6H, naphthalene H), 3.5 (s, 1H, aromatic CH), 1.6 (d, 3H, RCH-CH-), 3.5 (s, 3H, aromatic O-CH-), 4.9 (d, 2H, -COOCH-), 1.8-2.0 (m, 6H, -NCH-CH-CH-), 2.0-2.5 (m, 6H, -NCH-CH-CH-), 1.0 (s, 6H, -CH-) and m/z 399.	98.3 %

Table 4: *Elemental analysis of the synthesized prodrugs*

Prodrugs Code	Molecular formula	(%) Carbon		(%) Hydrogen		(%) Nitrogen	
		Calculated	Obtained	Calculated	Obtained	Calculated	Obtained
A-1	$C_{22}H_{21}NO_4$	72.71	72.89	5.82	5.81	3.85	3.82
A-2	$C_{22}H_{20}ClNO_4$	66.42	66.41	5.07	5.04	3.52	3.52
A-3	$C_{23}H_{23}NO_4$	73.19	73.17	6.14	6.15	3.71	3.70
A-4	$C_{23}H_{24}N_2O_4$	64.70	64.68	4.94	4.92	6.85	6.84
A-5	$C_{22}H_{20}FNO_4$	69.28	69.28	5.29	5.28	3.67	3.67
A-6	$C_{23}H_{23}NO_4$	71.52	71.53	7.37	7.37	3.79	3.80
A-7	$C_{18}H_{21}NO_4$	68.55	68.53	6.71	6.69	4.44	4.42
A-8	$C_{20}H_{25}NO_4$	69.95	69.92	7.34	7.35	4.08	4.08
A-9	$C_{20}H_{25}NO_4$	69.95	69.95	7.34	7.32	4.08	4.06
A-10.	$C_{24}H_{25}NO_4$	72.15	72.13	8.33	8.33	3.50	3.50

Table 5: *Evaluation of anti-inflammatory activity by carrageenan induced acute paw oedema in rats*

Groups	Dose mg/kg	Paw volume in mean ± SEM				
		Initial	1st hour	2nd hour	3rd hour	4th hour
1	100 mg/kg	0.550±0.003[a]	0.558±0.007[ns]	0.548±0.023[a]	0.498±0.009[a]	0.540±0.018[a]
2	100 mg/kg	0.557±0.012[a]	0.560±0.006[ns]	0.528±0.009[a]	0.510±0.003[a]	0.530±0.003[a]
3	100 mg/kg	0.540±0.002[a]	0.548±0.005[ns]	0.525±0.009[a]	0.475±0.012[a]	0.510±0.005[a]
4	100 mg/kg	0.548±0.007[a]	0.550±0.003[ns]	0.530±0.014[ns]	0.482±0.014[a]	0.526±0.006[a]
5	100 mg/kg	0.532±0.009[a]	0.542±0.004[ns]	0.475±0.006[a]	0.452±0.004[***]	0.528±0.003[ns]
6	100 mg/kg	0.542±0.011[a]	0.551±0.004[ns]	0.488±0.005[***]	0.463±0.021[***]	0.521±0.006[a]
7	100 mg/kg	0.525±0.006[a]	0.538±0.005[ns]	0.488±0.016[a]	0.471±0.008[a]	0.528±0.006[a]
8	100 mg/kg	0.543±0.006[a]	0.548±0.004[ns]	0.468±0.003[***]	0.436±0.007[***]	0.547±0.005[a]
9	100 mg/kg	0.531±0.007[a]	0.538±0.013[ns]	0.462±0.007[***]	0.462±0.013[a]	0.462±0.005[a]
10	100 mg/kg	0.542±0.004[a]	0.551±0.003[ns]	0.502±0.006[***]	0.482±0.016[a]	0.523±0.012[a]
Standard Naproxen	100 mg/kg	0.550±0.016[a]	0.605±0.005[ns]	0.580±0.024[ns]	0.538±0.004[a]	0.574±0.003[a]

*Significant differences with respect to control group was carried out using't' test followed by one way ANOVA, *p<0.05, **p<0.01, ***p<0.001 and NS – Non significant*

Ulcerogenic activity of the synthesized prodrugs was carried out by pyloric ligation method in rats. The activity of synthesized prodrugs was compared with the Naproxen at dose of 100 mg/kg. The prodrugs A-2 and A-5 showed reduced ulcerogenic activity when compared to Naproxen. The prodrugs A-1, A-3 and

A-4 showed moderately reduced ulcerogenic activity compared to Naproxen. The results are as shown in Table 6. This activity might be due to the various substituents on secondary and tertiary amides in naproxen prodrugs.

Table 6: *Evaluation of reduced ulcerogenic activity by pyloric ligation method in rats*

S. No	Treatment	Dose	Total volume	pH	Ulcer index	Total Acidity	Free acidity
1	Control	5% CMC	5.20±0.23	2.1±0.03	0.25±0.20	37.5±0.16	24.0±0.16
2	A-1	10 mg/kg	5.68±0.24ns	1.18±0.09*	1.67±0.21*	38.6±0.019*	26.0±0.12**
3	A-2	10 mg/kg	5.32±0.26 ns	1.48±0.12***	1.42±0.27**	41.0±0.13***	30.4±0.14**
4	A-3	10 mg/kg	5.37±0.22*	1.30±0.03**	1.50±0.22**	38.6±0.17**	26.3±0.12**
5	A-4	10 mg/kg	5.69±0.28ns	1.28±0.06*	1.52±0.22**	39.0±0.22**	27.6±0.18**
6	A-5	10 mg/kg	5.2 8±0. 19 ns	1.46±0.08***	1.33±0.22***	42.2±0.13***	31.5±0.19***
7	Standard Naproxen	10 mg/kg	5.75±0.003*	1.07±0.052*	2.67±0.003*	51.5±0.034*	35.1±0.008*

*Significant differences with respect to control group was carried out using't' test followed by one way ANOVA, *p<0.05, **p<0.01, ***p<0.001*

ACKNOWLEDGMENT

We express our cordial thanks to Mrs. Grace Rathnam, Principal and Mrs. C.N. Nalini, Assistant professor, C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India, for supporting this work.

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