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Synthesis and Evalution of Amide Prodrugs of Diclofenac

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Abstract:
"Prodrugs Approach" is a versatile approach in solving the problems associated with drug molecules. Diclofenac is a pain reliever in variety of painful conditions but it has some side effects i.e. absorption, toxicity, distribution, instability, formulation etc. These side effects can be reduced by "Prodrugs Approach". In the present research work some amide Prodrugs of Diclofenac have been synthesized via acid amine coupling of Diclofenac and ester derivatives of amino acids using HOBT, NMM and EDC.HCl in dichloromethane medium. These newly synthesized Prodrugs were analyzed by NMR and IR spectroscopy. These newly synthesized Prodrugs were analyzed by NMR and IR spectroscopy. All the compounds were evaluated for analgesic activity by acetic acid induced writhing and anti-inflammatory activity by Carragennan Induced Rat hind Paw method.

Key Words: *NMM, EDC.HCl, HOBT, GI and NSAID*

Introduction:

"Prodrugs Approach" is an integral part of drug development. This strategy increases the selectivity of drugs for intended target and there is a lower chance for attack on healthy cells which reduce side effects. Prodrug refers to a pharmacologically inactive compound that is converted to an active drug by a metabolic transformation[1]. The term "Prodrug" was first introduced by Albert's. This bioconversion or transformation may take place during and after absorption with in the body[2-4]. Now it is an established part of drug development[10]. It emphasizes on improving the desirable properties of drugs and decreasing the side effects[9]. Today many successful prodrugs have been developed with enhanced therapeutic efficiencies[4-7] Prodrug approach is very effective and helpful in decreasing the problem related with solubility, absorption, distribution, site specificity, instability, toxicity, formulation and bioavailability problem[3-7]. Among various type of prodrugs, ester and amide prodrugs are most common type[11]. In body, these prodrugs break in to parent drug and coupled moiety. Stomach is mainly responsible for the breaking down the large molecules into smaller molecules which are easily absorbed by the small intestine. Prodrugs are frequently applied to mask polar and ionizable group of a drug molecule with the aim to improve membrane permeability and oral absorption[20].

Diclofenac is one of the most important NSAID which effect on a variety of inflammatory mediators. It is an effective analgesic and anti-inflammatory agent with a good tolerability profile. But unfortunately like other NSAIDs it has some side effects like it affect GI system[19], dyspepsia and low bioavailability[22]. The gastric side effects of Diclofenac is due to free carboxylic acid group. A possible way to solve this problem is to derivatize the free carboxylic functional group of Diclofenac to produce prodrug. Amide based Amidase sensitive prodrugs system has been used for Prodrugs of acids and amines[8]. Chlorzoxazone ester prodrugs of some acidic NSAIDs induced very little irritancy in the gastric mucosa[21]. Nebumetone is a prodrug, cause fewer GI ulcer than conventional NSAIDs[25]. Diethyl carbonate prodrug of Ibuprofen and naproxen undergo rapid transformation as compared to parent drugs[24]. Morpholino alkyl ester of naproxen was found more bioavailability and less irritating to gastric mucosa than parent drug[26].

The result from the literature survey indicated that prodrugs have good chemical stability towards hydrolysis and more bioavailability.

Major side effect is that prostaglandin (which is necessary for mucosal defense) synthesis could be blocked by NSAIDs[19].

The result from the studies indicated that prodrugs had good chemical stability.

Keeping in view the wide ranging biological and pharmacological importance of prodrugs,

It was considered worth while to synthesize some new amide Prodrugs of diclofenac having biological and pharmacological interest. In the present research work, we synthesize some amide Prodrugs of Diclofenac by treating the parent drug with amino acid derivatives. Diclofenac was made to react with derivatives of amino acids, via, proline methyl ester, glutamic acid methyl ester, phenyl alanine methyl ester and sarcosine ethyl ester, in presence of Hydroxybenzotriazole (HOBT), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (EDC- HCl) and N-methylmorpholine, in dichloromethane. Chemical structures of compounds were determined by ^{1}H NMR and FT-IR. NMR spectra were recorded and peaks were interpreted. In this study, the amino acid derivatives chooses to mask the free carboxylic acid group of diclofenac because it is condition for prodrug that the premoiety should be nontoxic and prodrugs formed could show varying degree of lipofilicity and less side effects. These amino acids are not harmful for living system.

Objectives:

1. Design, synthesize and characterization novel Prodrugs including Diclofenac and amino acid, which could improve the physiochemical and pharmacological properties of drug.
2. To increases the utility of Diclofenac by "Prodrugs Approach"

Material and Method:

Chemicals and Apparatus:

Chemicals obtained from S.D. fine and E Merck was of reagent grade used as such without further purification unless otherwise specified. Solvent used were double distilled on rota vapor. ^{1}H NMR spectra were recorded on a Brucker 400 MHz spectrometer in $CDCl_3$ solution. IR were recorded with resolution 4.00 cm^{-1}

Animals:

Animals were housed in-group of 5 per cage under standard laboratory conditions, temperature and humidity. All experimental procedures were carried out under standard guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animal and were approved by Institutional Animal Ethics Committee. 45 Albino mice (18-22 g) five in each group were used for analgesic activity while 45 Wistar rat (120- 170 g) five in each group were used for anti-inflammatory activity.

Method:

To a ice-water cooled solution of diclofenac (500 mg, 1.69 mmol) in dichloromethane (8.5 ml, 5.0 ml/mmol), Hydroxybenzotriazole (251 mg, 1.86 mmol) and 1-Ethyl-3-(3 dimethylaminopropyl) carbodiimide-HCl (EDC-HCl) (356.2 mg, 1.86 mmol) and N-methyl morpholine (341.4 mg, 3.38 mmol) was added and reaction mixture was stirred at 0°c for 5 minutes. A cooled solution of amino acid derivative (1.69 mmol) in dichloromethane (3 ml) was then added, and mixture was stirred at 0°c for additional 10 minutes. Removed the ice water bath and stirred the reaction mixture for 6-8 hrs. at room temperature.

Reaction completion was checked by TLC using Ethyl acetate-Hexane (1:1) as an eluting solvent. Reaction was quenched by addition of water (10 ml). The organic compound was extracted with ethyl acetate (3x20ml). Organic layer was washed with brine (3x20ml), dried over anhydrous Na_2SO_4 and concentrated in vacuum.

Purification was done by column chromatography using Silica (100-200) in ethyl acetate-Hexane.

General structure and scheme of the desired prodrug molecule

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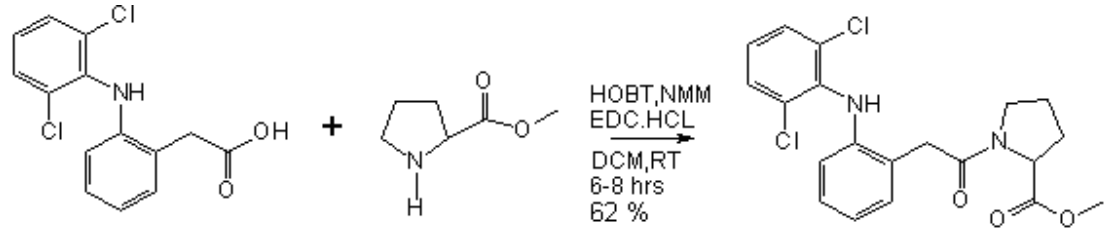
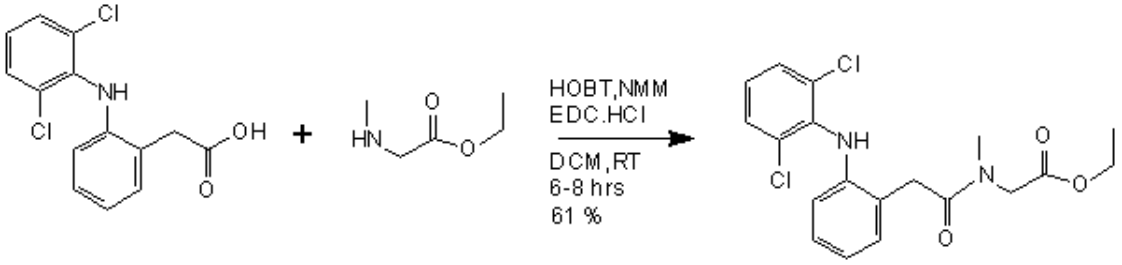
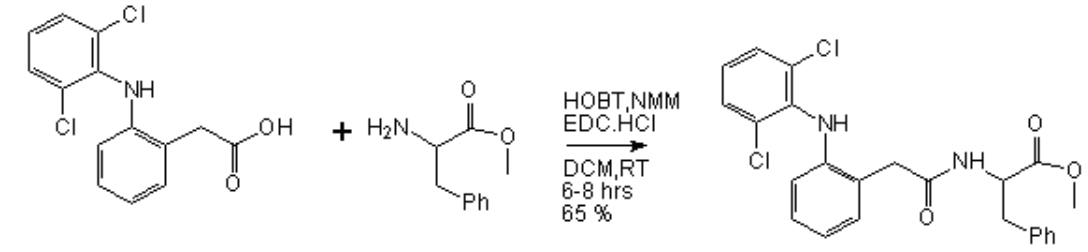


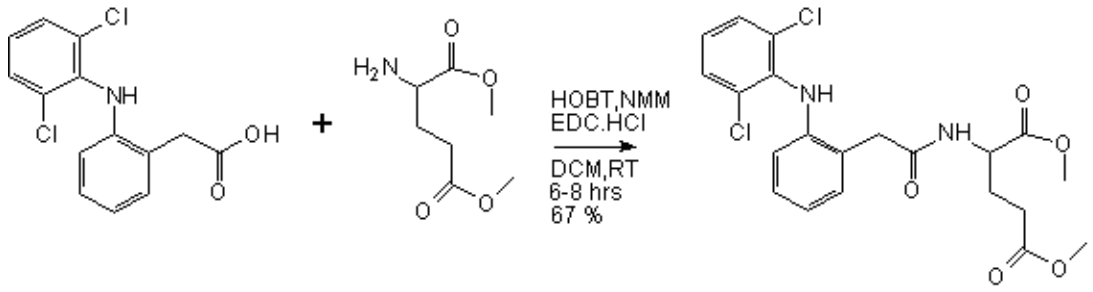
Scheme-i



Scheme-ii



Scheme-iii



Scheme-iv

Study Design for Pharmacological study (in vivo) of Synthesized Prodrugs:

Diclofenac as well as the Synthesized Prodrugs were evaluated for anti-inflammatory and analgesic activity

Acetic acid – induced writhing

The animals were pre-treated with drug 45 minute before induction of writhing. The drug Diclofenac (100 mg/kg, i.p.) served as reference standard drug. Analgesic activity of synthesized derivatives of Diclofenac (100 mg/kg, i.p.) was accessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.). The number of writhes per animal was counted for the next 10 minutes. Percentage protection against abdominal constriction was taken as an index of analgesia.

It was calculated as:

$$\frac{\text{Number of writhing in control group} - \text{Number of writhing in treated group}}{\text{Number of writhing in control group}} \times 100$$

Carragennan Induced Paw edema

Experimental inflammation was induced according to the method described by Winter et al. (1962). Carrageenan (0.1 ml of 1%) was injected in to the right hind paw of each rat under the planter aponeurosis. The test groups of rats were treated with Derivatives of Diclofenac 100 mg/kg body weight, i.p. one hour before Carrageenan injection. At the same time the control group was administered the reference group was administered i.p. with an ethanolic solution of Diclofenac at a dose of 100mg/kg body weight. The displacement technique using the plethysmometer (IITC 520, USA) immediately and 1 and 2 h did the measurements of paw volume after the injection of Carrageenan. The inhibitory activity was calculated according to the formula

$$\text{Percentage inhibition} = (1 - Vt/Vc)\ 100$$

Where Vt = Mean edema volume in the drug treated and

Vc = Mean edema volume in the drug control groups.

Results and Discussion:

Characterization of the Synthesized Prodrugs

Scheme-I: Preparation of methyl 1-(2-(2-(2, 6-dichlorophenylamino)phenyl)acetyl) pyrolidine-2-carboxylate

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, J=4.4, 8.1, 2H, Ar H), 7.18 (dd, J=1.4, 7.5, 1H, ArH), 7.11-7.06 (m, 1H, ArH), 6.95 (dt, J=2.3, 8.0, 1H, ArH), 6.90 (dt, J=1.2, 7.5, 1H, ArH), 6.51 (d, J=8.1, 1H, ArH), 4.55 (dd J=3.8, 8.4, 1H, NCH_2), 3.87 (s, 2H, CH_2-Ph), 3.80 (t, J=7.3, 2H, NCH_2), 3.70 (s, 3H, $COOCH_3$), 2.13-2.10 (m, 2H, ringCH_2), 2.05-2.02 (m, 2H, ringCH_2),

IR: 3340 cm^{-1} ($^{}$NH), 1743 cm^{-1} ($COOCH_3$), 1668 cm^{-1} (amide)

Scheme-II: Preparation of 2-(2-(2,6-dichlorophenylamino)phenyl)-N-(2-ethoxyallyl)-N-methylacetamide

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, J=3.8, 8.1, 2H, ArH), 7.18(dd, J=1.3, 7. 5, 1H, ArH), 7.11-7.08 (m, 1H, ArH), 6.96 (dt, J=4.8, 8.0, 1H, ArH), 6.92-6.89 (m, , 1H, ArH), 6.52 (d, J=7.9, 1H, ArH), 4.21 (q, 7.1, 2H, OCH_2), 4.20-4.15 (m, 2H, NCH_2), 3.91 (s, 2H, Ph-CH_2), 3.26 (s, 3H, NCH_3), 1.23 (t, J=7.2, 3H, CH_3)

IR: 3332 cm^{-1} (NH), 1738 cm^{-1} ($COOCH_3$), 1668 cm^{-1} (amide)

Scheme-III: Preparation of methyl 2(2-(2-(2,6-dichlorophenylamino)phenyl)acetamido) -3-phenylpropanoate

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J=1.6, 1H, ArH), 7.33 (s, 1H, ArH), 7.27 (d, J=1.5, 1H, ArH), 7.24 (dd, J=2.6, 6.0, 1H, ArH), 7.20 (dt, J=1.3, 4.7, 1H, ArH), 7.15 (dt, 1.6, 7.7, 1H, ArH), 7.05 (dd, J=1.4, 7.8, 1H, ArH), 7.00(d, J=8.0, 1H, ArH), 6.98-6.96 (m, 2H, ArH), 6.66 (bs, 1H, HN-C=O), 6.56 (d, J=8.0, 1H, ArH), 6.41(d, J=7.8,

1H, ArH), 4.87 (dd, J=2.1, 5.9, 1H, CH*), 3.86 (s, 2H, CH_2-Ph), 3.72 (s, 3H, $COOCH_3$), 3.13-3.02 (m, 2H, CH_2-Ph),
IR: 3330 cm^{-1} (NH), 1734 cm^{-1} ($COOCH_3$), 1668 cm^{-1} (amide)
Scheme-IV: 4-(methoxycarbonyl)-2(2-(2-(2, 6-dichlorophenyl)amino)phenyl)amino) phenyl)acetamido)butanoate
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, J=3.8, 8.1, 2H, ArH), 7.18 (dd, J=1.3, 7.5, 1H, ArH), 7.12 -7.08 (m, 1H, ArH), 6.95 (dt, J=2.6, 8.0, 1H, ArH), 6.92-6.88 (m, 1H, ArH), 6.51 (d, J=7.8, 1H, ArH), 4.58 (dt, J=5.2, 7.8, 1H, CH*), 3.87 (s, 2H, CH_2-Ph), 3.65 (s, 3H, OCH_2), 3.60 (s, 3H, OCH_3), 2.33 (t, J=7.6, 2H, CH_2), 2.17 (dt, J=7.5, 14.1, 1H, CH_2), 1.92 (dt, J=7.9, 14.2,1H, CH_2)
IR: 3336 cm^{-1} (NH), 1735 cm^{-1} ($COOCH_3$) 1668 cm^{-1} (amide)

Physiochemical Properties of the Synthesized Prodrugs

Table 1: General data for various Amide Prodrugs of Diclofenac

Compound	Molecular Formula	Molecular Weight	Percentage Yield
Diclofenac	$C_{14}H_{11}O_2NCl_2$	296	-
Proline ester derivative	$C_{20}H_{20}O_3N_2Cl_2$	407	62
Sarcosine ester derivative	$C_{19}H_{20}O_3N_2Cl_2$	395	61
Phenyl alanine ester derivative	$C_{24}H_{22}O_3N_2Cl_2$	457	65
Glutamic acid ester derivative	$C_{21}H_{22}O_5N_2Cl_2$	453	67

Table 2: Solubility data for various Amide Prodrugs of Diclofenac

Solvent	Water	Ethanol	Chloroform	Acetone	Diethyl ether
Proline ester derivative	insoluble	soluble	soluble	soluble	soluble
Sarcosine ester derivative	insoluble	soluble	soluble	soluble	soluble
Phenyl alanine ester derivative	insoluble	soluble	soluble	soluble	soluble
Glutamic acid ester derivative	insoluble	soluble	soluble	soluble	soluble

Pharmacological study (in vivo) of Synthesized Prodrugs:

Table 3: Table for Analgesic Activities by Acetic acid – induced writhings

	Dose	Number of Writhes (Mean + SEM)	Percent Inhibition
Control (Ethanol)	-	72 ± 0.063	-
Diclofenac Standard	100 mg	24 ± 0.059	66.66
I	100 mg	56± 0.062[1]	28.27
II	100 mg	35 ± 0.028[2]	51.38
III	100 mg	48 ± 0.039[1]	33.33
IV	100 mg	48 ± 0.058[2]	30.65

0: indicates Non significance with relative to Control; 1: indicates 0.05 < p with relative to Control; 2: indicates 0.01 < p with relative to Control

Table 4: Anti-inflammatory Activies by Carrageenan-induced rat Paw edema

	Dose	**½ hour**	**1 hour**
Control (Ethanol)	-	0.88 ± 0.086	1.1+ 0.02
Diclofenac Standard	100 mg	0.56± 0.028	0.46+ 0.3
I	100 mg	0.52 ± 0.36^{1}	$0.45+ 0.02^{1}$
II	100 mg	045 ± 0.08^{2}	$0.41+ 0.18^{2}$
III	100 mg	0.40 ± 0.16^{2}	$0.39+ 0.16^{2}$
IV	100 mg	0.46 ± 0.02^{2}	$0.42+ 0.16^{2}$

0: indicates Non significance with relative to Control; 1: indicates 0.05 < p with relative to Control; 2: indicates 0.01 < p with relative to Control

Conclusion:

In the present study, amide Prodrugs of Diclofenac were successfully synthesized and the structures were confirmed by NMR and UR analysis. The free carboxylic group of Diclofenac was temporarily masked by amino acid derivatives to prevent the direct exposure of the mucous layer of the stomach to this free carboxylic acid group. Four amide Prodrugs of Diclofenac were synthesized by selecting corresponding amino acids viz. proline methyl ester, sarcosin ethyl ester, phenyl alanine methyl ester and glutamic acid methyl ester by direct coupling in the presence of NMM, HOBT and EDC-HCL. The selection of the amino acids was done in such a manner that the Prodrugs with varying degree of lipophilicity could be obtained. High solubility in various organic solvents was observed for the newly synthesized prodrugs indicating the lipophilic behavior. The physicochemical and solubility data are recorded in Table 1 and 2. All the Synthesized Prodrugs were found pharmacologically active as compared to control and were quantitatively less active than standard Diclofenac.

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