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SYNTHESIS AND EVALUATION OF PRODRUG OF NSAIDS FOR REDUCTION OF ULCEROGENECITY

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ABSTRACT

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, the 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.

Key words: NSAIDs**,** prodrug**,** antioxidants**,** umbelliferone**,** analgesic activity**,** anti-inflammatory activity**.**

1 INTRODUCTION

Numbers of drugs possess some undesirable and poor organoleptic, physicochemical and biological properties. Their therapeutic efficacy can be improved by minimizing or eliminating the undesirable properties while retaining the desirable ones. This can be achieved through biological, physical or chemical means. The biological approach is to alter the route of administration, which may or may not be acceptable to the patient. The physical approach involves modification of the design of the dosage form such as controlled delivery of drugs, while the chemical approach emphasizes on the enhancement of selectivity to minimize toxicity A Prodrug is a chemically modified inert drug precursor which upon biotransformation liberates the pharmacologically active parent compound[1].

1.1 Prerequisites of Ideal Prodrug

An ideal prodrug should possess the following properties:

- Pharmacological inertness.
- Rapid transformation, chemically or enzymatically, into the active form at the target
- Non-toxic metabolic fragments followed by their rapid elimination[2].

1.2 Applications of Prodrug Approach

1.2.1 Taste Masking

One of the reasons for poor patient compliance particularly in case of children is the bitterness, acidity or causticity of the drug. Two approaches can be utilized to overcome the bad taste of drug.

- Reduction of drug stability in saliva.
- To lower the affinity of drug for taste receptors, thus making the bitterness orcausticity

imperceptible[3].

1.2.2 Odour masking

The odor of a compound depends upon its vapour pressure. Low boiling point liquids will have a strong odour. e.g. Ethyl mercaptan. Foul smelling liquid of boiling point 35°C useful in the treatment of leprosy is converted into its phthalate ester, diethyl isophthalate, which has higher boiling point and odorless[4] .

1.2.3 Change of physical form of the drug

Some drugs which are in liquid form are unsuitable for formulation as a tablet especially if their dose is high. The method of converting such a liquid drug into solid prodrug involves formation of symmetrical molecules having a higher tendency to crystallize e.g. P-Acetamidobenzoic acid ester[5].

1.2.4 Reduction of G.I. irritation

Several drugs cause irritation and damage to the gastric mucosa through direct contact, increased stimulation of acid secretion or through interference with protective mucosal layer . The NSAIDs, salicylates lower the gastric pH and induce or aggravate ulceration. This can be overcome by use of prodrug approach e.g. Salsalate[6].

1.2.5 Reduction of pain on injection

Intramuscular injections are painful when the drug precipitates or penetrates into surrounding cells or when the solution is strongly acidic, alkaline, alcoholic or poor solubility of drugs e.g. intramuscular injection of antibiotic like Clindamycin and anticonvulsant drug like Phenytoin are responsible for pain on injection. This can be overcome by making phosphate ester prodrugs respectively and maintaining the formulation at pH 12.

1.2.6 Enhancement of solubility and dissolution rate (Hydrophilicity) of drug

Hydrophilicity or water solubility is desired where dissolution is the rate limiting step in the absorption of poorly aqueous soluble agents or when parental or ophthalmic formulation of such agents is desired. Many drugs in the pipeline in recent drug development are hydrophobic in nature (BCS Class-II) and possess poor bioavailability. Prodrug approach can be applied for rectification of the solubility problem. Drugs with hydroxyl functional group can be converted into their hydrophilic forms by use of half esters such as hemisuccinate, hemiglutarates or hemipthalates. The other half of these acidic carriers can form sodium, potassium or amine salts and render the moiety more water soluble. Phenolic drugs and some alcohols, as in the case of steroidal drugs such as Cortisol, Prednisolone, Betamethsone and Dexamethasone, the sodium succinate salts, have poor chemical stability and hence phosphate esters are preferred. Glycosidic prodrugs of some agents and L-lysine ester of benzodiazepines are also water soluble[7].

1.2.7 Enhancement of chemical stability

A drug may destabilize during its shelf life stability. The commonest conventional approach is to lyophilize the solution into a powder, which can be reconstituted before use . The prodrug design of such agents is a good alternative to improve stability e.g. Antineoplastic drug- Azacytidine. The aqueous solution of azacytidine is readily hydrolyzed but the bisulfite prodrug shows stability to such degradation at acidic pH and is also more water soluble than the parent drug. The prodrug gets converted to active drug at the physiological pH [8]

1.2.8 Site specific drug delivery

After its absorption into the systemic circulation, the drug is distributed to the various parts of the body including the target site as well as the non-target tissue. Such distribution pattern has several disadvantages like undesirable toxic effects in the non-targeted tissue (especially if its therapeutic index is low), decreased concentration at target site, drug accumulation in lipoidal tissues and development of tolerance due to excess exposure of drug to receptors.These problems can be overcome by targeting the drug specifically to its site of action by altering its disposition characteristics. There are several approaches for drug targeting including prodrug design. The prodrug is converted into its active form only in the target organ/tissue by utilizing either specific enzymes or a pH value different from the normal pH for activation e.g. 5- amino salicylic acid[9].

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 10 mmol of sesamol was added. Then 10mmol of DCC were added to the reaction mixture at 0-80C, which is then stirred for 5 minutes at 0-80C and 3hr at 20- 250C.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. The collected crude ester purified by recrystallization. Before the recrystallization the product was treated with alcohol to remove the excess of sesamol. If the

product after synthesis was sticky, it was treated with petroleum ether two or three times. The same procedure was used for the synthesis of meclofenamic acid-methylumbelliferone prodrug[MU] and meclofenamic acid -thymol prodrug [MT].

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 is very 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, 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.[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, 1H 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 1H 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 3g 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.

2.3.1.1 Procedure

10 mg of the prodrug was in 90ml of the SGF and SIF and 15 ml of the solution was withdrawn and transferred to 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].

2.3.2 Simulated intestinal fluid (pH 7.4)

2.3.2.1 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 1000ml 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 1000ml by distilled water[15].

2.3.2.2 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 5ml of the supernatant was taken and monitored the free concentration of drug.

2.4 Pharmacological Evaluation

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

2.4.1 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.

2.4.2 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 measured by the vernier caliper at 0.5, 1, 2, 4 and 6 h. The percentage inhibition of paw edema was calculated by the equation

$$\text{Percentage inhibition} = (1\text{-}Va/Vb)\ 100$$

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

2.4.3 Ulcerogenic activity

2.4.3.1 Exerimental 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 groups treated 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 after the 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. The lesions 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 as grade 1: less than1 mm erosions, grade 2: 1-2 mm erosions and grade 3: more than 2 mm erosions, The UI was calculated as:[18]

UI = [1× (number of lesions of grade 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, 1H NMR spectrometry.

3.1.1 Solubility profile

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

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

Code	Water	0.1 M HCl	0.1 M NaOH	MeOH	EtOH	$CHCl_3$	Benzene
Meclofenamic acid	--	--	++++	++	++	++	++
MS	--	--	++	+++	+++	++++	++++
MU	--	--	++	+++	+++	++++	++++
MT	--	--	++	+++	+++	++++	++++

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

3.1.2 Physical-chemical properties of the synthesized prodrugs

Table 2: Physical-chemical properties of the synthesized prodrugs

Prodrug	Colour	Melting point (^0C)	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

3.1.3 Spectral data analysis

- **MS**:FTIR(cm^{-1}, KBr): 3317(-NH), 3024(aromatic C–H), 2853(Aliphatic C-H)and1684 (C= O ester); 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(d,1H), 7.30(t,1H), 7.10(t, 1H).
- **MU**: **FTIR** (cm^{-1}, KBr): 3364 (N-H stretching), 2,980 (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 H), 2921 (Aliphatic C-H) and 1671 (C=O ester); 1,071 (C–O, ester).
- **^{1}H-NMR** ($CDCl_3$): 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 ester linkage from the NSAIDs and antioxidants.

3.2 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 pH and intestinal pH. 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 intestinal pH and that is up to above 86 percentages.

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

3.3 *In vivo* Study

3.3.1 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.

3.3.2 Anti ulcerogenic activity of meclofenamic acid and prodrugs

The ulcer formation in the different treated rodents was visually monitored and the 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 4: Anti-inflammatory activity of drug and prodrugs

Group	Prodrug	Anti-inflammatory activity (%)				
		0.5 h	1 h	2 h	4 h	6 h
I	Healthy	-	-	-	-	-
II	Meclofenamic 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 a very effective method in the drug development and that produced synergistic therapeutic profile and decrease in the unwanted effects.

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