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EVALUATION OF SOME AMIDES AND GLYCOLAMIDE ESTER OF MEFANAMIC ACID AS ANTIINFLAMMATORY AGENTS

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ABSTRACT

Some amides and Glycolamide ester of Mefanamic acid have been evaluated for the anti-inflammatory activity in albino rats. Compounds 5, 1, 3, 2, 4 have shown enhanced anti-inflammatory and analgesic activity. Amides and Glycolamide ester of Mefanamic acid as prodrugs were have been evaluated for anti-inflammatory activity for using carrageenan induced rat paw edema model. Mefanamic acid (250 mg/kg) was used as standard. Compounds 5, 1, and 3, exhibited significant enhancement in anti-inflammatory activity in carrageenan induced rat paw edema model. Compound 5, was found to possess maximum anti-inflammatory and analgesic activity.

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INTRODUCTION

NSAIDs, though generally effective in the management of inflammation, are used with the development of several gastrointestinal complications especially in stomach. The direct damage to the lower GI tract is however, unusual[1] several approaches have been made to overcome the GI complications like co-therapies with sucralfate[2], H_2 Antagonists[3] and prostaglandin inhibitors[4] have been tried. In recent years NSAIDs based on COX-2 selective compounds have also been used[5].

Survey of literature revealed that (N-2, 3 xylyl anthranilic acid) gained importance on account of its antiinflammatory and analgesic properties. Mefanamic acid has been mainly indicated for analgesia and symptoms associated with primary dysmennorhoea[6]. Some polymorphs and amino-acid analogues as prodrug of Mefanamic have been reported as to exhibit improved biological activity and stability. Mefanamic acid is the only fenamate, which displays a central as well as peripheral effect and inhibits cyclooxygenase unlike other aspirin like drugs. It was thus envisaged to study its glycol amide ester and a few selected amino-acid analogues as prodrugs and evaluate for analgesic and antiinflammatory activities[7]. Compound 1 is L-Phenyl alanineethyl ester HCl, Compound 2 is Beta

Alanine ethyl ester HCl, Compound 3 is L-Valine ethyl ester HCl, Compound 4 is Glycine ethyl ester HCl and Compound 5 is Glycolamide Ester of Mefanamic acid and are shown in Figure 1. Compounds were tested by the carrageenan induced rat paw edema model[8, 9]. The above amides were studied as prodrugs[10-14] for their anti-inflammatory and analgesic activity.

EXPERIMENTAL

(i) Synthesis of Ethyl Ester Hydrochlorides Of Amino Acids.

A solution of thionyl chloride (100 mmol) in dehydrated ethanol (250 ml) was placed in an ice bath and the amino acid (100 mmol) was added slowly with stirring. The reaction mixture thus obtained was refluxed for about four hours and the solvent was removed under reduced pressure to get the ethyl ester hydrochloride. The crude solid hydrochloride was triturated carefully with several portions of cold ether (220 ml) to remove dimethyl sulfite and finally allowed to dry in a vacuum dessicator. The product was recrystallized from hot ethanol (25 ml) by slow addition of ether (200 ml) followed by cooling in an ice bath. The crystals were collected, washed twice with the solution of ether in ethanol (5:1) and once with ether and finally dried under high vacuum.

(ii) Synthesis Of Mefenamic Acid Chloride

Mefenamic acid (6g, 0.024 mol) was mixed with chloroform (300 ml) in a round bottomed flask and then and thionyl chloride (3.57 g, 2.18 ml, 0.19 mol) was added dropwise to the above solution with gentle shaking. The reaction mixture was refluxed at 60-70^{0}C. Untill, the contents become dark brown in colour. The solvent and excess of thionyl chloride were removed under reduced pressure. The dark brown mefenamic acid chloride was collected, washed with solvent ether and preserved carefully. Yield 6.0322g.

(iii) Synthesis Of The Prodrugs [Modified Shotten-Baumann Reaction]

1. Synthesis of N-(2-o-xylylamino-1-benzoyl)L-phenyl alanine Ethylester

The ethyl ester hydrochloride of phenylalanine (5.74 g, 0.025 mol) was added in small portion to the cold aqueous solution of potassium carbonate (50 ml, 10%). The solution was allowed to stir until it became clear. Mefenamic acid chloride (6.49 g, 0.025 mol) was added in small portions to the alkaline solution of the amino acid ester. While, the contents were stirred maintaining the temperature at 10^{0}C for 2 hours. The solid was separated and dried in air. It was then washed with cold sodium hydroxide solution (0.5%) and recrystallized from chloroform. The Physical data are shown in Table 1 (Prodrug-I)

2. Synthesis of N-(2'o-xylylamino-1-benzoyl) ß-Alanine Ethyl Ester

The same method using ß – alanine ethyl ester hydrochloride (3.84 g, 0.025 mol) was followed maintaining the same conditions as for the Prodrug-I.The physical data are shown in Table 1 (Prodrug-II).

3. Synthesis of N-(2'o-xylylamino-1-benzoyl) valine Ethyl Ester

The same method using L-valine ethyl ester hydrochloride (4.54 g, 0.025 mol) was followed maintaining the same conditions as for the prodrug-I.The physical data are shown in table 1 (Prodrug-III).

4. Synthesis of N-(2'o-xylylamino-1-benzoyl) Glycine Ethyl Ester

The same method uding glycine ethyl ester hydrochloride (3.48 g, 0.025 mol) was followed maintaining the same conditions as for the Prodrug-I.The physical data are shown in Table 1(Prodrug-IV)

5. Synthesis of Glycolamide Ester of Mefenamic Acid

Pyridine (4.55 mml, 0.05 mol) was added to the cold solution of morpholine (4.36 mol, 0.05 mol) in ethyl acetate (20 ml) was added slowly with constant gentle shaking to the pyridine containing

morpholine solution, the reaction mixture thus obtained was filtered to obtain a clear solution of chloroacetyl morpholine (0.04 mol). The solution of chlolroacetyl morpholine (0.04 mol) treated with mefenamic acid (9.75 g, 0.04 mol) in ethyl acetate containing pyridine (3.6 ml, 0.04 mol), sodium iodide (0.59 g, 0.004 ml) and refluxed for three hours. The reaction mixture was allwed to filter upto cooling. The filtrate was washed with sodium thiosulphate (2%), sodium bicarbonate (2%) and water respectively After drying over Na_2So_4 the ethyl acetate was removed under reduced pressure to get glycolamide ester. The physical data are show in Table 1 (Prodrug-V).

BIOLOGICAL EVALUATION

Analgesic activity

The analgesic activity of the prodrugs was determined by thermal stimulus using tail flick method. A hot water analgesiometer was used for the determination of the pain threshold of the albino rats. Cold water was circulated through the water jacket of the instrument to avoid the heating of the area near the hot wire. Albino rats (100-200 grams) were divided into seven groups, each group containing three animals. The rat was placed in the holder through which the tail of the animal protruded out. The normal reaction which is the time taken for the tail to flick was noted. The current was so adjusted that the 90% of the rats reacted with the tail flick within a range of 5-9 seconds.The prodrug (100 mg) was suspended in an aqueous solution of Sodium carboxymethyl cellulose was (10 ml, 0.5% w/v) and the volume of this suspension containing the dose equivalent to the body weight (250 mg/kg) was given orally to the experimental animals.The results are shown in Table 3.

Anti-inflammatory activity

The anti-inflammatory activity of the prodrugs was determined by hind paw edema method utilizing carrageenan as phlogistic agent (0.1 ml, 0.2 mg). Albino rats (100-200 grams) were divided into seven groups, each group containing three animals including the standard and the control group. Initial volumes of right hind paw of albino rats were measured by plethysmometer without administration of the drug.The prodrug (100 mg) was suspended in an aqueous solution of Sodium carboxymethyl cellulose was (10 ml, 0.5% w/v) and the volume of this suspension containing the dose equivalent to the body weight (250 mg/kg) was given orally to the experimental animals. After 30 minutes of administration of the test prodrugs, carrageenan (0.1 ml, 0.2 mg) was injectedinto the planar surface of the right hand paw of each animal as a phologistic agent. Three hours after the volume of swelling of each animal paw was measured using the same technique.Animals of the control group were given saline orally. The mean increase in volume of the right hind paw of the animals was compared with the standard and test groups.The results are shown in Table 4.

RESULTS AND DISCUSSION

All the prodrugs were tested for analgesic and antiinflammatory activity. Thermal stimulus method was adopted for the screening of analgesic activity. Mefenamic acid (250 mg/kg) was given animals as standard. It was found to increase the mean time before the dug and thereafter the drug administration as compared to the parent drug mean time was increased in comparison to that the parent drugs.

Compound 5>Compound 1>Compound 3>Compound 2>Compound 4> Mefanamic Acid

Carrageenan induced rat paw edema method was followed for anti-inflammatory screening. The effects were compared to the control. Mefenamic acid (250 mg/kg) was given animals as standards was given in equimolar ratio of the drugs and were found Compound 4>Compound 1>Compound 3>Compound 2>Compound 4>Mefanamic Acid

The Glycolamide was found to inhibit maximum percent edema inhibition 90% whereas other

prodrugs like phenylalanine analogues were (75.94%), beta alanine (67.44%), valine (70.68%) and glycine (64.92%) have shown satisfactory results than the parent drug. The drug Mefanamic acid inhibited 60.56 % edema.

CONCLUSION

The present study includes synthesis and evaluation of amides and Glycolamide ester of Mefanamic acid for the anti-inflammatory activity in albino rats. Compounds 5, 1, 3, 2, 4 have shown enhanced anti-inflammatory and analgesic activity. Amides and Glycolamide ester of Mefanamic acid as prodrugs were have been evaluated for anti-inflammatory activity for using carrageenan induced rat paw edema model. Compounds 5, 1, and 3, exhibited significant enhancement in anti-inflammatory activity in carrageenan induced rat paw edema model. Compound 5, was found to possess maximum anti-inflammatory and analgesic activity.The present study opened the way in favour of detailed study of more amides and glycolamides of non-steroidal anti-inflammatory agents.

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REFERENCES

1. Peterson, S.P. and Killraina, I., Postgraduate Medicine, 1996, 100, 131.
2. Agrawal, N. M., Roth, S. and Grahm, D. Y., Ann. Intern. Med., 1991, 115., 195
3. Robinson, M. G., Griffin, J.W. and Bowers, J., Dig. Dis. Sci., 1989, 34, 424.
4. Grahm, D. Y., White, R. H. and Moreland, L.W., Ann. Intern. Med., 1993, 119, 257.
5. Roth, S. H., Tindall, F. A. and Jain A. K., Arch. Intern. Med., 1993, 153, 2565
6. Khoobchandani, R. P., Ghatikar, K. N., Keny, S. and Usgankar, N. G., J. Amer. Pharm. Intern.,1995, 43, 514.
7. Hsieh, H. K., Tsao, L.T., Wang, J.T. and Lin, C. N., J. Pharm. Pharmacol., 2000, 52, 163.
8. Sobottka, A. M., Werner, W., Blaschke, G., Kiefer, W., Nowe, U., Dannhardt, G., Schapovan, E. E., Schenkel, E. P. and Scriba, G.K., Arch. Pharm., 2000, 333, 205.
9. Vagdevi, H. M., Latha, K. P., Vaidya, V. P., Vijaya Kumar, M. L. and Pai, K. S. R., Indian J. Pharm. Sci., 2001, 63, 279.
10. Singh, G., Wadhwa, L. K. and Sharma, P. D., J. Sci. Indian Research, 1996, 55, 497.
11. Khan, M. S. and Khan R. M., Indian J. Chem., 2002, 41, 2172.
12. Khan, M. S. and Khan R. M., Indian J. Chem., 2002, 41, 1055.
13. Sharma, P. D., Singh, K. J., Gupta, Chandiran, S. K., Indian J. Chem., 2004, 43, 636.
14. Shanbhag, V. R., Crider, A.M., Gokhale, R., Harpalani, A., and Dick, R. M., J. Pharm. Sci., 1992, 81, 149.

Tables & Figures

Table 1: Physicochemical data of synthesized compounds

S. No.	Comp. code	Physical State	Molecular formula	Yield (%)	M. P. range (°C)
1.	**1**	Solid amorphous/	C25H26N2O3	74.34	214-215
2.	**2**	Solid amorphous	C20H24N2O3	71	182-183
3.	**3**	Solid amorphous	C22H28N2O3	74.63	185-186
4.	**4**	Solid amorphous	C19H22N2O3	68.52	195-196
5.	**5**	Solid flakes	C21H24N2O4	56.24	93-34

Table2: Spectral data of compounds

Comp. code	IR (KBr/cm^{-1}) observed	Indication	Inference
1	3350	NH stretching vibration	Presence of secondary amide group
	1640	C=O stretching vibration	Presence of ester linkage
	1500	CH_3 CH deformation	Presence of CH_3 groups
	1410	CH_3 CH deformation	
	1160	C-O Streching	Presence of higher ester linkage
	780	CH out of plane deformation (due to 3 adjacent H atoms)	Presence of 1, 2, 3 trisubstituted aromatic ring
	740	CH out of plane deformation (due to 4 adjacent H atoms)	Presence of 1,2 disubstituted aromatic ring
	700	CH out of plane deformation (due to 4 adjacent H atoms	Presence of mono substituted phenyl ring
2	3310	NH stretching vibration	Presence of secondary amide group
	1650	C=O absorption	Presence of amide I
	1500	CH_3 CH deformation	Presence of CH_3 groups
	1440	CH_3 CH deformation	
	1330	N-H deformation	Presence of aryl NH linkage
	1160	C-O Streching	Presence of higher ester linkage
	780	CH out of plane deformation (due to 3 adjacent H atoms)	Presence of 1, 2, 3 trisubstituted aromatic ring
	760	CH out of plane deformation (due to 4 adjacent H atoms)	Presence of 1, 2 disubstituted aromatic ring
3	3400	NH stretching vibration	Presence of secondary amide group
	1550	CH_3 CH deformation	Presence of CH_3 groups
	1400	CH_3 CH deformation	
	1320	N-H deformation	Presence of aryl NH linkage
	1160	C-O Streching	Presence of higher ester linkage
	755	CH out of plane deformation (due to 3 adjacent H atoms)	Presence of 1, 2, 3 trisubstituted aromatic ring
	740	CH out of plane deformation (due to 4 adjacent H atoms)	Presence of 1, 2 disubstituted aromatic ring
4	3300	NH stretching vibration	Presence of secondary amide group
	1640	C=O streching	Presence of ester linkage
	1160	C-O Streching	Presence of higher ester linkage
	1500	CH_3 CH deformation	Presence of CH_3 groups
	1440	CH_3 CH deformation	
	1330	N-H deformation	Presence of aryl NH linkage
	780	CH out of plane deformation (due to 3 adjacent H atoms)	Presence of 1, 2, 3 trisubstituted aromatic ring
	760	CH out of plane deformation (due to 4 adjacent H atoms)	Presence of 1, 2 disubstituted aromatic ring
5	3300	NH stretching vibration	Presence of secondary amide group
	1645	C=O streching	Presence of tertiary amine (Amide I)

	1580	N-H deformation	Presence of higher ester linkage
	1500	CH_3 H deformation	Presence of CH_3 groups
	1450	CH_3 CH deformation	
	1330	C-N vibration	Presence of secondary amine aromatic
	1260	C-O Streching	
	780	CH out of plane deformation (due to 3 adjacent H atoms)	Presence of higher ester linkage Presence of 1, 2 trisubstituted aromatic ring
	745	CH out of plane deformation (due to 4 adjacent H atoms)	Presence of 1, 2, 3 disubstituted aromatic ring

TABLE 3: COMPARATIVE STUDY OF THE ANALGESIC ACTIVITY OF THE PRODRUGS OF MEFENAMIC ACID

Prodrug	No. of Animals taken	Mean change in time (seconds)[a]
1	3	9.1 ± 0.0697
2	3	10.12 ± 0.0825
3	3	9.41 ± 0.0778
4	3	9.78 ± 0.0668
5	3	9.27 ± 0.0648
Control	3	-
Standard	3	8.71 ± 0.0573

[a]At the Dose of 250 mg/kg

TABLE 4: COMPARATIVE STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF THE PRODRUGS OF MEFENAMIC ACID

Prodrug	No. of Animals taken	Mean change in paw volume	% Edema Inhibition[a]
1	3	0.1783 ± 0.0218	75.94%
2	3	0.2413 ± 0.0238	67.44%
3	3	0.2173 ± 0.0698	70.68%
4	3	0.2600 ± 0.0214	64.92%
5	3	0.1045 ± 0.0209	85.90%
Control	3	0.7413 ± 0.0219	-
Standard	3	0.2923 ± 0.0170	60.56%

[a]At the Dose of 250 mg/kg

FIGURES

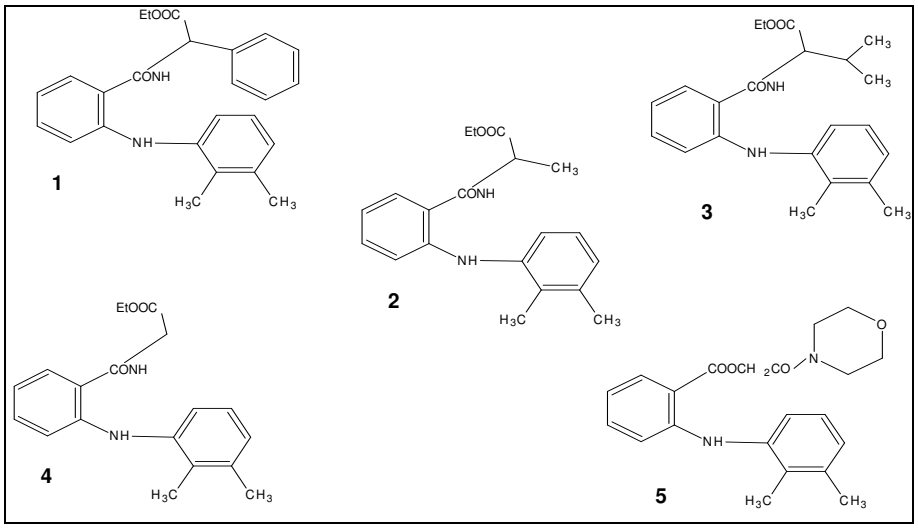

Figure 1: Structure of Amides and Glycolamide Ester of Mefanamic Acid

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