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Der Pharma Chemica, 2014, 6(6):241-245
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, spectroscopic characterization and activity of stable ester conjugates of mefenamic acid pro-drug as safer NSAID

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ABSTRACT

All NSAIDs are suffering from deadlier GIT toxicity. The free carboxylic group is thought to be responsible for this toxicity. The main aim of the present study was to develop new chemical entities as prodrug of potential anti-inflammatory agents with no gastric toxicity. Various substituted derivatives of Mefenamic acid were synthesized in good yield by esterification. All structures of the newly synthesized compounds were elucidated by elemental analyses, spectral data like infrared, proton magnetic resonance and mass spectroscopy. Prodrugs were also characterized by solubility studies, partition coefficient and hydrolytic studies. The synthesized derivatives are screened for their anti-inflammatory and Ulcerogenic study which show retention of anti-inflammatory activity with reduced Ulcerogenicity.

Keywords: Ester prodrugs, Anti-inflammatory activity, Ulcerogenicity, Mefenamic acid, Carrageenan.

INTRODUCTION

Non Steroidal Anti-Inflammatory Drugs (NSAIDs) is one of the most extensively used categories of therapeutic agents. NSAIDs are used in the management of pain and various acute and chronic inflammatory disorders like rheumatoid arthritis, inflammatory bowel disease etc [1-3]. The mechanism of pharmacological action of the most of the NSAIDs is by peripherally blocking the production of prostaglandins (PG) through non-selective inhibition of enzyme Cyclo-Oxygenases (COX-1 and COX-2) to varying extents [4]. In chronic conditions, NSAIDs are usually recommended in a long term dosage regimen. However, it is observed that the prolonged consumption of these drugs causes gastrointestinal and renal side effects. These effects are due to inhibition of constitutive COX-1 which is responsible for the production of PGs that are important for gastro-protection and vascular homeostasis. In addition, a direct contact of the drugs having acidic groups with gastric mucosa also plays a major role in the development of gastrointestinal lesions [5]. As a result, the gastric disturbance due to NSAIDs is probably a combination of local irritations produced by the free carboxyl group in NSAIDs and by inhibition of the cytoprotective PGs on gastric mucosa. Hence it is a challenge for the researchers to develop the NSAIDs that are biologically effective but devoid of the above side effects.

Kalgutkar et al [6-8] reported that ester and amide derivatives of certain NSAIDs like Indomethacin and Meclofenamic acid selectively inhibited COX-2 and showed that certain amide derivatives of Indomethacin were potent non-ulcerogenic anti-inflammatory agents in the acute inflammation studies. These common approaches to overcome the gastrointestinal side effects of known NSAIDs were also used by many other researchers over the years and has been reviewed recently. For example, 2-formylphenyl esters of Indomethacin, Ketoprofen and Ibuprofen were shown to have more potent anti-inflammatory activity than the parent drugs [9]. In addition,

morpholinoalkyl esters of Naproxen, Indomethacin and Diclofenac were reported to have better bioavailability and be less irritating to the gastric mucosa than the parent compounds [10,11]The proline amides of Ibuprofen were reported as neuroprotective agents and were shown to possess anti-inflammatory activity without ulcerogenic potential. The L-cysteine ethyl ester of a series of NSAIDs including Ibuprofen and Ketoprofen was also found to be a potent anti-inflammatory and antioxidant agent while demonstrating considerably reduced gastrointestinal toxicity [12]. Based on the above findings, it is evident that derivatization of the free carboxylic acid group in NSAIDs represents a suitable strategy for obtaining novel compounds with an improved therapeutic index and even the conversion of non selective COX inhibitor NSAIDs into selective COX-2 inhibitor drugs.

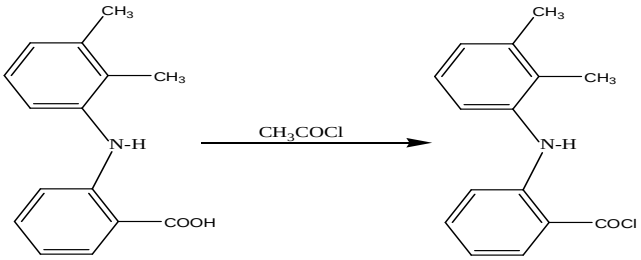
In the present study, an attempt was done to prepare ester derivatives of Mefenamic acid with increased pharmacological activity and low Ulcerogenicity.

MATERIALS AND METHODS

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. Mefenamic Acid (MA) was supplied by Micro Labs Bangalore. Melting points were determined by open capillary tube method on melting point apparatus LABIN and are uncorrected. The progress of reaction was monitored by thin layer chromatography (TLC) carried out on precoated silica gel aluminium plate 60 F_{254} from Merck and detection was done either by observing in UV light or exposure to iodine vapours as required. IR spectra (in KBr pellets) 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 "FTNMR VARIAN MERCURY YH-300" using tetramethyl silane (TMS) as internal standard. Mass Spectra were recorded on "Shimadzu GC-MS QP-5050" instrument by direct injection method. The synthetic strategies adopted to obtain the target compounds are depicted in **Scheme 1.**

Scheme 1:-

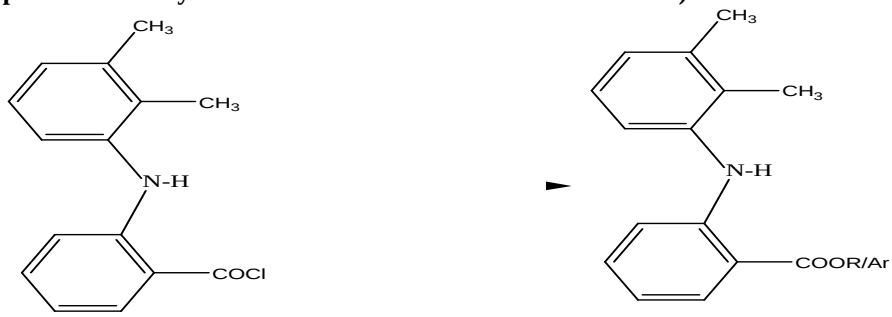
A] Synthesis of 4'-methyl biphenyl-2–carboxyl chloride:



Mefenamic acid **Mefenamic acid chloride**

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

B] Generalized procedure for synthesis of Mefenamic acid ester derivatives):-



Mefenamic acid chloride **Mefenamic acid ester derivatives**

In a three-necked flask fitted with a reflux condenser equipped with a calcium chloride drying tube and a dropping funnel, Phenols (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 hour under stirring with cooling in an ice bath. The mixture was stirred for an additional 30 minutes and then set aside protected by a

calcium chloride guard tube for approximately 10 hours. 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 sulphate. The drying agent was removed by filtration and the solvent was removed by distillation to give crude product which was recrystallized from acetonitrile / methanol.

VE6-Phenyl (2, 3-dimethylphenyl) Anthranilate:-
Yield 60% white colourless compound. R_f 0.54 (n-Hexane: Ethyl acetate) ; IR 1573 (C=C str), 3016 (C-H str), 752 (C-Hbend), aromatic ring; 2970 (C-H str),1444 (C-H bend), CH_3 Aliphatic; 1651 (C=O Str), 1157 (C-O str), COO ester; 3312 (N-H str), 1262 (Ar C-N str). ^{1}H NMR (CDCl3): δ 2.182 (s, 3H,-CH_3), 2.350 (s, 3H,-CH_3), 6.658-8.044 (m, 12H,-aromatic proton), 9.390(s, 1H,-NH), Mass: (70 eV) m/z 317.

VE7-3, 5 dimethylphenyl (2, 3-dimethylphenyl) Anthranilate:-
Yield 70% white colourless compound; R_f 0.50 (n-Hexane: Ethyl acetate); IR 1575 (C=C str), 3019 (C-H str), 752 (C-H bend), aromatic ring; 2977 (C-H str), 1450 (C-H bend), CH_3 Aliphatic; 1658 (C=O Str), 1157 (C-O str), COO ester; 3317 (N-H str), 1254 (Ar C-N str); ^{1}H NMR (CDCl3): δ 2.184 (s, 3H,-CH_3),2.338 (s,3H,-CH_3), 2.610 (s,6H,CH_3),6.660-8.036 (m,10H,-aromatic proton),9.386 (s,1H,-NH); Mass: (70 eV) m/z 345.

VE8-4-methylthiophenyl (2, 3-dimethylphenyl) Anthranilate:-
Yield 55% white colourless compound. R_f 0.64 (n-Hexane: Ethyl acetate) ; IR 1573 (C=C str), 3062, 3023 (C-H str), 752 (C-H bend), aromatic ring; 2981 (C-H str), 1449 (C-H bend), CH_3 Aliphatic; 1653 (C=O Str), 1157 (C-O str), COO ester; 3306 (N-H str), 1257 (Ar C-N str); ^{1}H NMR (CDCl3): δ 2.188 (s, 3H,-CH_3), 2.346 (s,3H,-CH_3), 2.718 (s,3H,-SCH_3), 6.651-8.059 (m,11H,-aromatic proton),9.384 (s,1H,-NH); Mass: (70 eV) m/z 363.

RESULTS AND DISCUSSION

The prodrugs Mefenamic acid ester were synthesized by using aromatic phenols. Purity of synthesized prodrug was ascertained by melting point and thin layer chromatography (TLC). The synthesized compounds were confirmed by FTIR, 1HNMR

and Mass spectroscopy. IR spectra were recorded using KBr on “JASCO FT-IR 460 plus” instrument by DRIFT method. The characteristics peaks for Mefenamic acid derivatives of substituted molecules were observed. For aromatic ring C-=C str. at 1570-1578cm^{-1}, C-H str. at 2965-2981cm^{-1},C-H bend at 1442-1450cm^{-1};CH_3 aliphatic C-H str at2965-2981cm^{-1},C-H bend at 1445-1450cm^{-1};COO ESTER C=O str. at 1650cm^{-1},C-O str.1160;-NH- str at 3305-3420cm^{-1},Ar C-N str at 1254-1260cm^{-1}.

The 1H NMR spectrum of synthesized prodrugs shows the chemical shift value for the anticipated compounds. The mass spectroscopic analysis gives the parent peak confirming molecular weight of the targeted compounds. The synthesized ester prodrugs of Mefenamic acid were subjected to solubility studies. Prodrugs were found insoluble in water and in 0.1 M HCl. Solubility studies showed that the synthesized prodrugs were found slightly soluble in 0.1 M NaOH. It showed moderate to high solubility in various solvents such as methanol, ethanol, chloroform and benzene. The greater solubility of the standard drug MA in 0.1 M NaOH is mainly due to the presence of free carboxyl group, which forms a sodium salt and makes the compound ionic. But prodrug showed moderate to high solubility in various organic solvents, which indicates lipophilic behaviour of the compound.. The Partition coefficient of Mefenamic acid and prodrugs were found in octanol-aqueous buffer (pH 7.4) system. The result indicates that synthesized esters were found to be more lipophilic than parent drug, Mefenamic acid. (Table 1)

The *in vitro* hydrolysis studies were designed in a manner to mimic the gastrointestinal tract pH, hence as a primary requirement, calibration curve in the experimental pH value related for the study were made. The hydrolysis studies were carried out in simulated gastric fluid at pH 1.2 for representing the condition of stomach, in simulated intestinal fluid at pH 7.4 and in 80 % human plasma at pH 7.4 to simulate the conditions of physiological pH of blood. The results from the hydrolysis studies carried out in SGF and SIF and 80 % human plasma at pH 7.4 are presented in Table 2-4.

The minimum reversion was observed at gastric pH (SGF, pH 1.2) suggesting the stability of synthesized prodrugs in gastric pH. However at higher pH values i.e. in SIF representing intestine, the percentage reversion was significantly higher, thereby making the free drug available for absorption in the intestine. A much higher value was observed in 80 % human plasma due to the enzyme dependant hydrolysis taking place in blood. Also the process of reversion increases almost linearly with time at intestinal pH and physiological pH of blood.

Table1: Characterization data of compounds from VE series

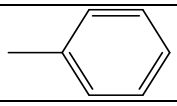
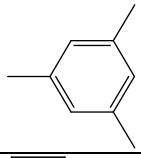
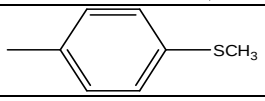
Sr. No.	Comp. Code	Amine	R/Ar	Molecular Formula	Mol. Wt.	M.P.°C	% Yield	R_f #	Partition coefficient
1	VE6	Phenol		$C_{21}H_{19}NO_2$	317	122-125	60	0.54	2.57
2	VE7	3,5 dimethyl Phenol		$C_{23}H_{23}NO_2$	345	145-148	70	0.50	2.81
3	VE8	4-methylthio Phenol		$C_{22}H_{21}NO_2S$	363	120-123	55	0.64	2.67

Table 2: Percentage release of Mefenamic acid on hydrolysis in SGF

Hr	0	0.5	1	2	3	4	5	6	7	8
VE6	0	2.74	5.06	10.12	11.13	13.16	14.17	17.20	20.24	22.26
VE7	0	3.85	7.08	11.13	15.18	18.22	20.24	24.29	27.32	30.36
VE8	0	3.30	6.07	8.10	14.17	16.19	19.23	23.28	26.31	29.35

Table 3. Percentage release of Mefenamic acid on hydrolysis in SIF

Hr	0	0.5	1	2	3	4	5	6	7	8
VE6	0	11.84	13.07	18.76	25.89	35.03	40.31	52.29	57.92	69.09
VE7	0	14.21	22.00	30.80	40.48	48.40	57.20	61.60	66.88	69.64
VE8	0	19.36	29.92	43.12	49.28	57.20	62.48	70.40	74.80	78.50

Table 4. Percentage release of Mefenamic acid on hydrolysis in human plasma

Hr	0	0.5	1	2	3	4	5	6	7	8
VE6	0	10.28	23.99	33.76	46.21	59.42	74.28	86.85	95.85	97.82
VE7	0	10.38	24.23	34.10	46.67	60.01	75.02	87.72	96.81	98.80
VE8	0	10.77	22.91	36.71	50.73	62.61	74.43	82.15	88.97	90.42

Synthesized prodrugs were evaluated for anti-inflammatory and Ulcerogenic activity. The following compounds were screened for anti-inflammatory activity of synthesized compound (ester derivatives) was determined by carrageenan-induced hind paw oedema method. This method indicated that prodrugs have comparable activity to parent drugs shown in (Table.5) The Ulcerogenic activity screened by One way ANOVA followed by Dunnets T Test. The synthesized prodrugs showed lower ulcer index value than Mefenamic Acid thus indicating decrease in gastrointestinal side effects through successful masking of free carboxylic group of drug (Table 6).

Table 5.Anti-inflammatory activity

Summary: MEAN DISPLACE MENT VALUE ± SEM

Code no	0 min	3hr	6hr	24hr
Control	1.89 ±0.041	2.88 ±0.071	3.93 ± 0.064	3.81 ±0.049
VE/6-20	1.93 ±0.070	2.68 ±0.031*	3.54 ±0.050**	3.64 ±0.046*
VE/6-40	1.91 ±0.054	2.70±0.032 000±0.036*	3.55 ±0.026**	3.57 ±0.044**
VE/7-20	1.82 ±0.049	2.73 ±0.025*	3.07 ± 0.096**	3.56 ± 0.026* 0.029**
VE/7-40	1.84 ±0.053	2.47±0.032 ±0.041**	3.42 ±0.034**	3.59 ± 0.026* 0.027**
VE/8-20	1.85 ±0.040	2.70 ±0.029*	3.71 ±0.024*	3.68 ±0.026*
VE/8-40	1.87 ±0.039	2.70 ±0.026*	3.59 ±0.033**	3.65 ±0.020**

Table:6 Ulcerogenic Activity of ester prodrug

Group	Treatment	Ulcer index (Mean ± SEM)
1	Mefenamic Acid control	16.16 ±0.600#
2	Diclofenac Control	14.00 ±0.577#
3	VE-6/40	11.00** ±0.365###
4	VE-7/40	06.83**±0.307###
5	VE-8/40	10.00**±0.365###

Values are expressed as Mean +SEM, (n=6), by unpaired t-test,(p<0.05) vs. Mefenamic acid.*

CONCLUSION

In present study Mefenamic acid ester prodrugs were synthesized by and characterized by Melting Point, Thin Layer Chromatography, Fourier Transform InfraRed Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy and Mass Spectroscopy to confirm its structure. Prodrug showed improved solubility in organic solvents which implies lipophilic character of ester prodrugs. The prodrugs were found chemically stable and bio-labile. The Mefenamic acid ester prodrugs showed comparable anti-inflammatory activities with reduced Ulcerogenicity. Retention of activity along with reduction in Ulcerogenicity may be due to prevention of direct contact of carboxylic group with gastric mucosa.

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

REFERENCES

[1] J.R. Vane, *Nature (London)*, **1971**, 231, 232-235.
[2] J.B. Smith, A.L. Willis, *Nature (London)*, **1971**,231, 235-237.
[3] M.C Allison, A.G. Howatson, C. J. Torrance, F.D Lee, R. I. Russell, *Engl. J Med*. **1992**, 327,749-754.
[4] T. Hla, K. Neilson *Proc. Natl. Acad.Sci. U.S.A.***1992**, 89, 7384-7388.
[5] V. Cioli, S. Putzolu, V. Rossi, P. Scorza Barcellona, C. Corradino, *Toxicol. Appl. Pharm.* **1979**, *50*, 283-289.
[6] A. S. Kalgutkar, B. C Crews, S. W. Rowlinson, A. B. Marnett, K. R. Kozak, R. P. Remmel, L. J Marnett. *P. Natl. Acad. Sci. USA* **2000**, *97*, 925-930.
[7] A. S Kalgutkar, A. B. Marnett, B. C. Crews, R. P Remmel, L. J. Marnett, *J. Med. Chem.* **2000**, *43*, 2860-2870.
[8] A. S. Kalgutkar, S. W. Rowlinson, B. C. Crews, L. Marnett, *J. Bioorg. Med. Chem. Lett.* **2002**, *12*, 521-524.
[9] E. A. Abordo, K. Bowden, A. P. Huntington, S. L Powell,. *Il Farmaco* **1998**, *53*, 95-101.
[10] V. K. Tammara, M. M. Narurkar, A. M. Crider, M. A. Khan,. *Pharm. Res.* **1993**, *10*, 1191-1199.
[11] V. K. Tammara, M. M. Narurkar, A. M. Crider, M. A Khan,*J. Pharm. Sci.* **1994**, *83*, 644-648.
[12] D. Galanakis, A. P. Kourounakis, K. C. Tsiakitzis, C. Doulgkeris, E. A. Rekka, A. Gavalas, C. Kravaritou, C. Charitos, P. N. Kourounakis, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3639-3643.
[13] E.K¨upeli, I.Tatli, I. Z. S. Akdemir, E.Yesilada, *J. Ethnopharmacol.* **2007**, *114*, 234-240.
[14] E. Yesilada, I. Gurbuz, *J. Ethnopharmacolww.* **2010**, *131*, 17-21.