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SYNTHESIS AND CHARACTERIZATION OF ESTER PRODRUG OF MECLOFENAMIC ACID FOR REDUCTION OF ULCEROGENECITY

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ABSTRACT

The research studies also reported that the of the ester prodrugs are for enhancing the anti-inflammatory activity and for reduction of ulcerogenic effects of NSAIDs. Also the studies attempted to evaluate the pharmacokinetics of the prodrugs by in-vitro methods. The inflammation induced by carrageenan that is an acute model. The carriers used in research work, sesamol, umbelliferone and thymol, also produced anti-inflammatory activity and antioxidant effects .Therefore, overall, synthesized products showed synergistic anti-inflammatory activity. The natural phenolic antioxidants for synthesizing the ester prodrugs are sesamol, 4- methyl umbelliferone and thymol and that itself showed various pharmacological activities.

Keywords: NSAIDs**,** ester**,** antioxidants**,** thymol**,** inflammation**,** anti-inflammatory activity**.**

1 INTRODUCTION

The prodrug approach has emerged as a tool in overcoming various obstacles to drug formulation and targeting such as chemical instability, poor aqueous solubility, inadequate brain penetration, insufficient oral absorption, local irritation and toxicity 6. It is justified by the fact that once the barrier to the use of parent compound has been overcome, these temporary forms can be converted to the free parent compound that can exert its pharmacological activity[1,2].

A prodrug is thus defined as a biologically inactive derivative of a parent drug molecule that usually requires a chemical or enzymatic transformation within the body to release the active drug, and possess improved delivery properties over the parent molecule. These attractive features render the prodrugs a well-recognized strategy to improve drug targeting, to enhance the physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically potent compounds, and thereby increase the usefulness of a potential drug[3,4].

2 METHODOLOGY

2.1 Synthesis of ester prodrugs of meclofenamic acid

To 10 mmol of meclofenamic acid in 25 ml of dichloromethane- anhydrous (DCM) and 10mmol of sesamol was added. Then 10mmol of DCC was added tothe reaction mixture at 0-8°C, which is then stirred for 5 minutes at 0-8°C and 3hr at 20-25°C. After the completion of the reaction, filtered and removed the precipitated urea. The filtrate was evaporated and again treated with 10ml DCM then it was extracted by using saturated sodium bicarbonate solution and then dried by magnesium sulphate. Thecollected crude ester purified by recrystallization. Before the recrystallization the product was treated with alcohol to remove the excess of sesamol. If the product after synthesiswas sticky, it was treated with petroleum ether two or three times.

The same procedure was used for the synthesis of meclofenamic acid-4-methylumbelliferone produg [MU] and meclofenamic acid -thymol prodrug [MT][5-7].

2.2 Characterization of drugs and synthesized prodrugs

2.2.1 Solubility studies

Solubility is a chemical property of a substance to dissolve in a solvent. Solubility test isvery much important that provide the valuable information about the biological activity assessment, structure optimization and pharmaco-kinetic properties etc. The procedure involved about 5mg of the solute from each synthesized prodrug and drug was treated with 5 ml of solvents at 37 ± 1ºC in glass test tubes. The solvent used for here was chloroform, methanol, water, DMSO, 0.1N sodium hydroxide, 0.1 N hydrochloric acid and 0.1N potassium hydroxide. If any insoluble fraction of solute was observed the known amount of solvent was again added to determine the solubility. The same procedure was used for determining the solubility of the prodrugs[8-10]

2.2.2 Thin Layer Chromatography (TLC)

TLC was done to check the reaction progress and purity of the synthesized compounds that was done on the pre coated silica G plates. The detection method was UV chamber. The solvent system used here was ethyl acetate: hexane 1:2[11].

2.2.3 Melting point

The melting point of the synthesized compound was found out by adding small amount of the sample to the fused capillary and placed in the melting point apparatus. Note the temperature when the solid started to melt and also the completion of melting[12].

2.2.4 Spectral data evaluation

The structure of the synthesized compounds was confirmed by the different spectral analysis such as UV, IR, ^{1}H NMR, spectra. The UV spectra provide the λ max value of the drugs and prodrugs. The IR spectrum provides the information about the functional groups. The ^{1}H NMR data provide the information about the protons[13]

2.3 Procedure of the in vitro hydrolysis study

2.3.1 Preparation of SGF (pH 1.2)

The SGF was made by treating 3 g sodium chloride in 1450ml of distilled water and then adjust the ph up to 1.2 by adding dilute hydrochloric acid and make up the volume with 1500 ml by adding more de-ionized water.

2.3.2 Procedure

10 mg of the prodrug was in 90 ml of the SGF and SIF and 15 ml of the solution was withdrawn and transferred to a centrifuge also make up the volume with methanol and this was continued up to 8 hours. After centrifugation 5ml of the supernatant was taken and monitored the free concentration of drug[14].

2.3.3 Simulated Intestinal Fluid (pH 7.4)

2.3.3.1 Preparation of SIF (pH 7.4)

Monobasic potassium phosphate (6.8gm) was treated with 250 ml of water and added 7 ml of 0.2 N sodium hydroxide. Transferred to 1000 ml of standard flask and add 300 ml of distilled water. A 10 g of pancreatin was mixed. Resultant solution was adjusted to pH 7.4 by adding 0.2 N sodium hydroxide. Then make up the volume to 1000 ml by distilled water[15].

2.3.3.2 Procedure

10 mg of the prodrug was in 90ml of the SGF and SIF and 15 ml of the solution was taken, centrifuged then diluted with methanol and this was continued up to 8 hours. After centrifugation 5 ml of the supernatant was taken and monitored the free concentration of drug.

2.3.4 Pharmacological evaluation

Drugs and the synthesized compounds were evaluated for anti-inflammatory activity and anti-ulcerogenic activity[16].

2.3.5 Experimental Animals

Albino wistar rats were purchased for the conducting the anti-inflammatory and anti-ulcerogenic activity. The animals were categorized in to six and each containing six animals. All experimental objects were properly housed according to the OECD guidelines.

2.3.6 Anti-inflamamtory screening methods

The anti-inflammatory activity was determined by hind paw edema method using carrageenan (0.1 ml, 1 % w/v) as inducing agent. All the compounds were dissolved in the 0.9% normal saline and it also act as control. The compounds were administered through orally and the dose is equivalent to the meclofenamic acid. After 30 min of oral administration of the compounds, carrageenan solution in normal saline was injected into the sub plantar surface of right hind paw of each group. The paw thickness of each animal was measuredby the vernier caliper at 0.5, 1, 2, 4 and 6 h. The percentage inhibition of paw edema

was calculated by the equation

$$\text{Percentage inhibition} = (1\text{-}V_a/V_b)\ 100$$

Where V_a—mean relative change in paw edema volume in test group, V_b—mean relative change in paw edema volume in control group[17].

2.4 Ulcerogenic Activity

2.4.1 Experimental method

The wistar albino (150-200g) rats were categorized in to the compounds treated groups and each containing six animals including healthy and standard group. The control group was treated with normal saline and other groupstreated orally the corresponding standard and test compounds as suspension in 0.5% acacia respectively to each group. The treatment was continued until five days and afterthe last dose was completed the rats were sacrificed by cervical dislocation after six hours. Then the stomach was separated and thoroughly washed with distilled water. Thelesions produced by the gastric mucosa were monitored visually using binocular magnifier. Ulcers greater than 0.5 mm were noted. The mean ulcer index was calculated on the basis of the severity of the lesion in the gastric mucosa and that can be divided asgrade 1: less than1 mm erosions, grade 2: 1-2 mm erosions and grade 3: more than 2 mmerosions , The UI was calculated as[18]

UI = [1× (number of lesions ofgrade 1) + 2× (number of lesions of grade 2) + 3× (number of lesions of grade 3)]/10

3 RESULTS AND DISCUSSION

3.1 Characterization of ester prodrugs of meclofenamic acid

The synthesized ester prodrugs were examined by physical-chemical characterization and spectral characterization. The physical and chemical characterization included the solubility, melting point, thin layer chromatography, etc and spectral characterization included the UV data, IR spectroscopy, ^{1}H NMR spectrometry.

3.1.1 Solubility profile

The solubility studies in different solvents showed better solubility in the non-polar solvents than in polar solvents that indicating the lipophilic nature Table 1.

3.1.2 Physical-chemical properties of the synthesized prodrugs

Physical-chemical properties are shown in Table 2.

3.1.3 Spectral data analysis

MS: FTIR(cm^{-1},KBr): 3317(-NH), 3024(aromatic C–H), 2853(Aliphatic C-H), 1684 (C= O ester) and 1071 (C–O, ester).

^{1}HNMR: ($CDCl_3$,): 2.31(S,3H), 2.15(S,3H), 5.99(S,2H in the ring), 6.80(d, benzene ring 1H), 6.73(t,1H), 6.82(d,1H), 8.15(d1H), 7.30(t,1H), 7.10(t, 1H).

MU: **FTIR:** (cm-1 KBr): 3364 (N-H stretching), 2980 (C–H), 1718 (C=O ester), and 1071 (C–O, ester).

^{1}H NMR ($CDCl_3$,δppm): 8.17(d,1H), 7.67(d,1H,), 7.33(t,1H,), 7.28(s,1H), 7.22(d,1H), 7.15(d,1H,), 7.12 (t,1H,), 7.05(d,1H,), 6.77(d,1H,), 6.75(t,1H,), 6.29(1H), 2.46(s,3H), 2.32(s,3H), 2.18(s,3H).

MT: FTIR(cm^{-1},KBr): 3324(-NH), 3070(aromatic C–H), 2921(Aliphatic C-H), 1671 (C=O ester) and 1,071 (C–O, ester).

^{1}HNMR: ($CDCl_3$,): 9.23(S,1H), 8.22(d,1H), 7.32(t,1H), 7.25(t, ,1H), 7.19(d,1H), 7.10(d,1H), 7.08(d,1H), 6.95 (s,1H), 6.83(d, J=8.55,1H), 3.10(m,1H), 2.35(s,3H), 2.31(s,3H), 2.17(s,3H).

The IR spectra of ester prodrugs showed absorption bands at 1684 -1671 cm^{-1} indicated the C=O stretching in the ester linkage and that is absent in the IR spectra of mefenamic acid. Also, prodrugs showed the peaks at 3317-3375(-NH stretching), 3100 - 3000(aromatic C–H) and 2853(Aliphatic C-H) that confirmed the formation of esterlinkage from the NSAIDs and antioxidants.

Table 1: Solubility of meclofenamic acid and its derivatives in different solvents

Code	H_2O	0.1 M HCl	0.1 M NaOH	MeOH	EtoH	$CHCl_3$	C_6H_6
Meclofenamic acid	--	--	++++	++	++	++	++
MS	--	--	++	+++	+++	++++	++++
MU	--	--	++	+++	+++	++++	++++
MT	--	--	++	+++	+++	++++	++++

Practically Insoluble = -, Sparingly soluble = ++, Soluble = +, Freely Soluble = ++++

3.1.4 In vitro hydrolytic study in SGF and SIF of the prodrugs

The in vitro hydrolysis study in SGF and SIF was done for understanding the percentage release of the drugs in the gastric p^H and intestinal p^H. The hydrolysis of the produgs were given in the table. The results showed the considerable stability of the prodrugs in the stomach and the release of the drug was elevated in the intestine. The results concluded that the

synthesized ester prodrugs showed enhanced release in the intestinalp^H and that is upto above 86 percentages (Table 3).

Table 2: Physical-chemical properties of the synthesized prodrugs

Prodrug	Colour	Melting point (^{0}C)	Yield (%)	Rf value
MS	Yellowish white	190-193	86	0.62
MU	Yellowish	170-172	79	0.56
MT	Yellowish	178-181	75	0.64

Table 3: Percentage hydrolysis of meclofenamic acid prodrugs in SGF and SIF

Time (hour)	Prodrug Hydrolyzed In SGF (%)			Prodrug Hydrolyzed in SIF (%)		
	MS	MU	MT	MS	MU	MT
0	0	0	0	0	0	0
1	6.10	5.83	5.70	16.55	12.20	12.55
2	9.80	9.00	8.70	26.50	22.10	21.65
3	13.70	12.80	12.60	35.40	28.00	28.25
4	16.50	15.50	14.70	47.00	40.50	37.30
5	20.60	20.20	17.80	56.50	49.75	44.81
6	22.50	23.10	23.60	66.20	58.75	55.75
7	25.90	25.10	25.90	71.00	67.75	63.92
8	26..00	28.50	27.00	86.50	83.89	80.82

3.2 *In-Vivo* Study

3.2.1 Anti -inflammatory activity

The synthesized natural antioxidant linked meclofenamic acid was subjected to anti- inflammatory activity. The percentage anti-inflammatory activity of meclofenamic acid and its prodrugs were determined and are listed in Table (Table 4).

3.2.2 Anti ulcerogenic activity of meclofenamic acid and prodrugs

The ulcer formation in the different treated rodents was visually monitored andthe variable to indicate the ulcer production is mean ulcer index. The photographs of the different groups were given below with the mean ulcer index data (Table 5).

Table 4: Anti-inflammatory activity of drug and prodrugs

Group	Prodrug	Anti-inflammatory activity (%)				
		0.5 h	1 h	2h	4 h	6 h
I	Healthy	-	-	-	-	-
II	Meclo-fenamic acid	46.0 ± 1.2	43.0 ± 2.1	42.8 ± 1.9	42.3 ± 1.2	41.5 ± 1.4
III	MS	43.1 ±3.3	51.2 ± 1.1	63.0 ± 2.2	69.3 ±1.3	74.5 ± 2.4
IV	MU	45.0 ±1.2	63.6 ± 1.6	69.7 ± 1.5	73.9 ±1.3	76.4 ± 1.9
V	MT	44.0 ± 1.5	51.0 ± 1.7	59.8 ± 1.8	69.4 ± 1.1	78.7 ± 1.4

Table 5: Ulcerogenic activity of ester derivatives of meclofenamic acid

Group	Treatment	Ulcer index (Mean ± SEM)
1	Healthy	0.00
2	Meclofenamic acid	29.66 ± 0.614
4	MS	7.00 ±0.577
5	MU	08.43±0.494
6	MT	08.13±0.497

4 SUMMARY AND CONCLUSION

Meclofenamic acid is a NSAID and it showed difficulties in the formulation processes owing to the poor solubility, dissolution rate, sticky nature etc., Meclofenamic acid has many therapeutic actions like analgesic, anti-inflammatory and anti-pyretic etc by acting on cyclooxygenase and prostaglandins**.** Meclofenamic acid showed certain unwanted effects like all NSAIDs and that can be reduced by many drug development processes. The current research work also proved that the prodrug synthetic approach is avery effective method in the drug development and that produced synergistic therapeutic profile and decrease in the unwanted effects.

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