Int. J. Chem. Sci.: 12(3), 2014, 1033-1043
ISSN 0972-768X
www.sadgurupublications.com

SYNTHESIS AND EVALUATION OF AMIDE PRODRUGS OF MEFENAMIC ACID

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ABSTRACT

A series of amide prodrugs of mefenamic acid (a known non-steroidal anti-inflammatory drug) have been synthesized with an aim to obtain new compounds with potential analgesic and anti-inflammatory activity. The structures of all synthesized compounds were confirmed by means of infrared, proton magnetic resonance and mass spectroscopy. All compounds were evaluated for their anti-inflammatory activities by carrageenan-induced rat paw edema test. Most of the synthesized compounds induced significant reduction in the writhing response when compared to control. The anti-inflammatory activity of these compounds were evaluated and showed significant anti-inflammatory activity in comparison to control but their effect was weaker than mefenamic acid.

Key words: Amide prodrugs, Anti-inflammatory activity, Ulcerogenicity, Mefenamic acid, Carrageenan.

INTRODUCTION

Mefenamic acid (MA) is 2-[(2, 3-dimethylphenyl) amino] benzoic acid, belonging to the class of N-arylanthranilic acid. It is one of the newly developed non-steroidal anti-inflammatory agents, which act as cycloxygenase-I inhibitor, indicated in various inflammatory and rheumatic conditions. The main side effects of MA include GI disturbance, peptic ulceration and gastric bleeding. These gastroenteropathies are generally believed to be resulted from the direct contact mechanism appears to play a important role in the production of gastrointestinal lesions and it is due to local irritation produced by the free carboxylic acid group of NSAID's and local inhibition of cytoprotective action of prostaglandines on gastric mucosa[1,2]. An ideal prodrug retains to achieve such a pharmacological profile a prodrug should exhibit optimum physicochemical properties. The

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activity of the parent drug is shown while unwanted side effects are eliminated or notably reduced. The use of prodrugs to provisionally mask the acidic group of NSAIDs has been proposed as an approach to suppress the GI toxicity due to the direct contact effect. 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 aminoacids[3-13]. However, no attempts were made to develop amide prodrugs of NSAIDs using amino acid, which has been utilized as a major tool with other NSAIDs. The advantages of using amino acids for this purpose are due to their characteristics like normal dietary constituent, non toxic in moderate doses, healing effect on gastric toxicity, marked anti inflammatory activity and site specificity.

Temporary masking of the free carboxylic acid group of NSAIDs can improve their gastrointestinal tolerability[14-16]. The amide prodrugs of mefenamic acid have been designed to achieve this very objective and to study various physicochemical characters, anti-inflammatory activity and ulcer index as prodrugs.

EXPERIMENTAL

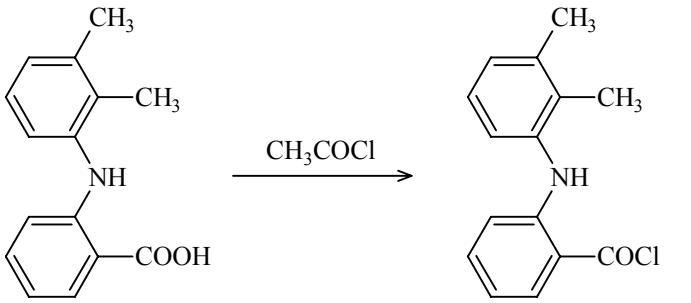
Material and method

IR spectra were recorded using KBr on "JASCO FT-IR 460 plus" instrument by DRIFT method. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution on "FT-NMR Varian Mercury YH-300" using tetramethyl silane (TMS) as internal standard. Chemical shift values are reported in δ, ppm. All reactions were monitored by thin layer chromatography (TLC) carried out on 'Silica Gel G' coated on laboratory micro slides prepared by dipping method or precoated silica gel G 60 F_{254} aluminium plate from Merck and visualization was done by observing in UV light. All the chemicals used were procured from Merck and purity of starting materials used for reactions was confirmed by checking their melting point or boiling point and by thin layer chromatography Mass Spectra were recorded on "Shimadzu GC-MS QP-5050" instrument by direct injection method.

General methods for synthesis of amide prodrugs

Preparation of acid chloride

In a dry round bottom flask equipped with reflux condenser and calcium chloride guard tube, Mefenamic acid (0.1 moles) and Acetyl chloride (0.2 moles) were refluxed for 5 hour. The viscous liquid was immediately poured on Petri dish and was vacuum dried to remove excess acetyl chloride. Yellow coloured crude mefenamic acid chloride was obtained (**Scheme 1**).

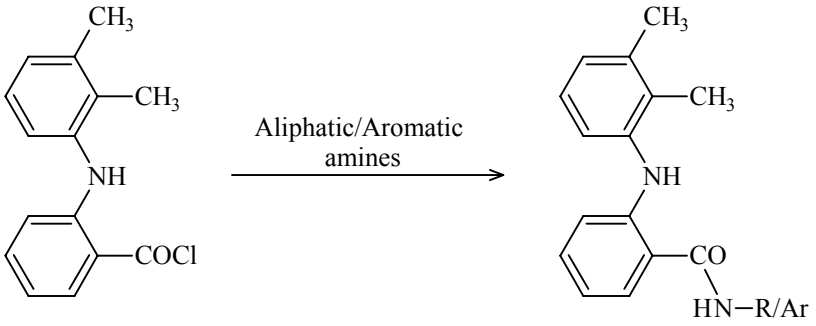


Mefenamic acid Mefenamic acid chloride

Scheme 1: Synthesis of 4'-methyl biphenyl-2–carboxyl chloride

Amidation

In a three-necked flask fitted with a reflux condenser equipped with a calcium chloride drying tube and a dropping funnel, Aliphatic/Aromatic amines (0.012 moles) dissolved in 25 mL of benzene were placed. Mefenamic acid chloride (0.01 moles) diluted with dry benzene was added gradually to it over the period of approximately 1 hr under stirring and cooling in an ice bath. The mixture was stirred for an additional 30 mins and then set aside protected by a calcium chloride guard tube for approximately 10 hrs. The progress of reaction was monitored by thin layer chromatography. The hydrochloride salt of phenol was removed by filtration with suction and washed with two 25 mL portions of benzene. The benzene layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was removed by distillation to give crude product, which was recrystallized from acetonitrile/ methanol (**Scheme 2**).



Mefenamic acid chloride 2-(2,3-dimethylphenylamino)benzamide derivatives

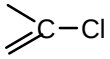
Scheme 2: Generalized procedure for synthesis of 2-(2,3-dimethylphenylamino) benzamide derivatives (Mefenamic acid amide derivatives)

The following compounds were synthesized by using the above procedure.

V-1) N1-(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-(2,3-dimethyl) aniline-1-(N-phenylbenzamide)

Yield 68%, White colourless compound. R_f 0.51 (n-Hexane-Ethyl acetate); IR 1573 (C=C str), 3043 (C-H str), 755 (C-H bend), Aromatic ring; 2977 (C-H str), 1446 (C-H bend), CH_3 Aliphatic; 1650 (C=O Str), C=O amide; 3313 (N-H str), 1257 (Ar C-N str).

V-2) N1-2,6-dichloro(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-2,6-dichoro (2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 61%, White colourless compound. R_f 0.72 (n-Hexane-Ethyl acetate); IR 1569 (C=C str), 3070 (C-H str), 755 (C-H bend), Aromatic ring; 2946 (C-H str), 1465 (C-H bend), CH_3 Aliphatic; 1646 (C=O Str), C=O amide; 3332, 3297 (N-H str), 1253 (Ar C-N str); 678 

V-3) N1-2-hydroxy (2,3-dimethylphenylamino)1-phenylbenzamide: -N1-2-hydroxy (2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 63%, White colourless compound. R_f 0.50 (n-Hexane-Ethyl acetate); IR 1565 (C=C str), 3050 (C-H str), 748 (C-H bend), Aromatic ring; 2985 (C-H str), 1442 (C-H bend), CH_3 Aliphatic; 1654 (C=O Str), C=O amide; 3343 (N-H str), 1249 (Ar C-N str); 1095 (-C-O str.of OH group).

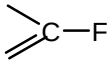
V-4) N1-3-hydroxy(2,3-dimethylphenylamino)1-phenylbenzamide:- N1-3-hydroxy (2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 52%, White colourless compound. R_f 0.59 (n-Hexane-Ethyl acetate); IR 1577 (C=C str), 3070 (C-H str), 759 (C-H bend), Aromatic ring; 2973 (C-H str), 1484 (C-H bend), CH_3 Aliphatic; 1646 (C=O Str), C=O amide; 3389 (N-H str), 1292 (Ar C-N str); 1079 (-C-Ostr.of OH group).

V-5) N1-3-methyl(2,3-dimethylphenylamino)1-phenylbenzamide:- N1-3-methyl(2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 71%, White colourless compound. R_f 0.66 (n-Hexane-Ethyl acetate); IR 1581 (C=C str), 3085 (C-H str), 759 (C-H bend), Aromatic ring; 2962 (C-H str), 1500 (C-H bend), CH_3 Aliphatic; 1645 (C=O Str), C=O amide; 3324 (N-H str), 1292 (Ar C-N str).

V-6) N1-4-fluro(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-4-fluro(2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 67%, White colourless compound. R_f 0.54 (n-Hexane-Ethyl acetate); IR 1573 (C=C str), 3039 (C-H str), 752 (C-H bend), Aromatic ring; 2977 (C-H str), 1446 (C-H bend), CH_3 Aliphatic; 1650 (C=O Str), C=O amide; 3316, 3251 (N-H str), 1257 (Ar C-N str). 1160 

V-7) N1-4-Hydroxy-(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-4-Hydroxy (2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 59%, White colourless compound. R_f 0.50 (n-Hexane-Ethyl acetate); IR 1573 (C=C str), 3070 (C-H str), 752 (C-H bend), Aromatic ring; 2985 (C-H str), 1457 (C-H bend), CH_3 Aliphatic 1646 (C=O Str), C=O amide; 3282, (N-H str), 1276 (Ar C-N str).1106 C-O str. of OH group.

V-8) N1-4-Methoxy-(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-4-Methoxy (2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 70%, White colourless compound. R_f 0.64 (n-Hexane-Ethyl acetate); IR 1573 (C=C str), 3070 (C-H str), 767 (C-H bend), Aromatic ring; 2981 (C-H str), 1461 (C-H bend), CH_3 Aliphatic 1640 (C=O Str), C=O amide; 3282, (N-H str), 1250 (Ar C-N str). 1106 C-O str. of Methoxy group.

V-9) N1-Napthyl-(2,3-dimethylphenylamino)1-phenylbenzamide:-N1-Naphthyl(2,3-dimethyl) aniline-1-(N-phenylbenzamide)

Yield 58%, White colourless compound. R_f 0.67 (n-Hexane-Ethyl acetate); IR 1577 (C=C str), 3050 (C-H str), 771 (C-H bend), Aromatic ring; 2938 (C-H str), 1457 (C-H bend), CH_3 Aliphatic 1627 (C=O Str), C=O amide; 3347, 3328 (N-H str), 1284 (Ar C-N str).

V-10) N1-Dimethyl-(2,3-dimethylphenylamino)1-phenylbenzamide:- N1-Dimethyl-(2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 47%, White colourless compound. R_f 0.58 (n-Hexane-Ethyl acetate); IR 1573 (C=C str), 3062 (C-H str), 759 (C-H bend), Aromatic ring; 2942 (C-H str), 1446 (C-H bend), CH_3 Aliphatic 1643 (C=O Str), C=O amide; 3486, 3401 (N-H str), 1253 (Ar C-N str).

V-11) N1-Ethyl-(2,3-dimethylphenylamino)1-phenylbenzamide:- N1-Ethyl-(2,3-dimethyl) aniline-1-(N-phenylbenzamide)

Yield 57%, White colourless compound. R_f 0.58 (n-Hexane-Ethyl acetate); IR 1580 (C=C str), 3051 (C-H str), 760 (C-H bend), Aromatic ring; 2978 (C-H str), 1485 (C-H bend), CH_3 Aliphatic 1650 (C=O Str), C=O amide; 3317 (N-H str), 1290 (Ar C-N str).

V-12) N1-Methyl-(2,3-dimethylphenylamino)1-phenylbenzamide:- N1-Methyl-(2,3-dimethyl)aniline-1-(N-phenylbenzamide)

Yield 73%, White colourless compound. R_f 0.62 (n-Hexane-Ethyl acetate); IR 1569 (C=C str), 3046 (C-H str), 755 (C-H bend), Aromatic ring; 2965 (C-H str), 1470 (C-H bend), CH_3 Aliphatic 1655 (C=O Str), C=O amide; 3380 (N-H str), 1295 (Ar C-N str).

Biological activity

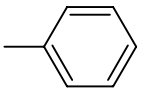
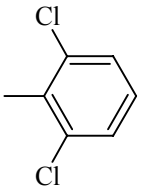
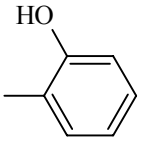
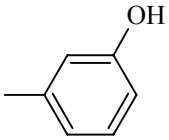
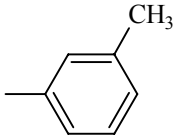
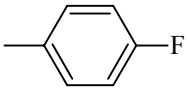
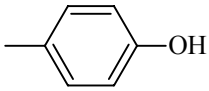
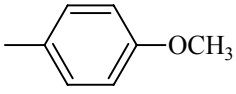
The following compounds were screened for ulcerogenic activity by One way ANOVA followed by Dunnets T Test. The anti-inflammatory activity of twelve synthesized compounds was determined by carrageenan-induced hind paw oedema method.

Ulcerogenic studies

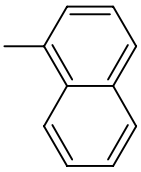
For comparison of Ulcerogenic potential of Mefenamic acid and synthesized prodrugs in laboratory animals, the fasted rat model was utilized. The animals were kept in standard laboratory conditions and fasted for 24 hr before dosing. However, water was given *ad libitum*. Rats were divided into six groups each consisting of six rats. Control and untreated group of animals was given 0.5% carboxy methyl cellulose (CMC) suspension. Compounds including mefenamic acid and amide prodrugs were administered orally as a 1 mg/Kg suspension in 0.5% CMC for the period of 14 days. All rats of respective groups were sacrificed after 60 mins after the last dosing. Stomach was removed and cut open from greater curvature to examine the mucosal surface for ulcers under illumination. Ulcer index was calculated using following scale: 1 x (number of ulcers level I i.e. ulcer area < 1 mm^2) + 2 x (number of ulcers level II ie ulcer area 1-3 mm^2) + 3 x (number of ulcers level III i.e. ulcer area > 3 mm^2). The ulcers can be scored depending upon the degree of ulcers produced as:

Number of hemorrhagic spots was also calculated and every five hemorrhagic spots were considered equivalent to 1 mm of ulcer[17] (Table 2).

Table 1: Physical data of compounds

Compd. code	Amine	R/Ar	Molecular formula	Mol. Wt.	M.P.* (^oC)	% Yield	$R_f^{\#}$
V1	Aniline		$C_{21}H_{20}N_2O$	316.4	112-114	68	0. 51
V2	2,6-Dichloro Aniline		$C_{21}H_{18}C_{l2}N_2O$	385.29	136-138	61	0.72
V3	2-Hydroxy Aniline		$C_{21}H_{20}N_2O_2$	332.4	127-131	63	0.50
V4	3- Hydroxy Aniline		$C_{21}H_{20}N_2O_2$	332.4	132-136	52	0.59
V5	3-Methyl Aniline		$C_{22}H_{22}N_2O$	330.42	160-165	71	0.66
V6	4-Fluoro Aniline		$C_{21}H_{19}FN_2O$	334.39	180-183	67	0.54
V7	4-Hydroxy Aniline		$C_{21}H_{20}N_2O_2$	332.4	138-142	59	0.50
V8	4-Methoxy Aniline		$C_{22}H_{22}N_2O_2$	346.42	152-156	70	0.64

Cont…

Compd. code	Amine	R/Ar	Molecular formula	Mol. Wt.	M.P.* (^oC)	% Yield	R_f #
V9	1-Napthyl Amine		$C_{25}H_{22}N_2O$	366.45	115-120	58	0.67
V10	Dimethyl amine	N, N-Dimethyl	$C_{17}H_{20}N_2O$	268.35	180-184	47	0.42
V11	Ethyl Amine	- CH_2CH_3	$C_{17}H_{20}N_2O$	268.35	167-169	57	0.58
V12	Methyl Amine	-CH_3	$C_{16}H_{18}N_2O$	254.33	129-132	73	0.62

*Uncorrected, # Solvent system: n-Hexane: Ethyl acetate (3:2)

Table 2: 14 days treatment: V series

Group	Treatment	Ulcer index (Mean ± SEM)
1	Mefenamic acid control	19.66 ± 0.614
2	Diclofenac control	17.00 ± 0.683#
3	V-2/20	11.00** ± 0.577###
4	V-4/20	09.33** ± 0.494###
5	V-9/40	08.83** ± 0.497###

Anti-inflammatory activity

The anti-inflammatory activity of twelve synthesized compounds was determined by carrageenan-induced hind paw oedema method. Rats of either sex with a body weight between 150 to 250 g were starved overnight. Drug was administered in the form of suspension prepared in 5% carboxy methyl cellulose. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 mL of 1% solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection and periodically at every 30 mins for

2 hours. Diclofenac was used as standard for the test. The results were analysized statistically by one way ANOVA method at 95% confidence interval[18] (Table 3).

Table 3: Anti-inflammatory activity of synthesized compounds

Code No.	0 min	3 hr	6 hr	24 hr
Cont	1.75 ± 0.041	3.00 ± 0.094	4.40 ± 0.171	3.17 ± 0.117
V/1-20	1.77 ± 0.034	2.85 ± 0.075	4.16 ± 0.088	3.32 ± 0.144
V/1-40	1.74 ± 0.035	2.95 ± 0.052	3.87* ± 0.077	3.61 ± 0.150
V/2-20	1.67 ± 0.028	2.38** ± 0.067	2.58** ± 0.136	2.67* ± 0.093
V/2-40	1.69 ± 0.034	2.07** ± 0.066	2.17** ± 0.057	2.66* ± 0.082
V/3-20	1.73 ± 0.037	2.91 ± 0.050	3.99 ± 0.074	3.13 ± 0.144
V/3-40	1.78 ± 0.052	2.70* ± 0.050	3.38** ± 0.167	2.63* ± 0.089
VE/4-20	1.88 ± 0.046	2.87 ± 0.050	3.94 ± 0.052	3.85 ± 0.056
VE/4-40	1.81 ± 0.040	2.91 ± 0.059	3.69 ± 0.030*	3.88 ± 0.055
VE/5-20	1.80 ± 0.044	2.97 ± 0.025	3.93 ± 0.046	3.83 ± 0.071
VE/5-40	1.78 ± 0.035	2.68 ± 0.031*	3.70 ± 0.049*	3.96 ± 0.024
VE/6-20	1.86 ± 0.025	2.95 ± 0.028	3.87 ± 0.063	3.82 ± 0.045
VE/6-40	1.79 ± 0.033	2.68 ± 0.034*	3.71 ± 0.063*	3.87 ± 0.063
V/7-20	1.76 ± 0.027	2.86 ± 0.04	4.02 * ± 0.052	3.33 ± 0.076
V/7-40	1.74 ± 0.024	2.78* ± 0.026	3.98* ± 0.033	2.88 ± 0.028
V/8-20	1.71 ± 0.018	2.77* ± 0.025	3.97* ± 0.059	3.09 ± 0.048
V/8-40	1.69 ± 0.017	2.79* ± 0.026	4.01* ± 0.067	3.14 ± 0.047
V/9-20	1.76 ± 0.018	2.79* ± 0.017	3.81** ± 0.047	2.84* ± 0.056
V/9-40	1.75 ± 0.032	2.70** ± 0.049	3.50** ± 0.133	2.62** ± 0.083
V/10-20	1.79 ± 0.024	2.78 ± 0.020	4.18 ± 0.065	3.04 ± 0.089
V/10-40	1.76 ± 0.014	2.80 ± 0.025	4.02 ± 0.037	2.95 ± 0.044
V/1120	1.79 ± 0.010	3.04 ± 0.045	4.38 ± 0.052	3.08 ± 0.074
V/11-40	1.74 ± 0.015	3.09 ± 0.052	4.29 ± 0.066	2.94 ± 0.041
V/12-20	1.78 ± 0.012	3.07 ± 0.052	4.34 ± 0.087	2.99 ± 0.046
V/12-40	1.76 ± 0.018	2.80 ± 0.023	4.08 ± 0.029	3.00** ± 0.063

RESULTS AND DISCUSSION

The objective of the present investigation was to synthesize prodrugs of mefenamic acid for temporary masking of the free carboxylic acid group by amide promoiety to avoid its contact with the gastric mucosa. A series of twelve amide prodrugs of mefenamic acid were synthesized with different aliphatic and aromatic amines for amidation. The varying degree of lipophilic characteristic was the basis behind the selection of phenols. The selected amines were aniline, 2,6-dichloroaniline, 2-hydroxyaniline, 3-hydroxyaniline, 3-methylaniline, 4-fluroaniline, 4-hydroxyaniline, 1-naphylamine, dimethylamine, ethylamine, methylamine. The amidation was done in two steps, *viz*. synthesis of acid chloride followed by amidation process.

The purity and homogeneity of all the synthesized compounds were confirmed by their melting point (uncorrected) & thin layer chromatography. The chemical structures were confirmed by infra-red absorption spectra of all the synthesized compounds. The aromatic Ar-stretching for all derivatives was found to be at the range of 3000-3100 cm^{-1}. The presence of N-H stretching was confirmed by the peak at the range 3200-3390 cm^{-1}. Also some ^{1}H NMR spectra were useful for some proton in the compounds such as 6-8 indicates the presence of phenyl ring protons and mass spectrum of the compounds gives mass of compounds.

ACKNOWLEDGEMENT

Authors are highly thankful to Principal, DSTS Mandal's, College of Pharmacy, Solapur, Maharashtra for providing the infrastructure required for the research work.

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Accepted : 30.06.2014