Research Article

Synthesis and Evaluation of Anti-inflammatory Activity of Mutual Prodrugs of Aspirin with Amino Acids

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are the first choice drugs used in treatment of pain, degenerative inflammatory joint diseases and rheumatoid disorders; they were found to be associated with undesirable side effects ranging from dyspepsia to symptomatic and complicated gastric and duodenal ulcers. The greatest degree of damage is generally caused by NSAIDs which contain a free carboxylic group. The present research work emphasizes reducing the side effect of aspirin by masking the carboxylic acid group with methyl ester of amino acids through the formation of amide linkage. The amino acid like alanine, tyrosine, tryptophan, arginine, cysteine, and glycine were chosen as a promoiety because they had broad spectrum of anti-inflammatory, cytoprotective and immunomodulatory properties and therefore would synergize the effect of prodrugs. We reported here design and development of prodrug of Aspirin with amino acids, for enhancing its anti-inflammatory potential and reducing the gastric side effect. The amide prodrugs were synthesized by coupling with DCC and its structures were confirmed by spectral analysis. The prodrug was extensively screened for anti-inflammatory activity, and increased anti-inflammatory activities of aspirin tryptophan prodrug were observed as compared to parent drug.

Keywords: NSAIDs, aspirin, mutual prodrugs, amide prodrugs, anti-inflammatory, conjugates.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAID) are commonly used in treatment of chronic inflammatory diseases[1].Currently available agents which is non-selective cox-1 and cox-2 inhibitors, shares the undesirable properties of producing effect to gastric and intestinal mucosa, resulting in erosion, ulcer and gastric bleeding and represent the major adverse reactions to the use of NSAIDs. The basic dual mechanism of gastric damage is their acidic nature damage the GI tract by changing the permeability of cell membrane, by allowing a back diffusion of hydrogen ions, causing cell damage. And also, nonselective inhibition of prostaglandin biosynthesis in the GI tract prevents the prostaglandin from exerting their protective mechanism on gastric mucosa[2].Aspirin is most important non-steroidal anti-inflammatory drug which affect on variety of inflammatory mediators but unfortunately like other NSAIDs it has gastro-intestinal side effects. The gastric side effect of aspirin is due to the presence of free carboxylic acidic group. Their therapeutic efficacy can be improved or eliminated the undesirable properties while retaining the desirable ones with the approach of drug design. This can be achieved by means of physical, chemical, biological modifications. The physical approach is to modify the design of dosage form, such as novel delivery system. The biological is to alter the route of administration of drug. The third is to improve the drug selectivity by minimizing the toxicity profile of drug.

Prodrug is one of the best chemical approaches to reduce the toxicity of drug[3].The term “prodrug” was first introduced by Albert’s[4]. Harper referred to this process as drug latentiation that is chemical modification of a biologically active compound to form a new compound that upon in vivo enzymatic attack will liberate the parent compound. A prodrug is a chemically modified inert drug

precursor, which upon biotransformation liberates the pharmacologically active parent compound. Chemical modification of drug via the attachment of promoiety generates the prodrug. The major objectives of prodrug design are improved formulation, improved chemical stability, improved patient acceptance, improved bioavaibility, prolonged action, selectivity and reduced toxicity. Prodrug design, therefore: aims to overcome numbers of barriers of the usefulness like taste and odor slow dissolution rate, poor solubility, Irritation or pain and sufficient oral absorption and short duration of action.[5-8]The selection of amino acid was done in such manner that, the prodrug with liphophilicity could be obtained. High solubility in organic solvents was observed for the newly synthesized prodrug indicating the lipophilic behavior. Hence, the aim of the present study is to reduce the gastric toxicity of Aspirin with its amino acids conjugates in relation to reduce gastro intestinal toxicity by masking the acidic group of aspirin with amide group of amino acids and to increase anti-inflammatory activity.

MATERIAL AND METHODS

Aspirin, amino acids, thionylchloride, N, N Dicyclohexylcarbodimide were commercially obtained from Loba chemicals Pvt. Limited, Mumbai, India. Diethyl ether, methanol, triethylamine, N, N diethyl formamide, N-hexane purchased from Merck Chemicals, Mumbai. Sodium sulphate, Sodium bicarbonate and Chloroform were commercially obtained from Research Lab Pune, India. Distilled water was used throughout the study. All other material used was of analytical grade. The purity of compound was checked using TLC technique & melting point. Infrared spectra were recorded on FTIR spectrophotometer 8400S, Shimadzu corporation. ^{1}H-Nuclear magnetic resonance spectra record on ^{1}H-NMR Varian Mercury 300 MHz with super conducting magnet at Garware Research Centre, University of Pune.

SYNTHESIS OF AMINO ACIDS CONJUGATES OF ASPIRIN

Synthesis of Amino acid Methyl Ester Hydrochloride[9]

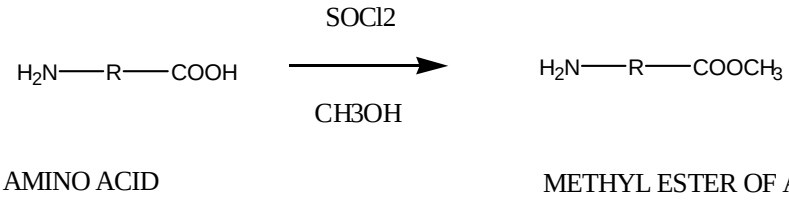
Freshly distilled thionylchloride 120mmole was slowly added to methanol 300ml with cooling and amino acid was added to it. The mixture was refluxed for 10 h at 60ºC to 70ºC with continuous stirring on magnetic stirrer. The solvent was distilled at 64ºC to 65ºC and the resulting product was collected and triturated with cold diethyl ether to remove the excess amount of dimethyl sulphite. Obtained crude product was recrystallized with hot methanol by adding 20 to 25ml diethyl ether followed by cooling at 0ºC.

Synthesis of Amino acid conjugates of Aspirin

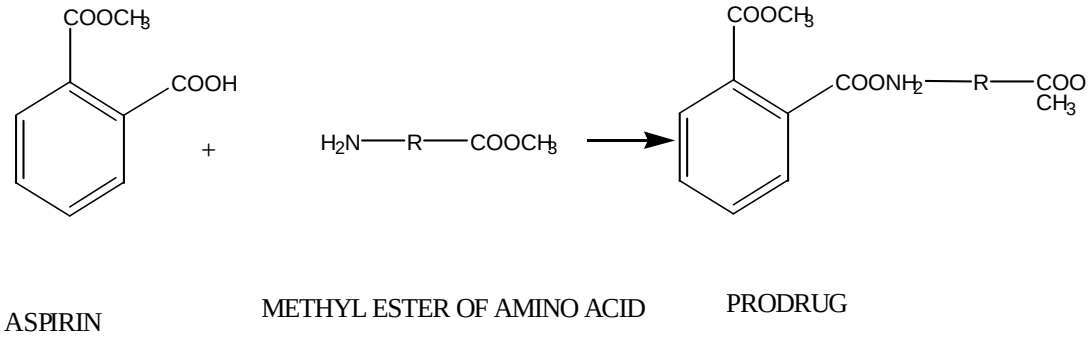
20 mmole Aspirin was dissolved in 60 ml Dimethylformamide (DMF) in conical flask A and 20 mmole N-N Dicyclohexylcarbodimide (DCC) was added with continuous stirring, 20 mmole of methyl ester hydrochloride of amino acid was dissolved in conical flask B and 42 mmole triethylamine was added at 0° c. The content of flask A were added to into B after stirring for 15 min on magnetic stirrer the mixture was filter and equal volume of distilled water was added to the filtrate followed by extraction of drug with ether. In ether layer 10 to 15 gm sodium sulphate (anhydrous) was added and the crude product was recrystallized with chloroform and washed with 10 % w/v dilute hydrochloric acid, saturated solution of sodium chloride and sodium bicarbonate sequentially. Anhydrous sodium sulphate was added to remove the water content, filtered and concentrated under rotary evaporator to obtained pure crude product. The yield was determined and the purity of compound was established by TLC, IR, NMR, Melting point.

A. Scheme for Synthesis Prodrug

Step I



Step II



Biological screening

All the experimental procedures and protocols used for pharmacological screening were reviewed and approved by Institutional Animal Ethics Committee (IAEC) of Sinhgad Institute of Pharmaceutical Sciences, Lonavala and were in accordance with the guidelines of CPCSEA, Government of India. The animal used for the study were housed under standard environmental condition of temperature 23 ± 1ºC and relative humidity of 50 ± 5%.A 12 h light/dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet.

Anti-inflammatory activities by carrageenan-induced rat paw edema method[10]

Wister rat of either sex having body weight 175–200 gm were used in this study. The animals were divided randomly in four groups with three rats per group. A freshly prepared solution of carrageenan (1%w/v, 0.1ml) solution was injected into the planter region of right hind paw of each rat. One group was kept as control and the animals of other group were pre-treated with test drug suspended in 1% CMC given orally 1hour before the carrageenan treatment. The paw volume measured using UGO Basil Plethysmometer 7140, Italy at 0 h, 1h, 3h, 6h, after carrageenan treatment. Percent inhibition was calculated according to following equation.

%inhibition= {1-[(Vd-Vp)/ (Vc-Vp)]} ×100

Where (Vd-Vp) is the difference in paw volume after carrageenan injection (Vd) and before carrageenan injection (Vp) in drug treated group and (Vc-Vp) is the difference in paw volume after carrageenan injection (Vc) and before carrageenan injection (Vp) in a vehicle treated group data was expressed as mean ±SEM.

RESULT AND DISCUSSION

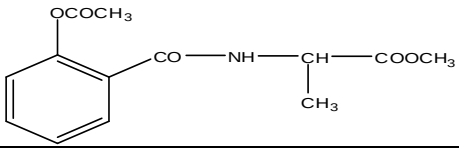
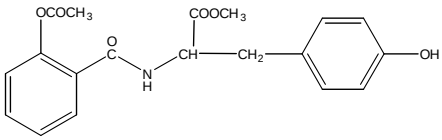
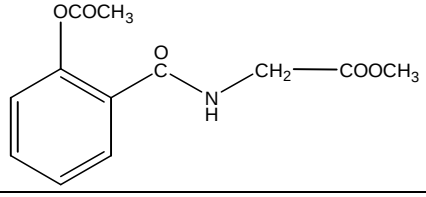
The synthesized prodrugs of aspirin were confirmed by IR, ^{1}H NMR. Moderate to high solubility in various organic solvent, were observed for the prodrug, indicating lipophilic behavior. Pharmacological investigations of the synthesized prodrugs were done for anti-inflammatory activity. The dose of prodrug administered was equivalent to 100 mg/kg body weight. The anti-inflammatory activity activities obtained after 1 h and 6 h of administration of standard drug aspirin were found to be 53 and 60 %. The anti-inflammatory activity after 1 h administration of prodrug AS1, AS2, AS3, AS4, AS5, and AS6 was 61, 63, 58, 78, 52,and 34 % respectively while after 6 h administration 68, 85, 86, 83, 74, and 87 % respectively were observed. The maximum anti-inflammatory activities of all prodrug were observed at 6 h and remain practically constant up to 8 h. It was observed that anti-inflammatory activity of aspirin decreased with time while those prodrugs increased with time. The statistical significance testing using one way analysis of variance of data showed that the anti-inflammatory activity of the prodrug were significant in comparison with aspirin.

The alanine, arginine, tyrosine, tryptophan, cysteine, glycine, containing aspirin amide prodrugs were successfully synthesized and the structure were confirmed by IR and NMR spectral analysis. The prodrugs show the excellent pharmacological response and increased anti-inflammatory activity in comparison to that of parent drug was observed. On the basis of above observation it is concluded that these prodrugs can be successfully applied to minimized gastro intestinal toxicity without loss of desired anti-inflammatory activity of drug. In summary, novel aspirin prodrugs has been developed based on drug amino acid conjugation and it provides a most beneficial and safer way for the delivery of drugs.

Statistical analysis

Statistical analysis of the pharmacological activity of the synthesized prodrugs on animals was evaluated using a one way analysis of variance (ANOVA). Student's t-test was applied for expressing the significance and the experimental data are expressed as mean ± SD (standard deviation).

Table 1: Structureas of all Synthesized Prodrugs

S.No	Amino acid used	Structure of Prodrug
1.	Alanine	
2.	Tyrosine	
3.	Glycine	

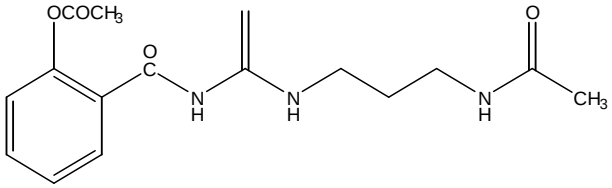
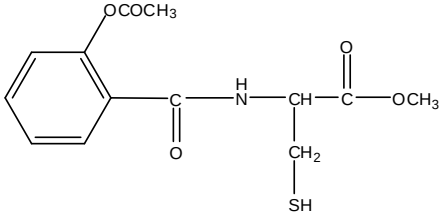
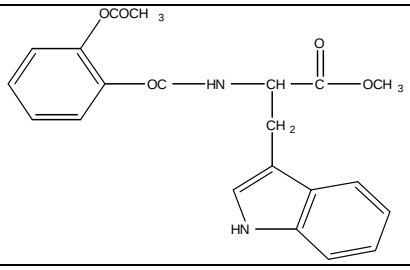
4.	Arginine	
5.	Cysteine	
6.	Tryptophan	

Table 2: General data for various amide prodrugs of aspirin

Prodrug	Molecular Formula	Molecular Weight	Elemental Analysis	Percentage yield	Color	Rf value	Melting point
Arginine (AS4)	C16H21N3O4	319.36	C,60.17:H,6.63:N,13.16:O,20.04	39.22	White	0.54	97ºC-99ºC
Alanine (AS1)	C14H17NO5	279.29	C,6.21:H,6.14,N,5.02:O,21.64	50.29	White	0.56	102°C-104°C
Tyrosine (AS5)	C19H19NO6	357.36	C,63.86:H,5,36:N,3.96:O,26.86	40.27	White	0.66	85°C-87°C
Glycine (AS3)	C12H13NO5	251.24	C,57,37:H,5.22:N,5.58;O,31.81	55.71	White	0.52	107°C-108°C
Tryptophan (AS6)	C21H20N2O5	380.39	C,66.31:H,5.30:N7.36:O.21.03	40.67	White	0.68	95°C-97°C
Cysteine (AS2)	C13H14O6S	298.31	C,52.34:H,4.73:O,32.18:S,10.75	51.38	White	0.71	78°C-80°C
Aspirin (AS)	C9H8O4	180.16	C, 60.00:H, 4.48:O,35.52	-	White	-	135°C-138°C

Mobile phase for TLC: chloroform: methanol (7:2)

Table 3: Solubility data for various amide prodrugs of aspirin

Solvent	Water	Ethanol	Chloroform	Acetone	Diethyl ether
Aspirin	Slightly soluble	Soluble	Soluble	Soluble	Soluble
L-Arginine ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble
D-Alanine ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble
L-Tyrosine ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble
Glycine ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble
Tryptophan ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble
Cysteine ester derivative	Insoluble	Soluble	Soluble	Soluble	Soluble

Table 4: Anti-inflammatory activities by carrageenan- induced rat paw edema

	Dose mg/kg	Paw Vol. in ml				Difference in paw vol.			% Inhibition		
Group		**Before**	**1hr**	**3hr**	**6hr**	**1hr**	**3hr**	**6hr**	**1 hr**	**3 hr**	**6 hr**
Control		0.78±0.09	1.46±0.54	1.77±0.20	1.54±0.39	0.67±0.51	0.99±0.15	0.76±0.48	…	…	…
Aspirin	100	0.77±0.31	0.96±0.39	0.95±0.26	0.9±0.21	0.18±0.10	0.18±0.07	0.12±0.10	53.48	58.10	60.68
Alanine (AS2)	100	0.87±0.23	1.1±0.17	1.12±0.12	1±0.15	0.23±0.13	0.24±0.19	0.12±0.08	61.61	73.17	68.68
Cysteine (AS6)	100	0.85±0.29	0.95±0.15	1.17±0.14	0.97±0.16	0.10±0.17	0.32±0.17	0.11±0.15	63.92	63.34	85.70
Glycine (AS4)	100	0.74±0.27	0.95±0.13	0.94±0.26	0.87±0.24	0.20±0.05	0.19±0.04	0.12±0.03	58.89	77.57	86.01
Arginine (AS1)	100	0.81±0.32	0.95±0.34	0.97±0.22	0.95±0.21	0.14±0.10	0.16±0.10	0.14±0.12	78.17	80.13	83.08
Tyrosine (AS3)	100	0.82±0.31	1.01±0.25	1.03±0.19	1±0.16	0.19±0.09	0.21±0.19	0.18±0.15	52.90	73.25	74.85
Tryptophan (AS5)	100	0.77±0.04	1.01±0.05	1.1±0.07	0.91±0.03	0.43±0.07	0.33±0.10	0.14±0.07	31.44	65.85	87.02

Each value represent the mean ±SD (n=3).Significance level p<0.05 for all values as compared with the respective control.

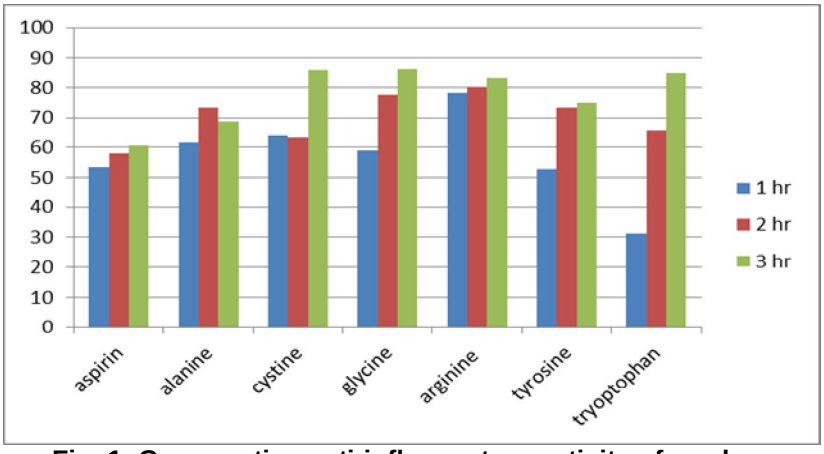


Fig. 1: Comparative anti-inflammatory activity of prodrug (percentage inhibition)

Table 5: Spectral Data of Synthesized Prodrugs

S. No	Prodrugs	IR (KBr, cm^{-1})	^1H NMR (δ, ppm) (CDCl3)
1	Aspirin arginine conjugate (AS1) [2 (1-(3 acetamidopropylamino vinyl carbomoyl) phenyl acetate]	3347(NH), 2926(CH), 1734(C=O), 1691(amide I), 1653(amide II) 1014(C-O ester).	8.10 (1H,NH),2.33(1H,NH), 8.08 (1H,NH),7.91(4H,ArH) 2.33 (1H,C=O),2.66(1H, CH2), 2.02(2H, C=O N),
2	Aspirin alanine conjugate (AS2) [Methyl 2-2 (acetoxybenzamido) propanoate]	3342(NH), 3005 (CH), 1755(C=O), 1691(amide I), 1681(amide II), 1012(C-O ester)	7.27(4H,Ar), 8.14(1H, NH),4.82(1H, CH), 1.66(1H, CH=CH), 2.08(3H, OCH3)
3	Aspirin tyrosine conjugate (AS3) [Methyl 2-2(acetoxybenzamido)-3-(4-Hydroxyphenyl) Propanoate]	3340(NH),2928(CH), 1730(C=O),1688(amide I), 1670(amide-II), 1014(C-O ester)	7.48(4H, ArH),8.08(1H,NH), 4.30(1H, CH),3.76(1H, CH=CH),6.97(4H, ArH), 4.30(1H, OH)
4	Aspirin glycine conjugates (AS4) [Methyl 2-(2-acetoxybenzamido) acetate]	3341(NH), 2931(CH), 1734(C=O), 1693(amide-I), 1674(amide -II), 1014(C-O ester)	7.25(4H, ArH), 8.06(1H,NH), 4.20(1H,CH=CH), 3.72(1H,OH), 2.06(1H,C=O)
5	Aspirin tyryptophan conjugate (AS5) [Methyl 2-(2-acetoxybenzamido)-3 *1H* Indol-3-yl) propanoate]	3342(NH), 2926(CH), 1734(C=O), 1691(amide-II), 1649(amide), 1012(C-O ester)	7.92(4H,ArH),8.04(1H,NH), 5.01(1H, CH), 6.93(4H, ArH), 5.08(IH, OH),
6	Aspirin cysteine conjugate (AS6) [3-Mercapto -1 Oxopropan-2-yl 2-acetoxybenzoate]	3340(NH), 2929(CH), 1751(C=O), 1693(amide-I), 1680(amide-II), 1014(C-O ester)	7.42(4H,ArH), 1.56(1H, SH), 4.27(1H,CH), 3.78(1H,CH=CH), 3.82(1H, OH),

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