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Synthesis, Pharmacological Screening and *In-Silico* Adme Prediction of Prodrugs of Aceclofenac

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ABSTRACT

Mutual Prodrugs of some non-steroidal anti-inflammatory agent NSAID i.e. Aceclofenac with Salicylamide, Thymol and Glucosamine, were synthesized with the aim of improving the therapeutic index through prevention of gastrointestinal implications and to check the efficiency of release of the parent drug in presence of spacer as well as without spacer. These mutual prodrugs were synthesized by direct condensation method using dicyclohexylcarbod iimide as a coupling agent and alanine as spacer. The title compounds were characterized by spectral techniques and the release of the parent drug from mutual prodrug was studied in two different non- enzymatic buffer solutions at pH 1.2, pH 7.4 and in 80% human plasma. All mutual prodrugs exihibited encouraging hydrolysis profile in 80% human plasma. Biological activity of title compounds was studied by carrageenan-induced rat paw edema method. From the results obtained, it was concluded that these compounds possess the Anti-inflammatory action.

Keywords: Non-steroidal anti-inflammatory, Mutual Prodrug, Aceclofenac, Salicylamide, Thymol, Glucosamine, Alanine, Insilico ADME.

INTRODUCTION

NSAIDs are among the most widely used of all therapeutic agents worldwide. Long-term high dose use of NSAIDs has been associated with gastrointestinal bleeding and nephro-toxicity. To reduce the toxic effects of such agents, they are derivatized into esters, amides, etc. For many years, several attempts have been made to develop bio-reversible derivatives or prodrugs of NSAIDs containing carboxylic acid function in order to depress upper GI-irritation and bleeding. Esterification/Amidation of the carboxylic acid moiety of NSAIDs would suppress gastro-toxicity without adversely affecting anti-inflammatory activity. Until now, except benorylate, mutual prodrug concept has not been employed to mask the free carboxylic group of NSAIDs to overcome their gastric irritancy. Mutual prodrug involves combining of two different pharmacophores with similar pharmacological activities to give synergistic action. But as the two molecules are being attached directly, there might be chances of stearic hindrance between two molecules which complicates the release of parent drug from the prodrug. So in the present research work an attempt is made to synthesize mutual prodrug of some widely used NSAIDs by direct coupling of NSAIDs and promoiety with and without the use of spacer [1, 2, 3].

EXPERIMENTAL SECTION

Materials

Melting points were determined by open capillary method and are uncorrected, which were further confirmed by visual melting point apparatus (Lab India). Progress of reaction was monitored and purity of components was checked by thin layer chromatography (TLC) on pre-coated silica gel GF_{254} plates (Merck) using UV light as detecting agent. The structures of the synthesized compounds were ascertained by spectral Analysis. UV spectra were recorded using UV/Vis spectro-photometer-1601 (Jasco V-30). IR spectra were recorded on FTIR-4200, Shimadzu spectrometer using KBr pellet method. 1H NMR spectra were recorded in $CDCl_3$ as solvent and TMS as internal standard on Jeol-FT-NMR-300mH spectrometer, Japan. Mass spectroscopy was carried out on GCMS-QP-2010-Schimadzu. Elemental analyses were performed using EA-112, Thermoquest CHN analyser. Aceclofenac& Salicylamide were obtained as a gift sample from Piramal Lifescience & Spectrochem Ltdrespectively and Glucosamine& Thymol are from Sisco Research Lab. All other chemicals used were of analytical grade procured from institutional store and solvents were of synthetic grade utilized after distillation. Human plasma was purchased from the Blood bank of KEM Hospital, Mumbai. Prior permission was taken for the conduction of In vivo studies in animals.

Experimental method

Part-I : **Synthesis and purification of mutual prodrugs of Flurbiprofen and Aceclofenac and their spectral characterization**.

Synthesis of mutual prodrug of Aceclofenac with Salicylamide (Acec-Sal)

Aceclofenac (3.54gm, 0.01mole) was dissolved in 50ml chloroform in a round bottom flask with calcium chloride guard tube attached to the neck and stirred for half an hour incold condition (0-4^{o}C). DCC (2.06gm, 0.01mole) was dissolved in 5ml ofN, N-dimethylformamide in another beaker and was then added drop wise to a solution of aceclofenac and stirred for 3 hrs in cold condition (0-4 ^{o}C).

Salicylamide (1.37gm, 0.01mole) was suspended in 30ml of chloroform in a beaker. 3-5ml of pyridine was added to dissolve the same. The mixture was stirred for one hour in cold condition (0-4 ^{o}C). The solution was added dropwise to the mixture of aceclofenac and DCC and stirred continuously. The reaction mixture was stirred for 4hrs in cold condition (0-4 ^{o}C) and 8hrs at room temperature. It was further filtered to remove the byproduct; precipitated dicyclohexylurea (DCU) The filtrate was washed with 0.05N HCl and saturated solution of $NaHCO_3$ to remove the unreacted salicylamide and dried under Vacuum. . The solid product was dissolved in acetone to remove remaining DCU, which was insoluble in acetone and was separated by filtration. The filtrate was dried under vacuum. The crude product was washed with absolute ethanol to remove any other impurities, if present and dried under vacuum. The following results obtained clearly depicted the formation of the title compound. (yield 82%). Scheme-1

IR: Frequency (cm^{-1}): 3319(N-H stretch, s. amine), 3277 (N-H stretch, amide)3095, 3068(Alromatic C-H stretch), 2970, 2866 (Aliphatic C-H stretch),1770 (–COO stretch, ester I), 1735 (–COO stretch, ester II),1658 (-CONH stretch, amide), 1589 (Aromatic stretch), 1151 (Aryl C-Cl stretch); 1H-NMR: (Acetone) δ(ppm): 6.4 – 7.55 (m, 12H, Aromatic Region and NH of $NHCOCH_3$), 7.885 (s, 2H, NH_2 of NH_2CO-), 4.77 (s, 2H, CH2 of –O-*CH2* – COO),3.98(s, 2H, CH2 of -*CH2* – COO)M^+Peak: 473.33**.**

Synthesis of mutual prodrug of Aceclofenac with Glucosamine (Acec-Glu)

This synthesis also carried out using similar methodology as mentioned for Acec-Sal (yield 88%)Scheme-1. IR: Frequency (cm^{-1}): 3566 (O-H stretch sec. alcohol), 3293 (N-H stretch, amide) 3110, 3067(Aromatic C-H stretch), 2984 (Aliphatic C-H stretch), 1739 (–COO stretch, ester), 1675 (-CONH stretch, amide I), 1614 (-CONH stretch, amide II),1647 (Aromatic stretch), 1211 (C-O stretch), 1296 (O-H bend., sec. alcohol) 1101 (Aryl C-Cl stretch); 1H-NMR: (DMSO) δ(ppm): 6.52-7.54 (m, 8H, Aromatic Region and NH of s.amine), 3.92 (s, 2H, CH2 of -CH2 – COO), 4.68 (s, 2H, CH2 of –O-CH2 –CONH), 9.18 (s, 1H, NH of -NHCO-), 2.18-2.20 (s, 4H, OH of Sec. alcohol), 2.54 (s, 2H, CH2 of CH2-OH), 3.28 – 6.30 (m, 5H, Tetrahydropyran) M^+ Peak: 515.35**.**

Synthesis of mutual prodrug of Aceclofenac with Thymol (Acec-Thy)

This synthesis also carried out using similar methodology as mentioned for Acec-Sal (yield 76%) Scheme-1. IR: Frequency (cm^{-1}): 3386 (N-H stretch, amide), 2971, 2964 (Aliphatic C-H stretch), 1764 (-COO stretch, ester I), 1737 (-COO stretch, ester II), 1301 (CO stretch), 1589 (Aromatic stretch), 1151 (Aryl C-Cl stretch); 1H-NMR: ($CDCl_3$) δ(ppm): 6.46 – 7.50 (m, 11H, Aromatic Region and NH of sec.amine and NH of $NHCOCH_3$),) 3.98(s, 2H, CH2 of

-*CH2* – COO), 4.76(s, 2H, CH2 of –O-*CH2* –COO), 1.20 – 1.21 (d, 6H,2CH3 of –$CH(CH_3)_2$), 3.21 – 3.32 (sep, 1H, CH of - $CH(CH_3)_2$Ar), 2.22 (s, 3H, CH3 of - CH3Ar).M^+ Peak: 486.40.

Synthesis of mutual prodrug of Aceclofenac with Salicylamide using alanine as spacer (Acec -ala- Sal mutual prodrug)

Boc-alanine (1.89gm, 0.01mole) was dissolved in 20ml dichloromethane (DCM) in round bottom flask with calcium chloride guard tube attached to the neck and stirred for half an hour in cold condition (0-4 ^oC). DCC (2.06gm, 0.01mole) was dissolved in 5ml of N, N-dimethylformamide in a beaker and was then added dropwise to a solution of Boc-alanine and stirred for 3 hrs in cold condition (0-4 ^oC).

Salicylamide (1.37gm, 0.01mole) was suspended in 20ml of dichloromethane (DCM) in a beaker and 3-5ml of pyridine was added to dissolve the same. The mixture was stirred for 1hr in cold condition (0-4 ^oC). The solution was added dropwise to the mixture of Boc-alanine and DCC and stirred continuously. The reaction mixture was stirred for 4 hrs in cold condition (0-4 ^oC) and 12 hrs at room temperature. It was further filtered to remove the byproduct, precipitated dicyclohexylurea (DCU). The filtrate was washed with 0.05N HCl and saturated solution of NaHCO3 to remove the unreacted salicylamide and dried under vacuum. The solid product was dissolved in acetone to remove remaining DCU, which was insoluble in acetone and was separated by filtration. The filtrate was dried under vacuum. The product of Boc-Ala-Sal was then stirred for 2 hrs at room temperature with 1:1 mixture of dichloromethane and trifluoroacetic acid (TFA) to break the Boc which is a protecting group for -NH_2 functional group in Boc-Alanine. After breaking Boc the compound obtained was found to be Alanine-Salicylamide (Ala- Sal). Aceclofenac (1.77 gm, 0.005mole) was dissolved in 5ml dimethyl formamide (DMF) in round bottom flask with calcium chloride guard tube attached to the neck and stirred for half an hour in cold condition (0-4 ^oC). DCC (1.03gm, 0.005mole) was dissolved in 5ml of dimethyl formamide in a beaker and was then added dropwise to a solution of Aceclofenac and stirred for 2 hrs in cold condition (0-4 ^oC). Ala- Sal (1.04gm, 0.005 mole) was dissolved in 10ml DMF in a beaker and stirred for 1hr in cold condition (0-4 ^oC). The solution was then added to the above mixture dropwise and stirred continuously for 4hrs in cold condition (0-4 ^oC) and 12 hrs at room temperature. The reaction mixture was further filtered to remove the byproduct, precipitated DCU. The filtrate was dried under vacuum. The crude product was purified by column chromatography using hexane: ethyl acetate: chloroform (2:0.5:0.25) as a mobile phase. The following results obtained clearly depicted the formation of the title compound.(Yield 69%).

IR: Frequency (cm^{-1}): 3319, 3280 (N-H stretch, s. amide), 3265 (N-H stretch, pri. amide) 3095, 3028 (Aromatic C-H stretch), 2937, 2866 (Aliphatic C-H stretch), 1766 (-COO stretch, Ester I), 1737 (-COO stretch, Ester II), 1658 (CO stretch, amide I), 1589 (CO stretch, amide II), 1577, 1510 (Aromatic stretch), 1247 (C-O stretch),1156 (Aryl C-F stretch); 1H-NMR: ($CDCl_3$) δ(ppm): 6.58-7.58 (m, 13H, Aromatic Region and NH of s.amine),4.08 (s,2H, CH2 of -*CH2* – COO), 4.86(s, 2H, CH2 of –O-*CH2* –COO), 8.0 (s, 2H, NH2 of NH2CO-), 8.38 (d, 1H, CH_3 of –CH-CH_3), 4.50 (s, 1H, CH of –CH $NHCOCH_3$), 1.28 (d, 3H, CH_3 of –CH $NHCOCH_3$).M^+ Peak: 544.43

Synthesis of mutual prodrug of Aceclofenac with Glucosamine using alanine as spacer (Acec -Ala- Glu)

This synthesis also carried out using similar methodology as mentioned forFlu-Ala-Para (yield 64%) Scheme-1.IR: Frequency (cm^{-1}): 3566, 3488 (O-H stretch),3336 (N-H stretch, s. amine),3293 (N-H stretch, amide),3110, 3067 (Aromatic C-H stretch),3010, 2984(Aliphatic C-H stretch), 1734(-COO stretch), 1675(CO stretch, amide I),1653(CO stretch, amide II), 1595, 1554 (Aromatic stretch),1296, 1161 (O-H bend, sec. alcohol)1109 (Aryl C-Cl stretch);1H-NMR: ($CDCl_3$) δ(ppm): 6.46-7.50 (m, 8H, Aromatic Region and NH of $NHCOCH_3$), 3.98 (s, 2H, CH2 of -CH2 – COO), 4.65 (s, 2H, CH2 of –O-CH2 –COO) 9.20 (D, 1H, NH of -NHCO-), 2.0 (s, 4H, OH of Sec. alcohol), 2.52 (s, 2H, CH2 of CH2-OH), 3.28-6.30 (m, 5H, Tetrahydropyran), 8.48 (d, 2H, NH of –CONHCH-CH_3), 4.47 (s, 1H, CH of –CH $NHCOCH_3$).M^+ Peak: 586.41

Synthesis of mutual prodrug of Aceclofenac with Thymol using alanine as spacer (Acec -Ala- Thy)

This synthesis also carried out using similar methodology as mentioned for Flu-Ala-Para (yield 66%) Scheme-1. IR: Frequency (cm^{-1}): 3319 (N-H stretch, s. amine),3277(N-H stretch, s. amide),3068 (Aromatic C-H stretch),2970, 2866 (Aliphatic C-H stretch), 1755 (-COO stretch, Ester I), 1737 (-COO stretch, Ester II), 1658 (-CONH stretch, amide), 1577, 1510 (Aromatic stretch), 1247 (-C-O stretch), 1153 (Aryl C-Cl stretch); 1H-NMR: ($CDCl_3$) δ(ppm): 6.55-7.33(m, 11H, Aromatic Region and NH of $NHCOCH_3$) , 4.0 (s, 2H, CH2 of -*CH2* – COO), 4.76(s, 2H, CH2 of –O-*CH2* –COO) 1.18(d, 6H, 2CH3 of –$CH(CH_3)_2$), 3.21(sep, 1H, CH of - $CH(CH_3)_2$Ar), 3.22 (s, 3H, CH3 of -CH3Ar), 8.48(d, 1H, NH of –CONH), 4.48 (s, 1H, CH of –CH $NHCOCH_3$).M^+ Peak: 557.55.

Elemental analysis of title compounds are given in the Table- 1.

Part-II: Invitro Hydrolysis kinetics Studies[4-9]

SynthesizedMutual prodrugswere subjected to chemical hydrolysis (using buffer of physiological pH) and enzymatic hydrolysis (using 80% human plasma). The reactions were monitored by UV for the increase in concentration of free drug with time.

In vitro hydrolysis studies in buffer solution of pH 1.2 (HCl buffer)

10mg of mutual prodrug was dissolved in 5ml chloroform in a 250ml beaker and 195ml buffer solution was added to it. The solution was stirred using magnetic stirrer and the temperature was maintained at 37^0C. 1ml aliquot was withdrawn from the beaker at the end of 0.5, 1, 2, 3 and 4 hours and sink conditions were maintained. The aliquots withdrawn were extracted with 5 ml chloroform. Chloroform layer was than extracted with 5ml of 0.05N NaOH solution. Absorbance of aqueous layer was recorded against 0.05N NaOH as blank treated similarly at λmax 278nm for aceclofenac. Table-2

The mutual prodrugs did not hydrolyze in buffer solution of pH 1.2 upto 4 hrs, As The absorbance of the aqueous layer was found to be negligible

In vitro hydrolysis studies in buffer solution of pH 7.4 (Phosphate buffer)

40mg of mutual prodrug was dissolved in 10ml chloroform and 1ml of the same was added to 99ml buffer solution in a 250ml beaker. The solution was stirred using a magnetic stirrer and the temperature was maintained at 37^0C. 1ml aliquot was withdrawn from the beaker at the end of 0.5, 1, 2, 3 and 4 hoursand sink conditions were maintained. The sample solution of pH 7.4 was acidified with 0.5ml of 9.6% phosphoric acid solution, prior to extraction. The aliquots withdrawn were extracted with 7ml chloroform. 5ml Chloroform layer was extracted into 5ml of 0.05N NaOH solution. Absorbance of aqueous layer was recorded against 0.05N NaOH as the blank treated similarly at λmax278nm for aceclofenac. Table-2

Plasma hydrolysis studies of mutual prodrugs

40mg of mutual prodrug of aceclofenac was dissolved in 10ml chloroform and 1ml of the solution was added to 99ml 80% human plasma in a 250ml beaker. The contents of the beaker were stirred continuously and the temperature was maintained at 37^0C. 1ml aliquot was withdrawn at the end of 0.5, 1, 2, 3 and 4 hours and sink conditions were maintained. The sample of pH 7.4 was acidified with 0.1ml of perchloric acid solution, prior to extraction. The aliquots withdrawn were extracted with 7ml chloroform. 5ml of Chloroform layer was extracted into 5ml of 0.05N NaOH solution. Absorbance of aqueous layer was recorded against 0.05N NaOH as the blank treated similarly at λmax278nm for aceclofenac. Table-3

Part III: Invivo pharmacological evaluation [10-17]

Anti-inflammatory activity:

The anti-inflammatory activity of the synthesized compounds was evaluated using Carrageenan-induced rat paw edema model. Albino wistar rats weighing 100- 200gm were divided into 7 groups of 6 rats each. Synthesized Mutual prodrugs were evaluated at single dose level; Aeclofenac was used as standard anti-inflammatory drug for comparison. 0.1 ml of 1% w/v Carrageenan solution was injected subcutaneously into the plantar region of the right hind paw of the rats to produce edema. The control animals were treated with vehicle (sodium CMC solution) instead of drugs. The dose administered of mutual prodrugs of aceclofenac was equivalent to 10mg/kg body weight of aceclofenac. The paw edema volumes were measured using digital plethysmometer at various time intervals like 0, 1, 2, 3, 4, 6 and 24hrs after carrageenan injection. The hind paw edema inhibition at doses of test drug and standard was calculated by comparing with vehicle treated control rats. The % inhibition of paw edema volume by the test compound or standard anti-inflammatory drug was calculated by the formula: (A – B)/A X 100, Where A represent control value and B represents the standard and/or test value. Fig -1

Analgesic activity:

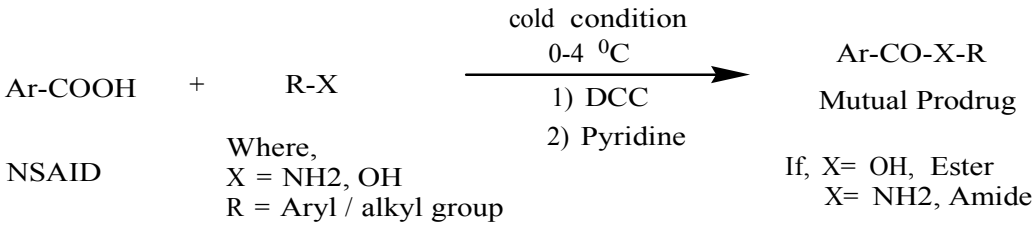
In this method, pain is produced by using radiant heat from the wire electrically heated in an apparatus called Analgesiometer (Mark II, Dolphin, cat no. 1125A). Animals were placed individually in the animal holder and the tail placed on the platform provided, on wire which was maintained at 55^oC ± 1^oC. The time in seconds taken by the animal to flick the tail was noted as the reaction time.

Female wistar rats weighing 30-35 gm were used for the study. The animals were segregated into different groups of six each.

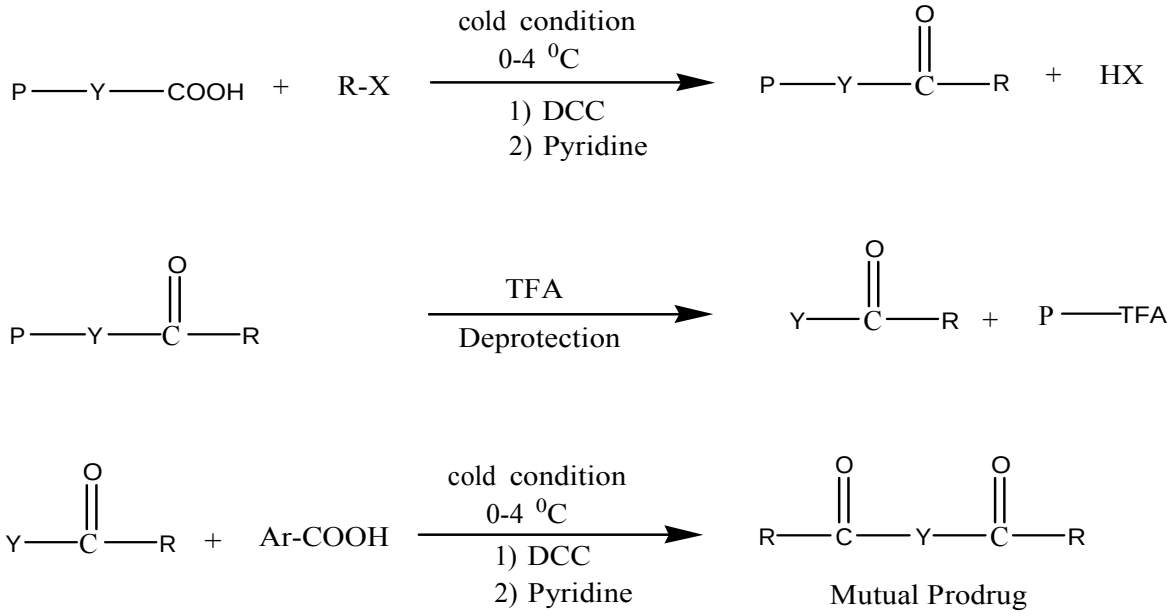
The results obtained for analgesic activity of the synthesized mutual prodrugs of Aceclofenac are depicted in **Fig. 2.**

Scheme-1 Mutual Prodrug of Aceclofenac with and without spacer

A] WITHOUT SPACER



B] WITH SPACER



P = Protecting group (tert-Butyloxycarbonyl) (BOC) Y = Spacer (alanine)

DCC= N, N-Dicyclohexylcarbodiimide TFA= Trifluro acetic acid

Ulcerogenicity:

Albino wistar rats weighing between 130-150 gms were selected. The animals were divided into different groups consisting of five each. Ulcerogenic activity was evaluated after oraladministration of the test compounds at a dose of 10 mg/kg body weight. Control group received only 0.5% CMC solution. After the drug treatment, animals were fed normal diet for 17 hrs and were then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. Mucosal damages were assessed for each stomach according to the following scoring system:

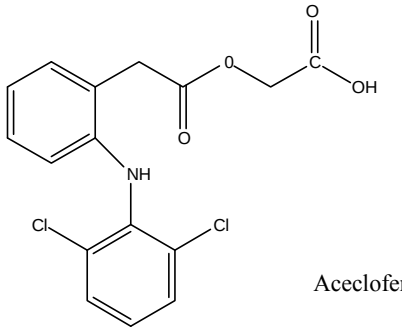
0.5: redness, 1.0: spot ulcer, 1.5: hemorrhagic streaks, 2.0: 3< ulcers ≤ 5, 3.0: ulcers > 5

Female wistar rats weighing 150-200 gms were used for the study. The animals were separated into different groups of the five each. The following groups containing five animals each were used for evaluating ulcerogenic potential of mutual prodrugs of Aceclofenac. The dose selected for this was the same as the one at which the compounds were screened for analgesic and anti-inflammatory activities (10 mg/kg body weight). Results are showed in Table 4

Insilico ADME predictions

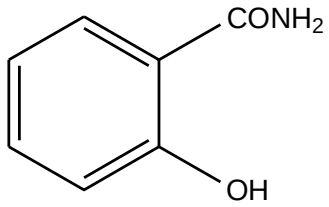
Software used: QikProp, version 3.0, Schrödinger, LLC, New York, NY, 2005. QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion(ADME) prediction program designed to predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. Table- 5

Ar-COOH =

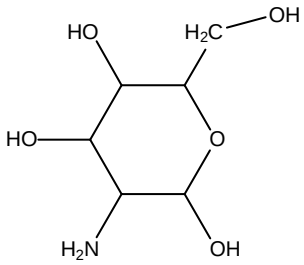


Aceclofenac

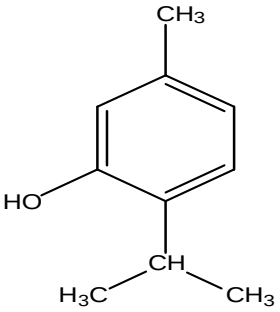
R – X =



Salicylamide



Glucosamine



Thymol

Table- 1: Elemental analysis of title compounds.

Sr. no.	Compound	Empirical Formula	Melting Point^{0}C	%C Cal.	% C found	%H Cal.	%H found	%N Cal	%N Found
1	Acec-Sal	$C_{23}H_{28}Cl_2N_2O_5$	180-182 ^oC	58.35	58.49	3.80	3.89	5.92	6.12
2	Acec-Glu	$C_{22}H_{24}Cl_2N_2O_8$	210-212 ^oC	51.26	51.10	4.66	4.45	5.44	5.52
3	Acec-Thy	$C_{26}H_{25}Cl_2N_1O_4$	104-106 ^oC	64.20	64.26	5.14	5.24	2.88	2.89
4	Acec-Ala-Sal	$C_{26}H_{23}Cl_2N_3O_6$	194-196 ^oC	57.35	56.97	4.23	4.98	7.72	7.57
5	Acec-Ala-Glu	$C_{25}H_{29}Cl_2N_3O_9$	132-134 ^oC	51.19	51.45	4.95	5.12	7.17	6.75
6	Acec-Ala-Thy	$C_{29}H_{30}Cl_2N_2O_5$	110-112 ^oC	62.48	62.68	5.39	5.05	5.03	4.70

Table -2 : Kinetic Profile at PH 7.4						
Mutual	**Time (in hrs.)**					
Prodrugs Prodrugs	**0**	**0.5**	**1**	**2**	**3**	**4**
Acec-Sal	**0**	**5.31**	**12.33**	**17.51**	**25.19**	**33.08**
Acec-Ala-Sal	**0**	**4.09**	**7.75**	**12.76**	**17.05**	**25.13**
Acec-Glu	**0**	**4.22**	**9.81**	**19.12**	**26.11**	**34.19**
Acec-Ala-Glu	**0**	**3.91**	**8.09**	**17.54**	**21.39**	**29.57**
Acec-Thy	**0**	**3.64**	**9.18**	**17.73**	**25.23**	**31.2**
Acec-Ala-Thy	**0**	**4.09**	**8.13**	**15.97**	**19.29**	**27.32**

Table -3 : Kinetic Profile at Human Plasma 80%						
Mutual Prodrugs	Time (in hrs.)					
	0	**0.5**	**1**	**2**	**3**	**4**
Acec-Sal	0	12.93	30.74	53.24	70.11	**88.56**
Acec-Ala-Sal	0	17.51	32.67	56.18	74.92	**92.33**
Acec-Glu	0	10.54	22.62	38.78	55.78	**76.87**
Acec-Ala-Glu	0	13.12	26.75	44.21	61.22	**82.87**
Acec-Thy	0	14.09	27.39	47.8	67.98	**83.96**
Acec-Ala-Thy	0	14.31	30.29	54.08	71.01	**87.19**

Table 4 :Ulcerogenicity of Mutual prodrugs of Acecloenac:

Group	Treatment and dose (mg/kg)	Score	Severity Index
1	Control	**0.0**	**0.0**
2	Aceclofenac (10.0)	**0.5**	**0.5**
3	Acec-Sal (13.4)	**0.0**	**0.0**
4	Acec-Glu (14.6)	**0.0**	**0.0**
5	Acec-Thy (13.7)	**0.0**	**0.0**
6	Acec-Ala-Sal (15.4)	**0.0**	**0.0**
7	Acec-Ala-Glu (16.6)	**0.0**	**0.0**
8	Acec-Ala-Thy (15.7)	**0.0**	**0.0**

Table-5: Value of Lipinski rule of Five of Mutual Prodrugs of Aceclofenac.

S.No	Title Compound	Mol. Wt.	Log Po/w	H-Bond acceptor	H-Bond donor	No. of Rotatable Bonds	Polar SA (PSA)
1	Aceclofenac	354.189	3.416	4.5	2	6	97.701
2	Acec-Sal	473.312	3.625	7.5	3	8	124.32
3	Acec-Thy	486.394	5.656	5.0	1	8	73.539
4	Acec-Glu	515.346	0.016	13.5	6	12	166.85
5	Acec-Ala-Sal	544.39	2.792	9.25	3.25	10	164.45
6	Acec-Ala-Thy	557.472	5.88	6.75	1.25	10	102.673
7	Acec-Ala-Glu	586.425	-0.286	15.25	6.25	14	197.75

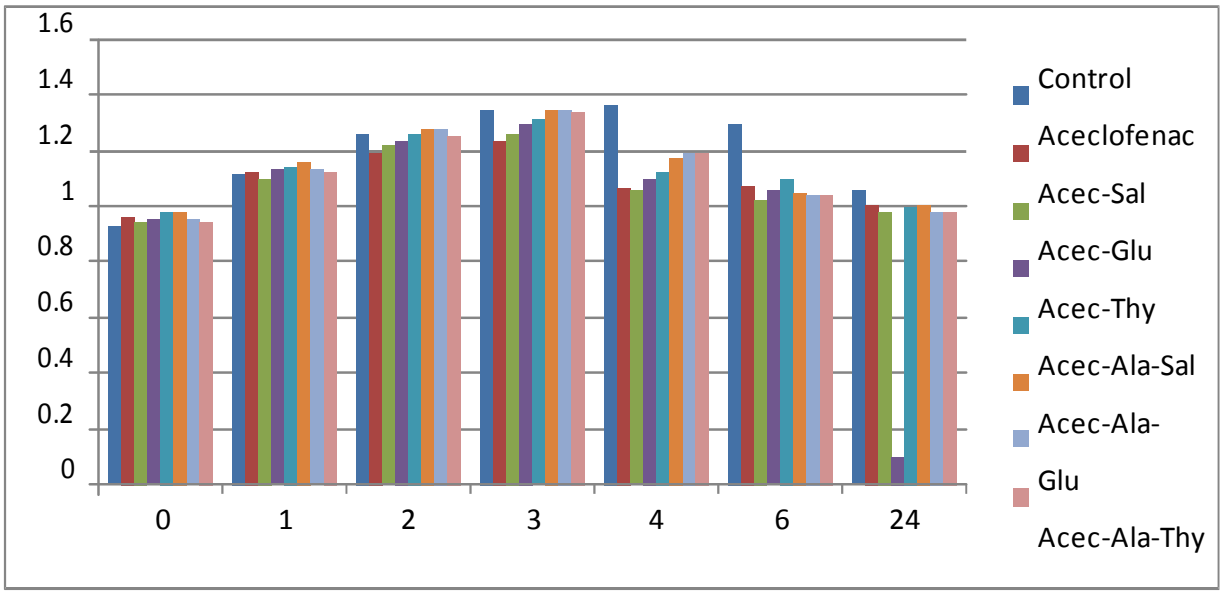


Fig.1: Plot of Paw volume Vs Time interval for test andstandard drugs

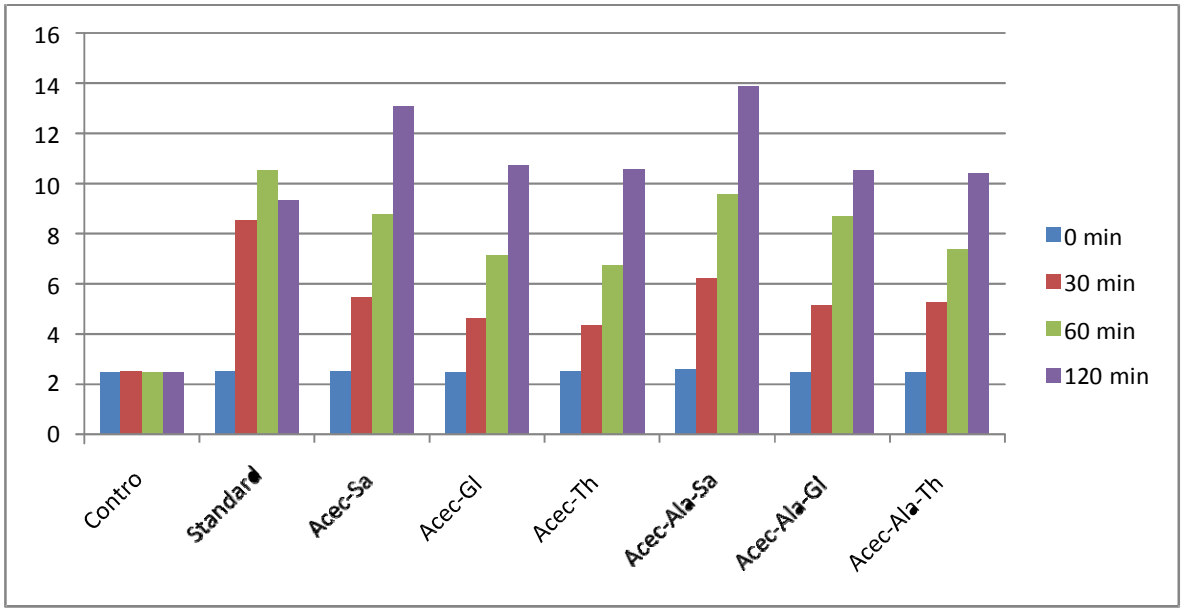


Fig. 2: Plot of Time intervalVs Mutual prodrugs andstandard drug.

RESULTS AND DISCUSSION

All the procedures utilized for synthesis of title compounds were standardized by varying important parameters to optimize the yield of product obtained. The structures of the title compounds were confirmed by spectroscopic techniques including UV, IR and 1H NMR and their purity were established by elemental analysis and TLC.

Hydrolysis studies revealed that all synthesized prodrugs were sufficiently stable at pH 1.2, so that no appreciable hydrolysis to the free acids might occur in the stomach. Hydrolysis of title compounds in phosphate buffer (pH 7.4) was found to be in the range of 25 – 34% over a period of 4 hours and follow first order kinetics. While in 80% human plasma, rate of hydrolysis of the title compounds was in range of 77 – 92% over a period of 4 hours .

Biological activity of synthesized compounds showed that the title compounds exhibited the encouraging anti-inflammatory activity. Title compounds showed good analgesic activity over the time. Further, from the results obtained, it was concluded that the title compounds did not show ulcerogenic properties.

Insilico ADME predictions of title compunds showed that almost all the properties were in range. Log Po/w (measure of lipophilicity) values revealed that they were more lipophilic than the parent drug results in oral bioavailability of title compounds.

CONCLUSION

In-vitro and In-vivo evaluation of the synthesized mutual prodrugs of aceclofenac with Salicylamide, Glucosamine and Thymol in the presence of spacer and without spacer revealed that the release of parent drug from the mutual prodrugs was improved with the incorporation of spacer between them. The mutual prodrugs retained the anti-inflammatory activity of parent drugs.

REFERENCES

[1] Sheha, M., khedr, A. and Elsherief, H., *Eur. J. Pharmaceutical Sci*., **2002**, 17, 121-130.
[2] Omar, F.A., *Eur. J. Med. Chem*., **1998**, 33, 123-131.
[3] Bhosle, D., Bharambe, S., Gairola, N. and Dhaneshwar, S.S., *Indian. J. Pharm. Sci*., **2006**, 68 (3), 286-294.
[4] Sheha, M., khedr, A. and Elsherief, H., *Eur. J. Pharmaceutical Sci*., **2002**, 17, 121-130.
[5] Wadhwa, L.K., Sharma P. D; *International J. of Pharmaceutics*; **1995**,118, 31 -39.
[6] Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, **1996**, vol. 2, A-144.
[7] Ayya, Manoj; M. Pharm Thesis; Mumbai University, Mumbai, **2005**.

[8] Zine, Sandip; M. Pharm. Thesis; Mumbai University, Mumbai, **2006**.
[9] A.S. Narendiran; M. Pharm. Thesis; Mumbai University, Mumbai, **2006**.
[10] Singh, H., Gosh, M. N*; J. Pharm. Pharmacol*; **1968**, Vol 20, 316-317.
[11] Sheetal M. Pharm. Thesis; Mumbai University, Mumbai, **2006**.
[12] QikProp 3.0, User Manual, Schrödinger Press, May, **2007**.
[13] Ajay; Bemis, G. W.; Murkco, M. A. Designing Libraries with CNS Activity. *J.Med. Chem*., **1999**, 42, 4942–4951.
[14] Duffy, E. M., Jorgensen,W. L., *J. Am. Chem. Soc*. **2000**, 122, 2878-2888.
[15] Abraham, M. H.,Andonian-Haftvan, J., Whiting, G. S., Leo, A., Taft, R. S. *J. Chem. Soc. Perkin Trans*. **1994**, 2, 1777-1791.
[16] Willet, P. Chemical Similarity Searching. *J. Chem. Inf. Comput. Sci*. **1998**, 38, 983-996.
[17] Vogel HG, In; Drug Discovery and Evaluation, New York: Springer-Verlag; **2002**, 696.