



ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2010, 2(6):225-233

Synthesis and evaluation of some amino acid conjugates of NSAIDS

Atul R. Bendale*¹, Sachin B. Narkhede², Anil G. Jadhav², G. Vidyasagar³

¹Suresh Gyan Vihar Universe, Jaipur, Rajsthan

²Smt. B. N. B. Swaminarayan Pharmacy College, Salvav (Vapi), Gujarat

³Dean (Pharmacy Department) Kutch University, Bhuj, Gujarat

ABSTRACT

The purpose of present research work is to improve solubility behavior by amino acid conjugation and to overcome general side effects of NSAID. Conversion of drug to prodrug is a chemically modified inert drug precursor, which upon biotransformation lead to activation and produce pharmacological action. Present prodrug approach stands for modification to overcome pharmaceutical barriers like solubility behavior. Though the amino acid conjugates comes under prodrug but it overcome the limitation of prodrug such as formation of unexpected metabolite and undesirable side effects. Tryptophan-Aceclofenac conjugate has maximum water solubility, while in methanol and chloroform solubility of remaining synthesized compounds shows greater result than parent compound. The partition coefficient of some the synthesized compound in octanol/water system was found to be more than the parent drug. Present research work indicates that conjugates synthesized with hydrophilic amino acid possess more water solubility. In future this approach can be applied to other NSAID having free carboxyl functional group as well as in vivo bioavailability study can be undertaken in animals and can be correlated in humans.

Keywords: Aceclofenac, Amino acid, Prodrug, conjugates and Synthesis.

INTRODUCTION

Drug discovery process can be defined as a process that starts with the identification of a disease and therapeutic target of interest and includes methodology and assay development, lead identification and characterization in-vitro, formulation, animal pharmacology studies, pharmacokinetics and safety studies in animals followed by phase I and II clinical studies.

When synthetic organic chemicals overtook the number of natural products, synthetic compounds offered opportunity to medicinal screening of compounds. Medicinal chemistry is

the discipline concerned with determining the influence of chemical structure on biological activity.

Without question, the drugs produced by the ethical pharmaceutical industry over the past century have changed the fabric of society improving both the individual quality of life and life expectancy. Bacterial infections like polio, smallpox, tuberculosis and related diseases and gastric ulcers that were once life threatening to major extent, became minor public health concern. [1]

The ethical pharmaceutical industry originated in Japan in 1600s, while the modern era of drug discovery has its root in the European and U.S.A. fine chemical industries of 19th century. The introduction of the NSAID aspirin as an antipyretic in 1889 to the establishment of a legacy within the pharmaceutical industry that survives to this day with the recent introduction of selective COX-2 inhibitors. [2]

The identification and characterization of cyclooxygenase (COX-2) in inflammatory cells in the early 1990's were the start of a race to the development of more selective non-steroidal anti-inflammatory drugs (NSAID), with reduced side effects (essentially gastro-intestinal toxicity). [3]

Disadvantages of present NSAID

Almost all the currently available agents which are non-selective COX-1 and COX-2 inhibitors, shares the undesirable properties of producing effect to gastric and intestinal mucosa, resulting in erosion, ulcers and gastric bleeding and represent the major adverse reactions to the use of NSAID. The acute and chronic injuries to gastric mucosa results in a variety of lesions referred to as Non-steroidal anti-inflammatory agents (NSAIA) 'gastropathy'.

NSAID induce gastric damage by dual insult mechanism, they are acidic in nature damage the GI tract by changing the permeability of cell membrane allowing a back diffusion of hydrogen ions, causing cell damage; on the other hand the nonselective inhibition of prostaglandin biosyntheses in the GI tract prevents the prostaglandin from exerting their protective mechanism on gastric mucosa

Prodrug approach [4]

Almost all drugs possess some undesirable physicochemical and biological properties. Their therapeutic efficacy can be improved or eliminating the undesirable properties while retaining the desirable ones with the approach of drug design.

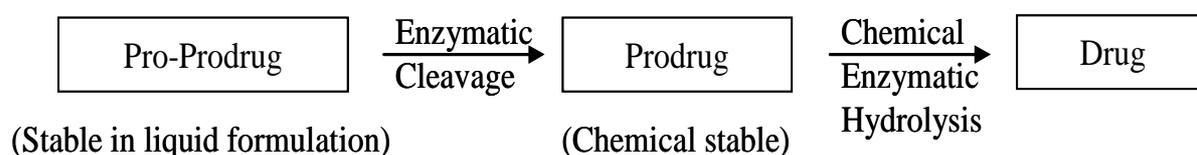
This can be achieved through biological, physical or chemical means. The biological approach is to alter the route of administration. The physical approach is to modify the design of dosage form, such as controlled drug delivery system. The third and best approach is to enhance drug selectivity while minimizing its toxicity is the chemical approach. Prodrug approach is one of the chemical approaches for optimizing the drugs therapeutics.

A prodrug is a chemically modified inert drug precursor, which upon biotransformation liberates the pharmacologically active parent compound. Chemical modification of a drug via the attachment of pro-moiety generates the prodrug. The properties of the prodrug enable it to cross the limiting barrier and it is designed ideally to be cleaved efficiently by enzymatic or non-enzymatic processes. This is followed by rapid elimination of the released pro-moiety.

The term is a chemically modified inert drug precursor, which upon biotransformation prior to eliciting a pharmacological response. These definition Includes metabolites if administered drugs that are true active drugs as well as latentiated drugs. [5]

Notari et al has defined prodrug as an inactive drug precursor formed by covalent bonding of a drug to an inert chemical by linkage, which may be broken enzymatically to release the parent drug. The other terms used interchangeably with prodrug include reversible or bio-reversible derivative and latentiated drug.

Soft drugs are pharmacologically active but undergo controlled and predictable conversion *in vivo*, generating non-toxic metabolites after having its therapeutic effect. Double-prodrug approach a more advanced prodrug design is applied to overcome the stability problems the seldom occur in the formulations of carriers linked prodrug. This is also termed as pro-prodrug.

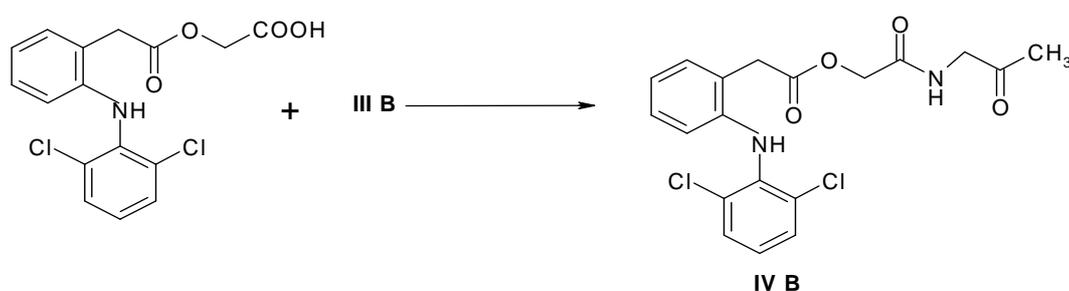
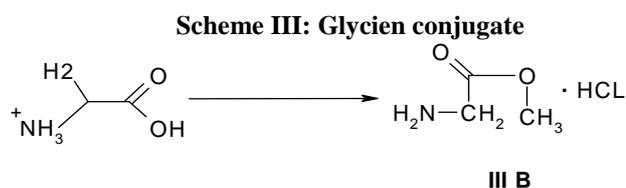
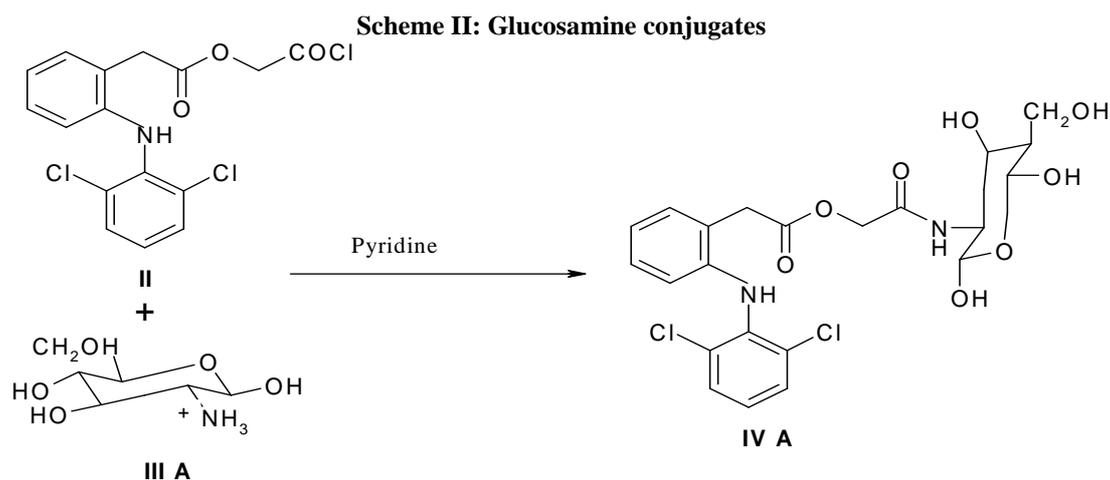
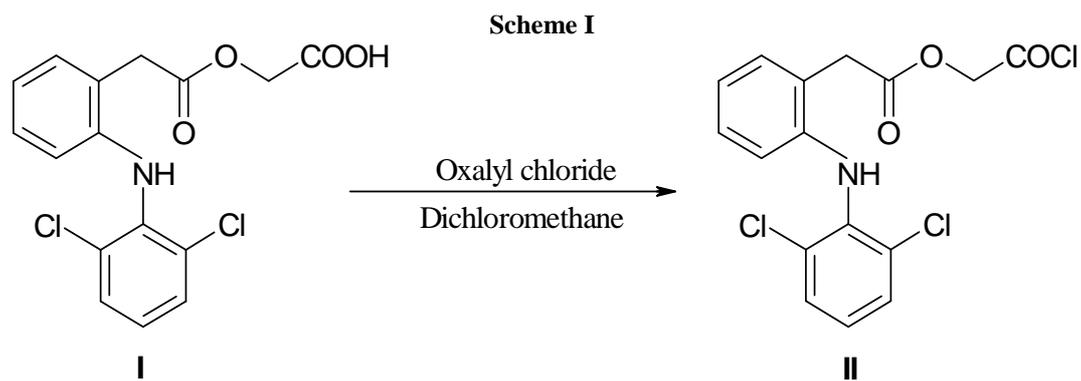


Objectives of prodrug design

The major objectives behind prodrug design are improved formulation, improved chemical stability, improved patient acceptance, improved bioavailability, prolonged action, selectivity and reduced toxicity. Prodrug design, therefore aims to overcome numbers of barriers of the drug usefulness like- Taste and odor, Slow dissolution rate, Poor solubility, Irritation/pain(Pharmaceutical barrier) and Insufficient oral absorption, Short duration, Pre-systemic metabolism, Unfavorable distribution, Non specificity (Pharmacokinetic barriers), Toxicity or side effects (Pharmacodynamic barrier)

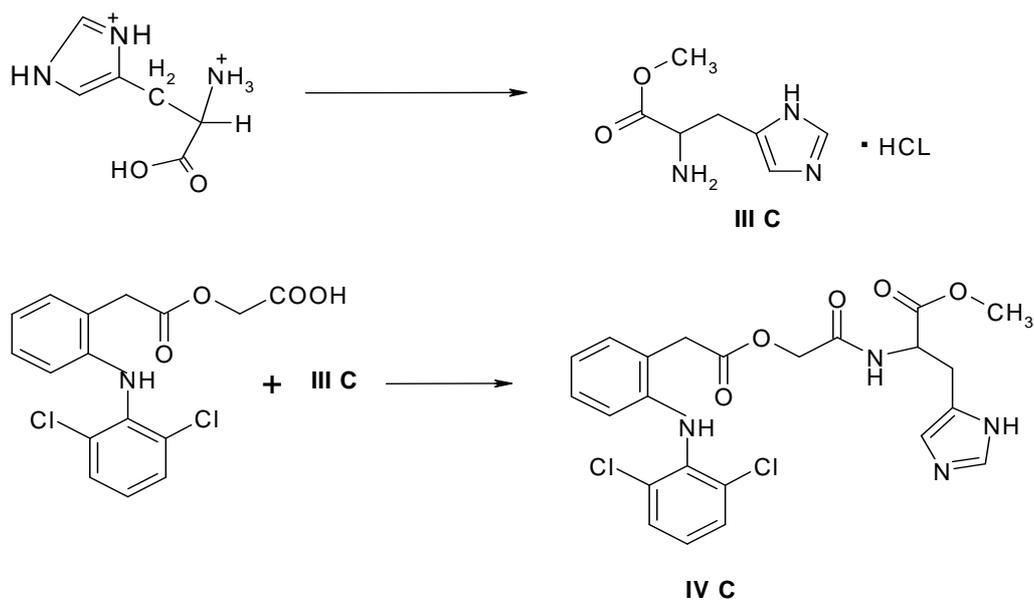
EXPERIMENTAL SECTION

All the research chemicals and solvents were procured from commercial sources and purified by standard procedures described in the literature. Aceclofenac, was procured as gift sample from Suyash Laboratories Ltd, Tarapur India, All the chemicals and solvents used in studies were of GR grade, dried and purified before use. Melting points were obtained using DBK programmed melting point apparatus and are uncorrected. The purification of synthesized compounds was performed by recrystallization with appropriate solvent system. The purity of the compounds was checked using TLC technique, spots were developed by exposure to iodine vapors and UV cabinet, ultraviolet spectra (λ max) were taken on UV 2401 (PC) S 220V double beam UV Spectrophotometer. Infrared spectra were recorded on FTIR spectrophotometer 8400S, Shimadzu corporation, (Tokyo, Japan). Mass spectra were recorded in QP-2010 PLUS GC-MS system (IICT, Hyderabad). Nuclear Magnetic Resonance spectra were recorded with AVANCE 300MHz (IICT, Hyderabad), using CDCl₃ and D₂O.

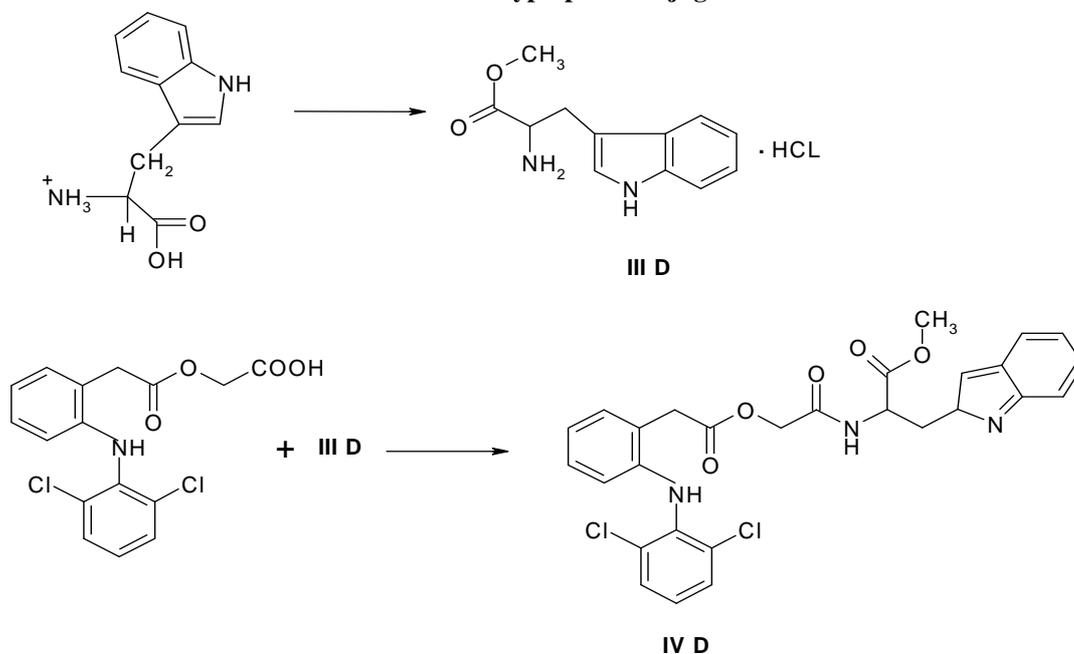


Synthesis of [2-(2, 6 Dichloro-phenyl) amino phenyl] acetic acid chlorocarbonyl methyl ester (II)

2-(2, 6 Dichloro-phenyl) amino phenyl] acetoxy acetic acid, (3.54 g) was taken in a flat bottomed flask; to it dichloromethane (6.5 ml) was added to form a suspension. To this suspension, oxalyl chloride (2.53 g) in 10 ml dichloromethane was added to form a clear solution, it was then stirred for 30 min at room temperature using magnetic stirrer. The solution was concentrated to give yellow solid of acid chloride of Aceclofenac (II), which was recrystallised with methanol and used for further reaction. [6]



Scheme V: Tryptophan conjugate



2-Oxo-2-(2, 5, 7-trihydroxy-6-(hydroxymethyl) oxocan-3-ylamino) ethyl 2-(2-(2, 6-dichloro phenylamino) phenyl) acetate (IV A)

Glucosamine HCL 1.1g was suspended in 10 ml methanol. Triethylamine 0.716 ml was then added drop wise to product-II to liberate free glucosamine. The mixture was stirred for two hrs at room temperature.

The acid chloride was slowly added drop wise at 10-15 °C and then it was filtered. The crude product (IV A) was recrystallised using methanol and dried in air. The reaction was monitored by TLC using chloroform: methanol (9:1) as solvent system. [7]

A. 2-Oxo-2-(2-oxopropylamino) ethyl 2-(2-(2, 6-dichlorophenylamino) phenyl) acetate (IV B). [Glycine conjugate],

B. 2-(2-(2-(2-(2, 6-Dichlorophenylamino) phenyl) acetoxy) acetamido)-3-(1H-imidazol-5-yl) propanoate (IV C) [Histidine conjugate],

C. Methyl 2-(2-(2-(2-(2,6-dichloro phenyl amino) phenyl) acetoxy) acetamido)-3-(2H-indol-2yl) propanoate (IV D) [Tryptophan conjugate],

Freshly distilled thionyl chloride [5ml] was slowly added to methanol [100ml] with cooling and appropriate amino acid (7.5 gm) were added to it. The mixture was refluxed for 6 hrs. at 60-70 °C with continuous stirring on magnetic stirrer. Excess of thionyl chloride and solvent was removed giving crude product. The crude product was triturate with 20 ml portion of cold ether at low temperature. The resulting solid product was collected and dried under vacuum to give crude product. It was recrystallised from hot methanol, followed by cooling. Crystals were collected on next day and washed twice with ether: methanol mixture (5:1) followed by pure ether and dried under vacuum to give pure product.

To the solution of product (12.6 g) dissolved in methanol, triethylamine (10.1g) was added slowly and stirred for 2 hrs. at low temp. The reaction mixture was then filtered and methanol distilled off to get sticky ester. The ester was then dried N,N', dicyclohexylcabodiimide (22.7 g) was added to solution of Aceclofenac (0.1M). and N- hydroxyl succiniimide (0.2 mol) in dry dichloromethane at low temp. Subsequently, ester (0.1 mol) in dichloromethane (100ml) was added to reaction mixture. It was stirred at low temp. for 2 hrs. and then at room temp. for 36 hrs. the reaction mixture was filtered to remove the precipitate and then filtrate washed with 1M HCl, 5% sodium bicarbonate, saturated solution of sodium chloride respectively. The organic layer was dried with anhydrous sodium sulphate to give respective Aceclofenac Amino acid ester. [8]

Table 1: Synthesized conjugates with physical constants

Compound	M.P. (°C)	Yield (%)	R _f Value	Mol. Wt.	Elemental analysis (%) (C,H,N)
IV A	208-210	52	0.68	545	53.05, 5.19, 5.16
IV B	144-146	58	0.60	409	55.76, 4.43, 6.84
IV C	180-182	64	0.55	492	54.66, 4.39, 11.09
IV D	222-224	60	0.74	455	60.66, 4.55, 7.58

Table 2: Mobile phase used for determination of R_f value of synthesized compounds.

Comp. No.	Mobile phase
I	Chloroform : Methanol : Acetic Acid (90 : 10 : 1)
II	Chloroform : Methanol : Ethyl Acetate (70 : 20 : 10)
IV A	Chloroform : Methanol (90 : 10)
IV B	Benzene : Ethyl Acetate (70 : 30)
IV C	Benzene : Ethyl Acetate (70 : 30)
IV D	Benzene : Ethyl Acetate (70 : 30)

RESULT**Spectral data**

A. [2-(2,6-Dichloro-phenyl amino) -phenyl]-acetic acid chlorocarbonyl methyl ester (II)
 λ_{\max} : 292 nm, I.R. (KBr, cm^{-1}): 3317.34 (N-H str), 2937.38 (C-H str), 1770.53 (C=O str), 1577.66(NH bend), 1452.30, 1417.58 (assymetrical bend, -CH₃), 748.33, 667.32.

B. 2-Oxo-2-(2,5,7-trihydroxy-6-(hydroxymethyl)oxocan-3-ylamino)ethyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate (IV A)(Aceclofenac-glucosamine conjugate)

λ max : 261 nm, I.R. (KBr, cm⁻¹): 3342 (OH str), 3292 (N-H, str), 3090, 3045 (aro. -CH str), 2943 (CH str -CH₃), 2923 (CH str -CH), 1653 (conjugated C=O str), 1618 (C=O str, amide), 1583 NH bend, sec. amide), 1446, 1421 (asymmetric bend, -CH₃), 1394 (symmetrical bend -CH₃), 1249 (OH bend, sec. OH), 1032 (in plane - CH bend, aromatic), 856, 773, 696., NMR (CDCl₃) :1.52 (d 3H methyl), 3.2-3.9 (s, 4H, tetrahydropyron), 3.92 (d, 1H, methane), 7.1-7.4 (m, 8H, Ar-H), 8.0 (d, 1H, sec amide), MS (m/z): 545, 431, 353, 337, 309, 277, 242, 214, 194, 178, 167, 151, 125, 107, 89, 55, 41.

C. 2-Oxo-2-(2-oxopropylamino) ethyl 2-(2-(2,6-dichlorophenylamino) phenyl)acetate (IV B) (Aceclofenac-Glycine conjugate)

λ max : 282 nm, I.R. (KBr, cm⁻¹): 3405 (-NH str), 1730 (ester carbonyl group), 1685 (amide carbonyl) 1446, 1421 (asymmetric bend, -CH₃), 1394 (symmetrical bend -CH₃), NMR (CDCl₃) :2.1(s,2H), 3.7(m, 3H), 4.10(m,2H), 5.0 (s, CO-N), 7.5-7.7 (M, 5H), MS (m/z): 409, 373, 339, 309, 277, 255, 242, 174, 127, 74, 46, 30.

D. 2-(2-(2-(2-(2,6-Dichlorophenylamino)phenyl)acetoxy)acetamido)-3-(1H-imidazol-5-yl) propanoate (IV C) (Aceclofenac-Histidine conjugate)

λ max : 284 nm, I.R. (KBr, cm⁻¹):3320 (-NHstr), 1734 (ester carbonyl group), 1620 (amide carbonyl bend), 1446, 1421 (asymmetric bend, -CH₃)1394 (symmetrical bend -CH₃), NMR (CDCl₃) : 3.20 (s, 2H), 3.90(d,1H),5.0(s, CO-N), 6.8(m, 6H), 7.6-7.8 (t, 5H), MS (m/z): 492, 431, 410, 353, 312, 277, 256, 242, 185, 167, 131, 110, 96, 82, 68.

E. Methyl 2-(2-(2-(2-(2, 6-dichlorophenylamino) phenyl) acetoxy) acetamido)-3-(2H-indol-2-yl) propanoate (IV D) (Aceclofenac-tryptophan conjugate)

λ max : 284 nm, I.R. (KBr, cm⁻¹):3320 (-NH str), 1734 (ester carbonyl group), 1620 (amide carbonyl bend), 1446, 1421 (asymmetric bend, -CH₃), 1394 (symmetrical bend -CH₃), NMR (CDCl₃): 3.20 (s, 2H), 3.90(d,1H),5.0(s, CO-N), 6.8(m, 6H), 7.6-7.8 (t, 5H), MS (m/z): 455, 419, 404, 319, 277, 242, 227, 219, 204, 161,132, 107, 89, 64, 56.

Solubility studies

The aqueous solubility of synthesized compounds were determined by stirring 200 mg accurately weighed compounds in water (10 ml) with a magnetic stirrer for 4 hrs. in a sealed flask. The solvent was filtered through Whatman filter paper No.42 and the portion of the filtrate was suitably diluted with water. The concentration of the compounds was determined by measuring the UV absorbance at λ max in water. The solubility was calculated mg/ml. [9]

Table 3: Solubility data of compounds in various solvents

Solvent	Water	Methanol	Ethanol	Chloroform
Compound	Solubility (mg/ml)			
IV A	12.48	21.45	26.56	41.28
IV B	13.32	24.98	26.08	40.92
IV C	11.47	17.42	23.82	49.67
IV D	16.43	27.62	26.42	41.04
Aceclofenac	1.02	10.47	13.93	33.48

Solubility in methanol, ethanol and chloroform

The solubility of the synthesized compounds were determined by stirring 500 mg accurately weighed synthesized compounds in methanol, ethanol and chloroform, respectively (5 ml) with a magnetic stirrer for 4 hrs. in a sealed flask. The solvent was filtered through Whatman

filter paper No. 42 and 1 ml filtrate was taken in the previously weighed china dish. The solvent was evaporated off and the weight of the residue determined. The solubility was calculated in mg/ml.

Determination of partition coefficient

The partition coefficients of the synthesized prodrugs were determined in three systems. A synthesized compound (100 mg) was added to 10 ml of aqueous phase and 10 ml of organic phase was added to it. This mixture was shaken for 30 sec in 10 min interval for 1 h and left for 2 h. Two layers were separated out using separating funnel. Concentration of the drug in aqueous phase was determined by measuring the UV absorbance at the λ max of the individual prodrug. [10]

Table 4: Partition coefficients data of the synthesized prodrug compounds

Compounds	Buffers		
	Octanol-Water (1:1)	Octanol-Hydrochloric acid (pH 1.2)	Octanol-Phosphate Buffer (pH 7.4)
IV A	13.73	20.82	5.35
IV B	11.92	18.53	7.69
IV C	10.98	15.37	9.25
IV D	10.26	16.19	7.76

DISCUSSION

In the present study conjugates in the form of esters and amide were synthesized by linking Glucosamine, Glycine, Histidine and Tryptophan with Aceclofenac as per the procedures described in schemes.

The parent drug Aceclofenac is practically insoluble in water, and literature revealed that the conjugates improve the water solubility. Thin layer chromatography was performed on silica gel G glass plates using suitable solvents systems (Table 2) to ascertain the purity of these compounds. The percentage yield, melting point and analytical data of the synthesized compounds are listed in Table-1. IR and NMR spectra confirmed the structure of the compounds. IR spectra exhibited characteristic absorption bands of N-H stretching, C=O stretching and C-H stretching functional groups vibrations. The acid chloride of Aceclofenac (II), showed IR absorption frequencies 3317.34 (N-H Str.) cm^{-1} , 2937.38 (C-H Str.) cm^{-1} , 1770.53 (C=O Str.) cm^{-1} synthesized compounds from IV A-D showed IR absorption frequency for N-H stretching in between 3566.54 to 3223.40 cm^{-1} , C-H stretching in between 2998.05 to 2918.05 cm^{-1} and C=O stretching in between 1770.53 1730.96

The solubility of compounds was determined in water, methanol, ethanol and chloroform. The parent drug Aceclofenac is practically insoluble in water, all the synthesized compounds showed better water solubility than the parent compound Aceclofenac. The compound IV D has maximum water solubility i.e. 16.43 mg/ml. While in methanol and chloroform solubility of synthesized compound is greater than parent compound.

A drug's partition coefficient is a measure of its distribution in a lipophilic /hydrophilic phase system, and is indicative of its ability to penetrate biological multiphase system. The partition coefficients of the compound were determined in three systems, i.e. Octanol /water, Octanol /hydrochloric acid (pH 1.2) and Octanol /phosphate buffer (pH 7.4).

The values of partition coefficient range from 10.26 to 13.73 in Octanol /water system. The value of partition coefficient range from 15.98 to 20.82 in Octanol /hydrochloric acid (pH 1.2) system and the value of partition coefficient range from 5.35 to 9.25 in Octanol /phosphate buffer (pH 7.4).

CONCLUSION

The conjugates of Aceclofenac were synthesized using simple synthetic route in good yields and their structure were confirmed by spectral analysis, the solubility of the compounds were found to be more in organic phase as compared to aqueous phase this indicates the lipoidal nature of the synthesized compounds, while compound IV B and highest solubility in water. The partition coefficients of the synthesized compounds found more in Octanol/ hydrochloric acid buffer (pH 1.2) as compared to Octanol /water and Octanol /phosphate buffer (pH 7.4). The partition coefficient of IV A, IV B and IV D were found to be remarkably high in two of the three systems as compared as to other conjugates.

The partition coefficient of all the synthesized compounds in Octanol /water system was found to be more than the parent drug. This study indicates that the synthesized compounds are more lipophilic than parent drug.

Future Scope

- a. The synthesized compounds can be subjected to in-vivo analgesic, anti-inflammatory and anti-ulcerogenic activity study.
- b. *In vivo* bioavailability study can be undertaken in animals and can be correlated in humans.
- c. *In vitro* plasma hydrolysis of the compounds can be done.
- d. Stability studies of the compounds as per ICH guideline can be performed.
- e. This approach can be applied to other NSAID having free carboxyl functional group.

REFERENCES

- [1] John H. B. and John M. B., Prodrugs and Drug Latentiation, Forrest, T. S. and Randall, C. C., Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry, 11th Edn., Lippincott Williams and Wilkins, **2004**,142-155.
- [2] David A. W. and Thomas L., Origin of Medicinal Chemistry, William. O. Foye, Foye's Principles of Medicinal Chemistry, 5th Edn., Lippincott William and Wilkins, **2002**,1-3.
- [3] Peter E., Gordon L., Clement B. and Testa B., *J. Med. Chem.*, **2004**, 47: 2393-2404.
- [4] Brahmanekar D. M. and Jaiswal S. B., Biopharmaceutics and Pharmacokinetics A Treatise, 1st Edi., Vallab Prakashan, Delhi, **1995**, 159.
- [5] Arienes E. J. and Simonis A. M., Strategy in Drug Research, Elsevier, Amesterdam, **1982**, 165.
- [6] Hutchison I., Sharon A., Jennings B., Rao V. and Andrew, W., *J. Med. Chem.*, **2001**, 45: 744-747
- [7] Niethammer A., Gerhard G., Holger N. L. and Wolfgang. W., *Bioconjugate Chem.*, **2001**, 12(3), 414-420.
- [8] Glodeshwar B. C., Pophalika R. N., Bhojani M. R., Deepoka N. and Sumeela, S. D., *Indian J. Pharm. Sci.*, **2004**, 66(6) : 773-777.
- [9] Doshi A., Samnt S. D. and Deshpande S. G., *Indian J. Pharm.*, **2001**, 66(5) : 440-444.
- [10] Pradip M., Lopamudra B. and Agrawal R. K., *Indian J. Pham. Sci.*, **1989**, 51 (1): 11-13.