

## SYNTHESIS OF PRODRUGS OF MEFENAMIC ACID AND THEIR *IN VIVO* EVALUATION

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### ABSTRACT

**Objective:** The purpose of the study was to synthesize prodrugs of mefenamic acid, to be used as Anti inflammatory drug with fewer adverse effects.

**Methods:** The drug was covalently bonded to PEG 1500 (polyethylene glycol) and PEG 6000 as such and with a linker glycine. The prodrugs were characterized by FT-IR and N.M.R. For the drug release studies, all the prodrugs were subjected to pH 1.2 and pH 7.2. For the anti inflammatory activity, Carrageenan induced rat paw edema method was followed and for Ulcer protecting activity, Pylorus ligation method was used, the prodrugs were administered to male Sprague-Dawley rats.

**Results:** The results suggested that the prodrugs of mefenamic acid, the drug release was higher at pH 7.2 than at pH 1.2. The result obtained for anti inflammatory activity was comparable to the standard drug of mefenamic acid. For ulcers, the prodrugs were found to possess Ulcer curing property higher than the standard drug.

**Conclusion:** The prodrugs thus synthesized possess anti inflammatory activity as well as good ulcer protecting activity, can be used instead of standard drug.

**Keywords:** Polyethylene glycol, Mefenamic acid, Ulcer protecting, Anti inflammatory, Prodrugs.

### INTRODUCTION

Mefenamic acid is an NSAID, derivative of N-aryl anthranilic acid belonging to class of fenamic acids. It is chemically N-(2,3-xylyl) anthranilic acid [1, 2, 3]. The drug is used for its analgesic, anti inflammatory activity [4], and primary dysmenorrhea. The dose of the drug used is 500 mg as initial dose followed by 250 mg every 6 hrs and not to continue more than a week. The mechanism of action [5, 6, 7] of mefenamic acid is inhibition of cyclooxygenase (COX) enzymes which are required for production of prostaglandins. The adverse effects of the drug include ulcer formation in the upper GI tract [2, 3].

### Prodrugs

Polymeric prodrug approach is one of the method where in a drug is covalently bonded to a polymer [8]. The requirements of prodrug are it should be chemically or enzymatically cleavable, non toxic and no pharmacological activity as such [9, 10]. Prodrugs are classified as Carrier linked prodrugs, Tripartite prodrugs, Mutual prodrugs, Polymeric prodrugs and Bioprecursor [10]. The use of polymers was first introduced by Prof. H. Ringsdorf [11]. Polymers have been used as carriers and backbone for many drugs which are biodegradable [12]. The commonly used polymers are polyethylene glycol, polyvinyl pyrrolidone (PVP). Polyethylene glycol is a non biodegradable polymer but, excreted easily [13]. Many drugs are conjugated to polyethylene glycol of varying molecular weights [14, 15]. Polyethylene glycol-acyclovir prodrugs [16] were synthesized and prodrugs of aspirin were synthesized using HEMA (Hydroxy ethyl methacrylate) as polymers [17]. PEG 6000 was used as polymer for preparation of solid dispersions of aceclofenac and was evaluated for particle size and dissolution rate [18]. Polymer-drug conjugates are synthesized covalently by ester, anhydride, amide linkages [19, 20]. Use of polyethylene glycol as polymeric backbone to drugs for drug delivery is known as PEGylation [21]. Mefenamic acid sustained release tablets have been formulated using sodium alginate to form water insoluble gel [22]. Mefenamic acid solubility

was increased using HPMC (Hydroxy propyl methyl cellulose) by spherical agglomeration technique [23]. Mefenamic acid mutual prodrug was synthesized using glucosamine [24]. Based on the facts, the thought for synthesizing prodrugs of mefenamic acid using polyethylene glycol 1500 and 6000 as polymeric backbone has turned up.

### MATERIALS AND METHODS

Mefenamic acid drug was obtained from Hetero Drugs, Hyderabad. PEG 1500, PEG 6000, DMF (di methyl formamide), DCC (dicyclohexyl carbodiimide), DMAP (dimethyl amino pyridine), Glycine and other solvents of reagent grade were purchased from SD Fine chemicals. Animal studies were done in department of Pharmacology, Geethanjali College of Pharmacy, Keesara, Ghatkesar and it was approved by Institutional Animal Ethics Committee, Regd no: 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013 for anti inflammatory and ulcer protecting activity.

### Synthesis of PEG 1500/6000-Mefenamic acid

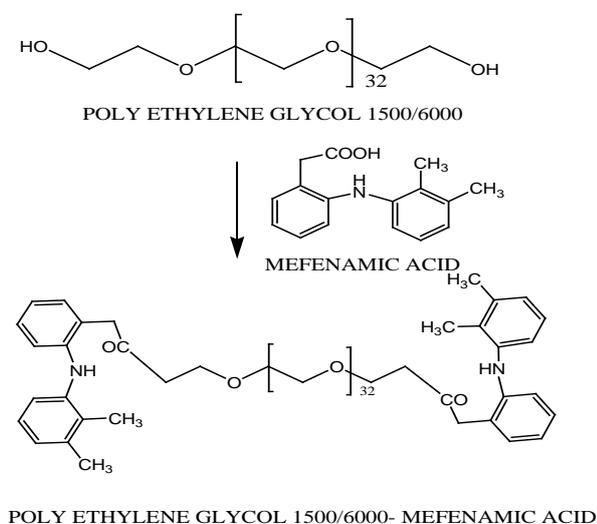
PEG 1500/6000 1.5 gms and 1.6 ml of pyridine were taken in a round bottomed flask, to it a solution of DCC 1gm and 0.6 gms of DMAP in 10 ml DMF was added. The flask was kept in an ice bath and temperature was maintained 0° C, to this mefenamic acid was added for 10 mins. The flask was then placed on a magnetic stirrer, attached with a condenser and was allowed for coupling reaction for 7 days at room temperature. The residue obtained was dissolved in DCM (dichloro methane) and reprecipitated by excess of cold diethyl ether [25]. The product obtained was subjected to TLC (thin layer chromatography) using mobile phase DCM: methanol 3:2. Finally structure was confirmed (Scheme I) by FT-IR and N.M.R.

### Synthesis of PEG 1500/6000 glycine

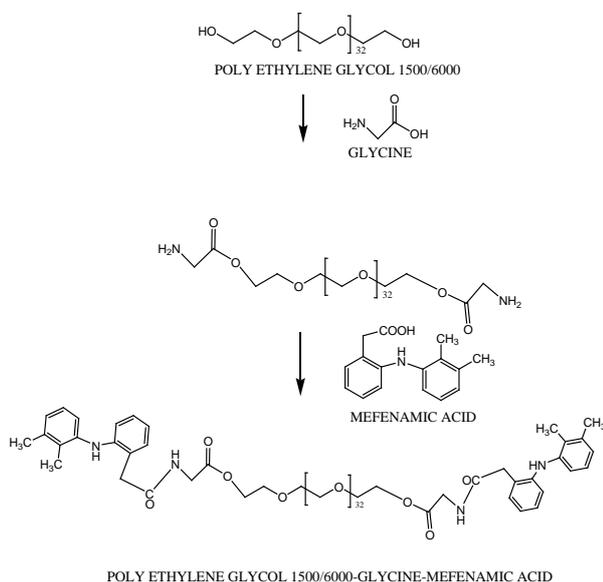
PEG 1500/6000 1.5 gms and 1.6 ml of pyridine were taken in a two necked round bottomed flask and 20 ml DMF was added and placed on a magnetic stirrer. To this 0.18 gms of glycine was added in small

portions for 3 hrs maintaining room temperature. Then the contents were refluxed by attaching a condenser, maintaining a temperature of 130° C for 21 hrs. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The formation of the product was subjected to TLC, mobile phase DCM: methanol 3:2 and structures (Scheme II) were confirmed by FT-I.R and N.M.R

Scheme I



Scheme II



## RESULTS

**Table 1: Physical properties of Mefenamic acid prodrugs**

S. No.	Compound	Solubility	Colour	Melting Pint	I.R Spectra	N.M.R Spectra
1	Mefenamic acid	Methanol	White	220-229° C	OH of COOH-2569, 2° NH-3348, C=O-1647.	2.1δ-2.4 δ (t, 6H) 2.5δ-(d, 2H) 6.7-7.5 δ (m, 7H) 7.9 δ (s, 1H), 9.5 δ (s, 1H).
2	PEG 1500-Mefenamic acid	Dichloro methane	White	200-208° C	2° NH-3327, C-O-C-1157, CH-str-2850, C=O-1627.	1.1-1.5δ-(m, 12H), 1.6-4.5δ (m, 44H), 5.6-7.7δ (m, 14H), 7.9δ (d, 2H).
3	PEG 1500-Glycine-Mefenamic acid	Dichloro methane	White	225-234° C	2° NH-3327, 3124, C-O-C-1157, CH-str-2850, C=O-1629.	1.1-1.5δ (m, 12H) 1.6-4.5δ (m, 44H) 5.6-7.5δ (m, 14H) 7.9δ(d, 2H).
4	PEG 6000-Mefenamic acid	Dichloro methane	White	224-233° C	2° NH-3327, C-O-C-1157, CH-str-2850, C=O-1626.	1.1-1.5δ-(m, 12H), 1.6-4.5δ (m, 44H), 5.6-7.7δ (m, 14H), 7.9δ (d, 2H).
5	PEG 6000-Glycine-Mefenamic acid	Dichloro methane	White	221-230° C	2° NH-3321, 2937, C-O-C-1161, CH-str-2852, C=O-1693.	1.1-1.5δ (m, 12H) 1.6-4.5δ (m, 44H) 5.6-7.5δ (m, 14H) 7.9δ(d, 2H).

## Synthesis of PEG 1500/6000-glycine-Mefenamic acid

A solution of DCC 1 gm in 10 ml of DMF and 0.6 gms of DMAP in 10 ml of DMF were taken in a beaker. The mixture was added drop by drop to another beaker containing a solution of 0.4 gms of PEG 1500/6000-glycine in 20 ml of DMF. Mefenamic acid 1 gm was added in portions to the above mixture at 0° C for 10 mins. The contents were transferred to a round bottomed flask fitted with a condenser and placed on magnetic stirrer.

The coupling reaction was carried out for 7 days at room temperature. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The product was subjected to TLC, mobile phase DCM: methanol 3:2 and structures (Scheme II) were confirmed by FT-I.R and N.M.R.

## In vitro Drug Release Studies

The synthesized prodrugs were checked for the drug release at pH 1.2 and 7.2, temperature was maintained at 37° C. The λ<sub>max</sub> was determined for mefenamic acid and found to be 285 nm, aliquots of 5 ml were collected at intervals of 0, 5, 10, 15, 30, 45, 60 mins, sink conditions were maintained. A standard graph was plotted and % drug release was found. A graph of time v/s cumulative drug release was plotted at pH 1.2 and 7.2.

## Anti inflammatory activity

Male Sprague-Dawley rats weighing 100-150 gms were divided into 6 groups. The method for this activity followed was Carrageenan induced rat paw edema. Ist group control, IInd group standard drug, IIIrd, IVth, Vth, VIth groups received mefenamic acid prodrugs, injected into the subplantar region in the left and right hind paws and the swelled volume was measured by Dolphin, India Plethysmometer. The measurements were taken at intervals of 1, 3, 6 hrs [26]. The % inhibition was calculated by

$$\% \text{ Inhibition} = 1 - \frac{(\text{vt} - \text{v0})_{\text{test}}}{(\text{vt} - \text{v0})_{\text{control}}} \times 100$$

## Ulcer protecting activity

For this activity, the method followed was Pylorus-ligation. The animals weighing 100-150 gms were fastened overnight, anaesthetized, incised 1 cm long in abdomen below the sternum. The stomach was exposed and a thread was passed round pyloric sphincter, a knot was tied. Abdomen was closed with sutures and animals were kept in separate cage, allowed to recover.

Ist group received control, IInd group received standard drug mefenamic acid 10 mg/kg, IIIrd, IVth, Vth, VIth groups were injected mefenamic acid prodrugs and after performing pylorus ligation kept in separate cages, after 4 hrs the animals were sacrificed and abdomen was cut open, stomach was removed and washed under running tap water, then placed on glass slide and observed on microscope at 10 X magnification for ulcers [27].

Ulcer Index was calculated by

$$\% \text{ Inhibition of Ulceration} = 1 - \frac{\text{Ulcer Index test}}{\text{Ulcer Index control}} \times 100$$



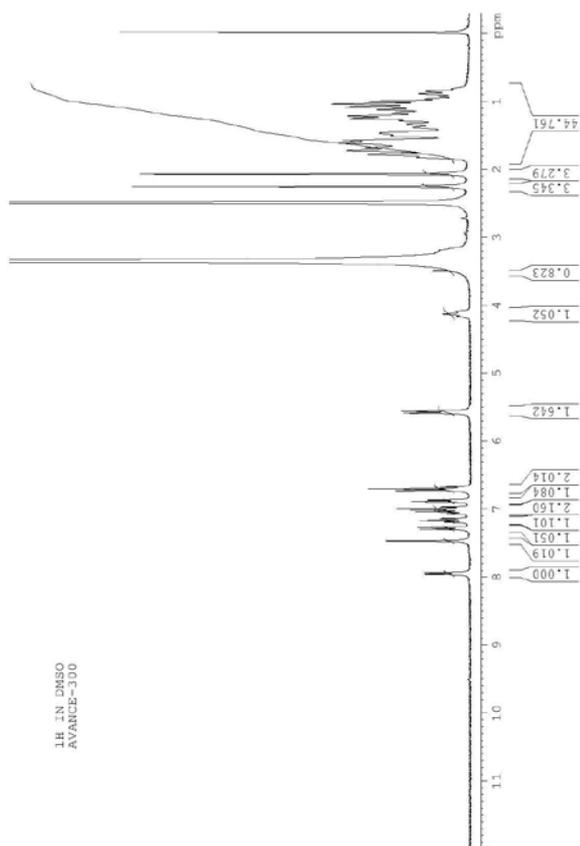


Fig. 5: N.M.R Spectra of PEG 1500/6000-mefenamic acid

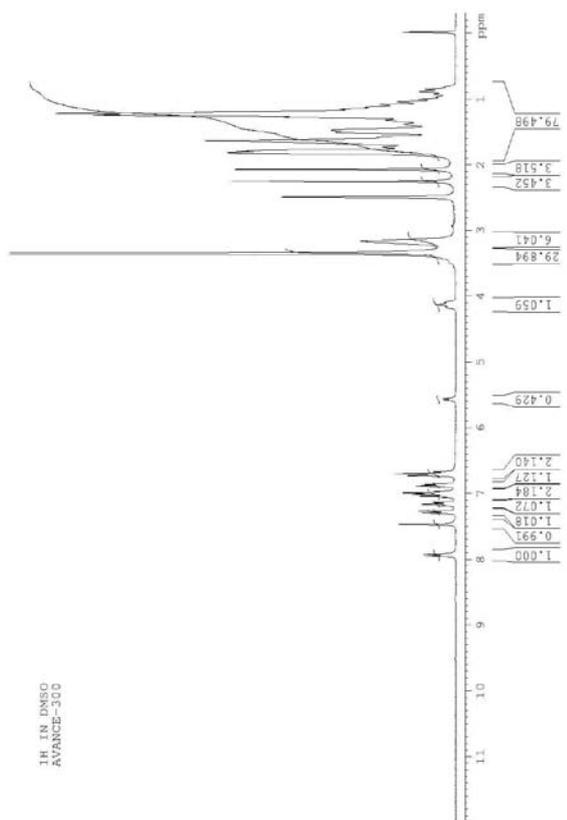


Fig. 6: N.M.R Spectra of PEG 1500/6000-Glycine-mefenamic acid

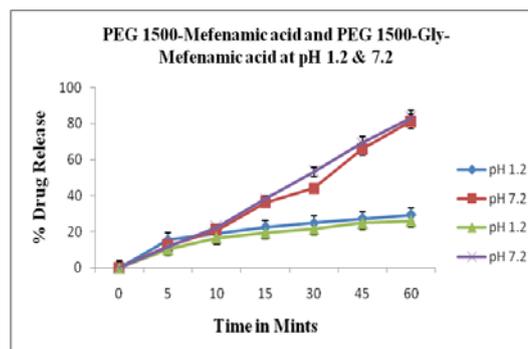


Fig. 7: In vitro drug release profile of PEG 1500 prodrugs at pH 1.2 and 7.2.

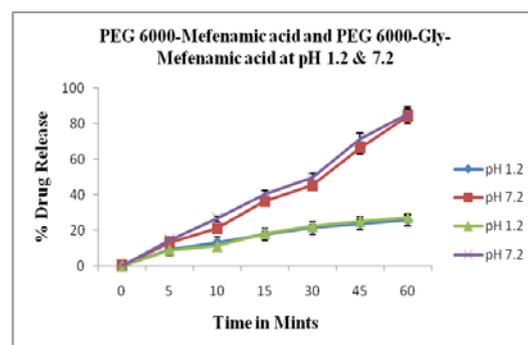


Fig. 8: In vitro drug release profile of PEG 6000 prodrugs at pH 1.2 and 7.2.

Table 2: Anti inflammatory Activity

Groups	Change in Paw volume (ml) mean±SEM & % Inhibition		
	1 hr	3 hr	6 hr
Control	0.52±0.02	0.61±0.02	0.65±0.03
Mefenamic acid	0.19±0.01 (63.46)	0.29±0.02 (52.46)	0.20±0.02 (69.23)
PEG1500-Mefenamic acid	0.27±0.02 (48.08)	0.41±0.01 (32.79)	0.22±0.01 (66.15)
PEG 1500-Gly-Mefenamic acid	0.21±0.03 (59.62)	0.42±0.01 (31.15)	0.23±0.01 (63.16)
PEG6000-Mefenamic acid	0.20±0.01 (61.54)	0.32±0.02 (47.54)	0.21±0.01 (67.69)
PEG6000-Gly-Mefenamic acid	0.24±0.02 (43.08)	0.36±0.02 (40.98)	0.24±0.02 (63.08)

Values are mean±SEM, n=6, one way ANOVA p<0.05 vs control

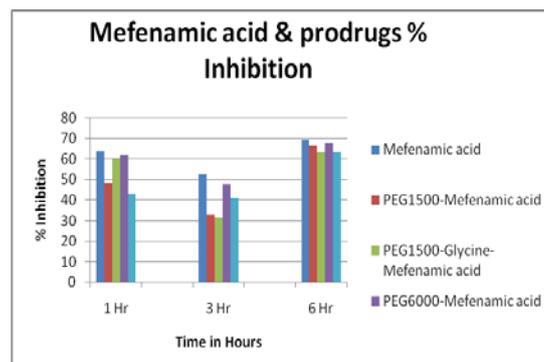


Fig. 9: Anti-inflammatory activity of mefenamic acid prodrugs

Table 3: Gross Ulcer Index of Mefenamic acid prodrugs

S. No.	Treatment	Ulcer Index
1	Control	10
2	Mefenamic acid	18
3	PEG1500-Mefenamic acid	16
4	PEG1500-Gly-Mefenamic acid	16
5	PEG6000-Mefenamic acid	19
6	PEG6000-Gly-Mefenamic acid	17

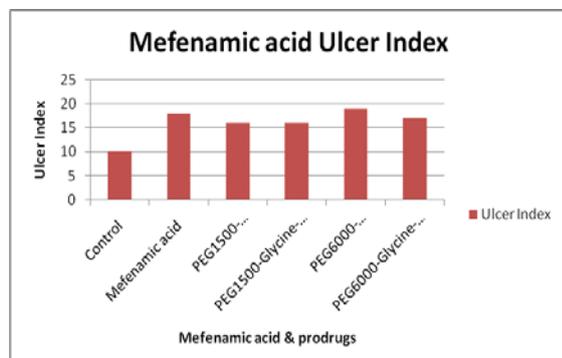


Fig. 10: Ulcer Index of mefenamic acid prodrugs

## DISUSSION

Prodrug approach is currently one of the popular approaches in the development of new drugs [28-35]. In this study, prodrugs of mefenamic acid were developed and investigated. The prodrugs were successfully synthesized. Upon synthesis, these were characterized for various parameters. The result obtained from spectral data (Sl.no 1 of table 1) indicated the standard drug had a COOH group by I.R and N.M.R. The structures of PEG 1500/6000-mefenamic acid (Sl.No 2 and 4) were confirmed by I.R and N.M.R, that COOH group of mefenamic acid had formed an ester, the structures were given in scheme I. The structures for PEG 1500/6000-glycine-mefenamic acid (Sl.No 3 and 5) by I.R and N.M.R, the carboxylic acid group of mefenamic acid had formed an amide linkage with the amino terminal of glycine, the structures were given in scheme II. Fig.1 and Fig.4 I.R and N.M.R spectras of standard drug mefenamic acid, Fig.2 and Fig.5 I.R and N.M.R spectras of PEG 1500/6000-mefenamic acid, Fig.3 and Fig.6 I.R and N.M.R spectras of PEG 1500/6000-glycine-mefenamic acid.

The drug release studies for the synthesized prodrugs were given in Fig.7 and Fig.8, the study revealed that drug release for PEG 1500-mefenamic acid, PEG 1500-glycine-mefenamic acid, PEG 6000-mefenamic acid and PEG 6000-glycine-mefenamic acid was more at pH 7.2 than at pH 1.2. Also the comparison study indicated that there is a role of spacer (glycine) in the drug release.

Table 2 and Fig.9 were the results obtained for anti inflammatory activity of the prodrugs synthesized. The results were taken for 1 hr, 3 hrs and 6 hrs for change in paw volume, out of the 4 prodrugs, 1 of the compound had better anti inflammatory activity than standard drug.

Table 3 and Fig.10 indicated ulcer protecting activity for the synthesized prodrugs. The ulcer index was measured and out of 4 prodrugs synthesized 3 of them were found to be more protecting towards ulcers than standard drug. The prodrugs which had more ulcer protecting activity are PEG 1500-mefenamic acid, PEG 1500-glycine-mefenamic acid and PEG 6000-glycine-mefenamic acid.

## CONCLUSION

The prodrugs of Mefenamic acid synthesized have retained their anti inflammatory activity similar to standard drug Mefenamic acid. An important finding of the result regarding ulcer protection that prodrugs of mefenamic acid have shown good ulcer protection than standard drug.

## CONFLICT OF INTERESTS

Declared None

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