Project of Graduation / Fifth stage

Synthesis and Study Ant inflammatory activity of Acetyl salicylic acid derivatives.

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Introduction

The non-steroidal anti-inflammatory drug (NSAIDs) are one of the most common therapeutic group of agent used worldwide for the treatment of pain, fever and inflammation [1,2]. However, the usefulness of these agents is limited due to higher incidence of the observed gastrointestinal damage that include gastric ulceration, perforations and their associated complication. [3]

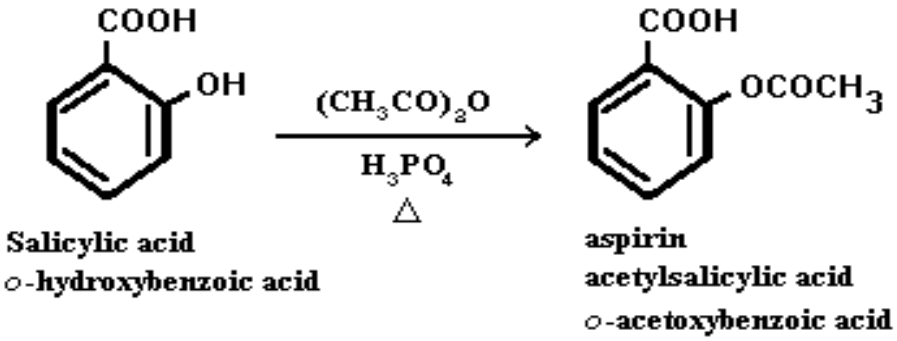
Mechanism of action of NSAIDs:

NSAIDS differ in their relative inhibitory potency against two iso form of COX: COX1 and COX2 the greatest degree of damage is generally caused by NSAIDS that are preferential COX1 inhibitor and contain a free carboxyl group e.g.: aspirins, indomethacin and ibuprofen etc. [4], so the major side effects of NSAIDS is the hemorrhage and ulceration which is due to inhibition of COX 1 which is responsible for the biosynthesis of cytoprotective prostaglandin E2. The PGE2 action include the increase in the blood flow ,increase the bio carbonate secretion, stimulate the mucus production and reduce the gastric acid secretion this protect the GI mucosa, while COX2 is inducible and its synthesis in response to pro inflammatory stimuli such as cytokine and growth factors [5-7] .

The objective of this study to synthesis a new anti-inflammatory derivative of aspirin as a potential selective COX2 inhibition with less ulcero-genic effect based on drug development. The conversation of carboxyl group of these drugs to carboxamide group and a conjugation with specify selected moiety of the heterocyclic compound may impart effect toward selective COX2 inhibitor with lower side effect because these conjugate will make aspirin is similar to that of isosteric functional group of previous coxibs and it is derivative with selective COX2 inhibitor.

Chemistry of aspirin:

One of the best known aromatic acetates is acetyl salicylic acid, or aspirin which is prepared by the esterification of the phenolic hydroxyl group of salicylic acid [8,9], as shown in scheme 1.



Scheme 1: Synthesis of aspirin.

Abstract about the biological activity of aspirin:

Salicylic acid and salicylates, obtained from natural sources, have long been used as medicaments. Salicylic acid was chemically synthesized in 1860 and was used as an antiseptic, an antipyretic, and an antirheumatic. Almost 40 years later, aspirin was developed as a more palatable form of salicylate. Soon after, other drugs having similar actions to aspirin were discovered, and the group was termed the "aspirin-like drugs" (also now termed the non-steroidal anti-inflammatory drugs [NSAIDs]). Twenty-five years ago, it was proposed that the mechanism of action of NSAIDs was through their inhibition of prostaglandin biosynthesis. Since then, there has been general acceptance of the concept that these drugs work by inhibition of the enzyme cyclooxygenases (COX), which we now know to have at least two distinct isoforms: the constitutive isoform, COX-1, and the inducible isoform, COX-2. COX-1 has clear physiologic functions. Its activation leads, for instance, to the production of prostacyclin, which when released by the endothelium is antithrombogenic and when released by the gastric mucosa is cytoprotective. COX-2, discovered 6 years ago, is induced by inflammatory stimuli and cytokines in migratory and other cells. It is therefore attractive to suggest that the anti-inflammatory actions of NSAIDs are due to inhibition of COX-2, whereas the unwanted side-effects, such as irritation of the stomach lining, are due to inhibition of COX-1. Drugs that have the highest COX-2 activity and a more

favorable COX-2: COX-1 activity ratio will have a potent anti-inflammatory activity with fewer side-effects than drugs with a less favorable COX-2: COX-1 activity ratio.

EXPERIMENTAL SECTION

Chemicals and Reagents

All chemicals and reagents were obtained from E. Merck (India) limited and Sigma-Aldrich. Reactions were monitored and the homogeneity of the products was checked by TLC (Thin layer chromatography).

General synthesis of Amide

Acetyl salicylic acid was reacted with thionylchloride to get 2-acetoxy-benzoyl chloride. Acid chloride was further reacted with different amino acid in hexane to get aspirin amides given in scheme (2). Amide prodrugs so synthesized were characterized with the FT-IR technique.

Synthesis of the 2-acetoxy-benzoyl chloride (1)

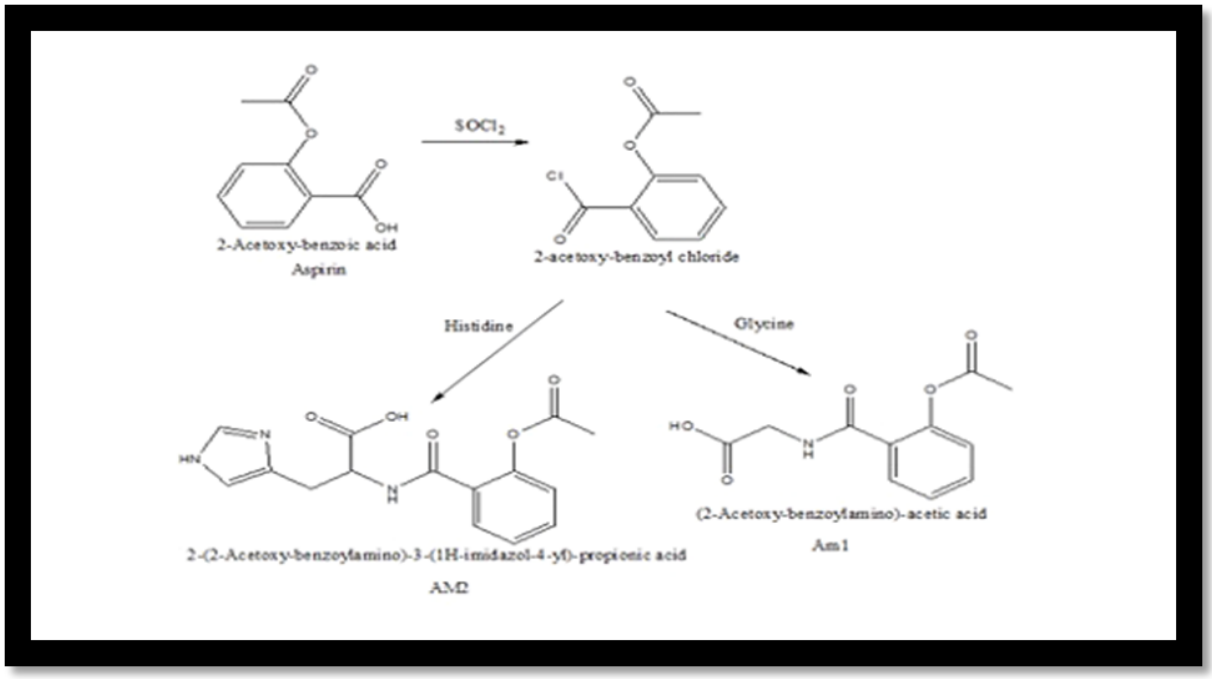
In a 250 mL flask, acetyl salicylic acid (1.8g, 0.01mol.), hexane (10 mL) stirrer the solution to 1 h at room temperature, then slowly added thionylchloride [11] (5.95 mL, 0.05mol) to prevent a rise of heat degree over 30 °C to avoid vapors of SO_2. After ending the adding, the temperature increases to 60-70°C with refluxing with stirrer to get rid of SO2 vapor to 30 mints. Then turn off reflux and stirring to 24 h, to obtained acid chloride (1) as shown in scheme (2).

General synthesis of amides:

(2-Acetoxy-benzoylamino)-acetic acid (2) and

2-(2-Acetoxy-benzoylamino)-3-(1H-imidazol-4-yl)-propionic acid (3)

To a mixture reaction of solution of 2-acetoxy-benzoyl chloride**1** (0.01mol) in hexane (10 mL) was added with stirring solution of **2** (0.01 mol, 0.75 gm) glycine or **3** (0.01 mol, 1.55 gm) histidine, and reflux to 2 h at 60 °C. The solid obtained was washed with hot distilled water then by 10% $NaHCO_3$ and centrifuged with ether (30 ml) for 10 min. three times to extract impurities and obtained purified product.



Scheme 2: Synthesis of amides compounds

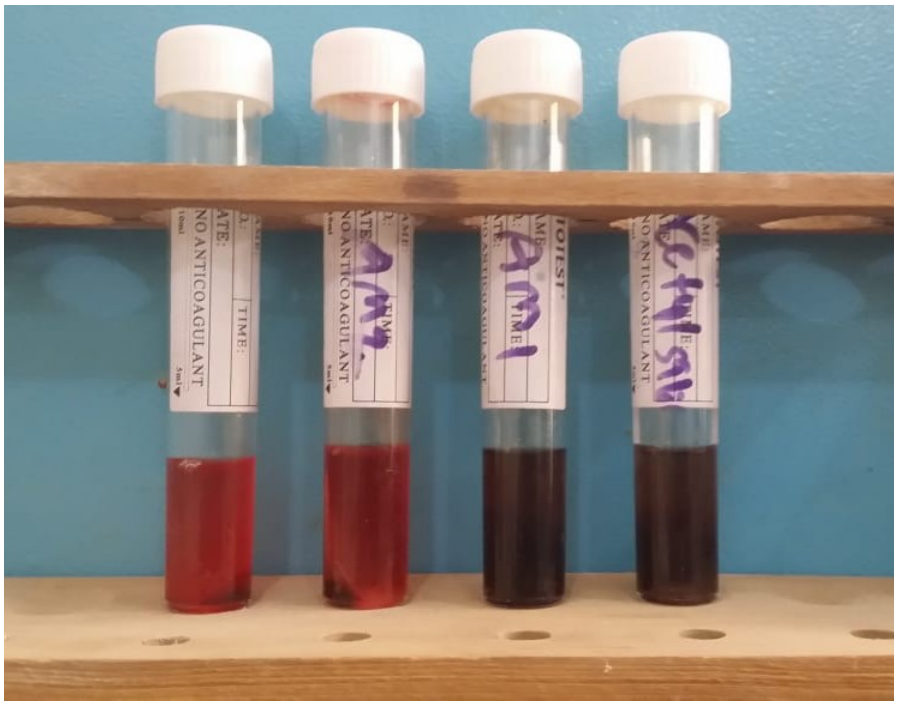
In vitro the anti-inflammatory activity

HRBC method used to estimation anti-inflammatory activity in vitro. Blood was collected from healthy volunteers and was mix with equal volume of sterilized Alsever's [10] solution. The blood solution was centrifuge at 3000 rpm and the packed cells were separate. The packed cells were wash with isosaline solution and 10% v/v suspension was complete with isosaline. Alsever's solution were prepare of 2.05% glucose, 0.42% NaCl, 0.8% trisodium citrate, 0.055% citric acid, all dissolved in water. This solution was using for storage RBC. Other solution were using in this method Hypo-saline (0.7% NaCl), Isosaline (0.9% NaCl), phosphate buffer (pH 7) and ethanol.

All the assay mixtures were incubate at 37ºC for 30 minutes and centrifuged at 3000 rpm for 10 min. The supernatant liquid was decanted and haemoglobin

content was estimate by spectrophotometer at 560 nm. The percentage hemolysis was estimate by assuming the hemolysis produced in the control as 100%, according to following equation.

- ***Percentage of protection=(Absorbance of sample/Absorbance of control) x100%***



Picture 1: Samples and control after incubation.

Table (1) The physical properties of amides.

Symbol	MP °C	Color	yield %	TLC	R_f
AM1	147-149	Greyish brown	55%	DCM:MeOH 2.5:2.5	0.83
AM2	81-83	Yellow	63%	DCM:MeOH 2.5:2.5	0.33

DCM: Dichloromethane, MeOH: Methanol

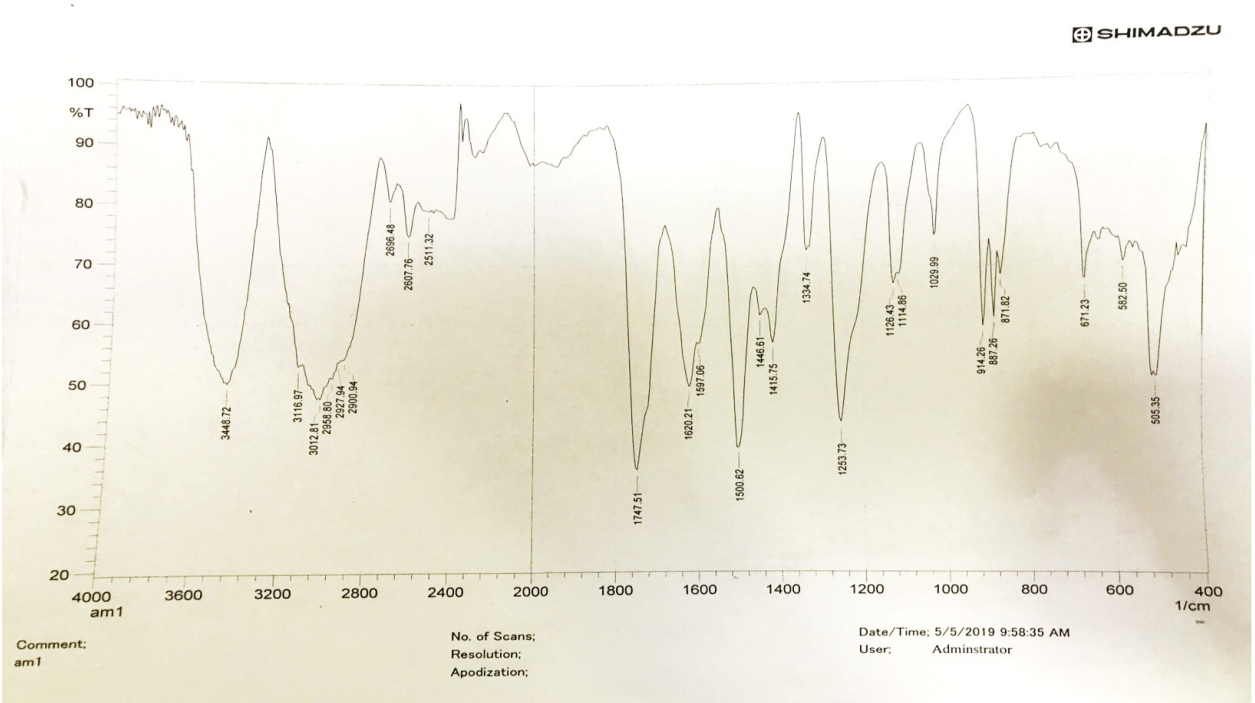


Figure 1: FTIR spectrum of AM1 compound

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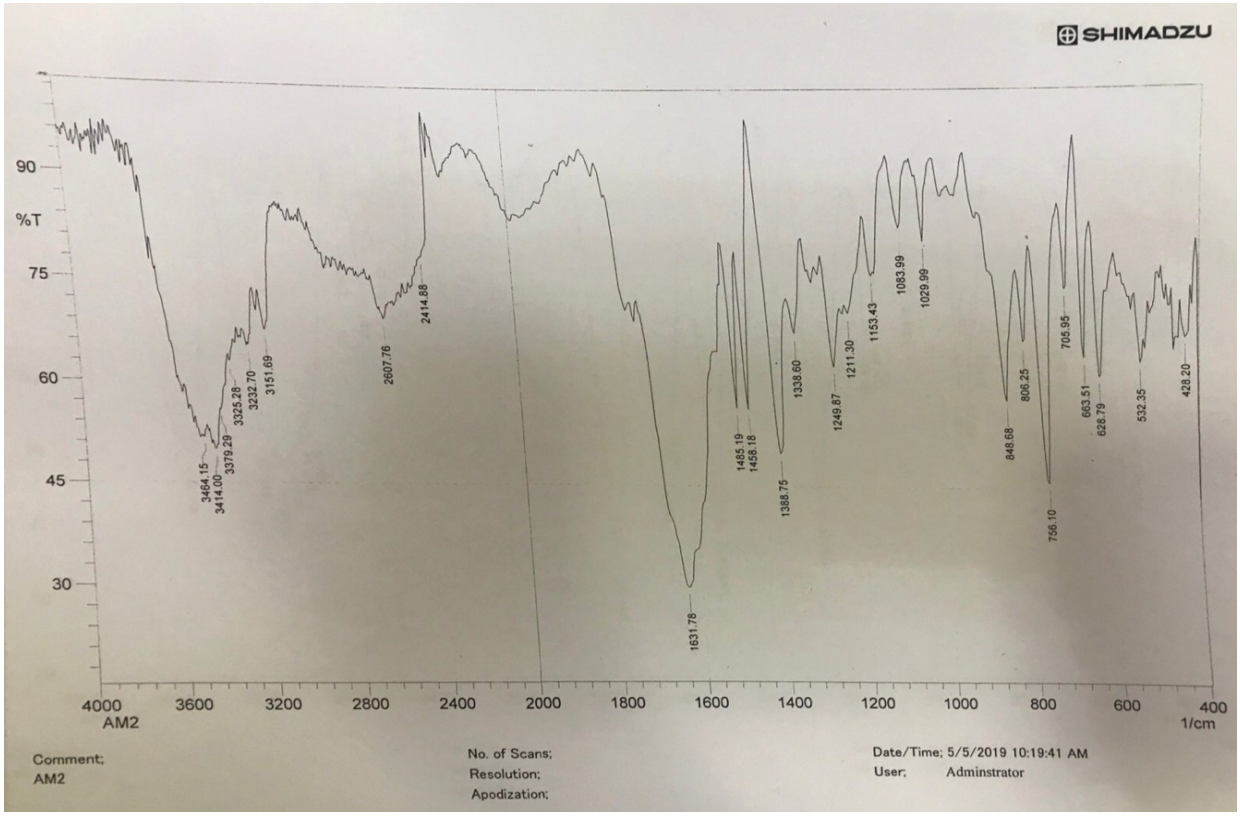

Figure 2: FTIR spectrum of AM2 compound.

Table (2) the main peaks of FT-IR spectra (cm^{-1}) of Amides compounds.

Symbol	*C=C*	*Ar-H*		*N-H Amide*	*Alphatic C-H*	*C=O Amide*	*-OH*
	(Str.)	Str.	Bend. O.O.P	(Str.)	Str.	Str.	(Str.)
AM1	1597 1500	3012	871	3448	2900 2927	1747	___
AM2	1458 1485	3010	848	3464	2800 2950	1710	___

Table 3: The steps of adding solution to sample and control.

	Test	Control
Sample	50mg	
Water or ethanol	1ml	1 ml
Buffer	1ml	1ml
Hypo-saline	2ml	2ml
HRBC	0.5ml	0.5ml

Table 4: Result of anti-inflammatory activity of Amide compounds.

Compound	Percent of protection %
Acetyl salicylic acid	64.56
AM1	67.34
AM2	27.88

DISCUSSION

The FTIR spectra [12] of this compounds, Figure (1) and Figure (2) showed the presence of the amide group (CO-NH) band at (3448 cm^{-1}) of AM1and (3464 cm^{-1}) of AM2 being broad due to intermolecular hydrogen bonding. Its IR spectrum also showed a band at the range (2900, 2800) cm^{-1} for C-H aliphatic. The main bands of FTIR are listed in Table 2.

Aspirin containing free carboxylic group has been modified into various amide derivatives using different amino acid, resulted in masking of the carboxylic

moiety. The newly synthesized prodrugs were evaluated for their anti-inflammatory activity in vitro. Amide (AM1) show a more anti -inflammatory activity 67.34% than amide (AM2) 27.88% compared with Aspirin 64.56% as see by prevent the leakage of hemoglobin from RBC. We obtained prodrug with low gastric side effect.

CONCLUSION

This study were contains the design and synthesis of new non-steroidal anti-inflammatory agents (NSAIDs) to accomplish better activity and low gastric side effects.

Two compounds have been designed and synthesized as potential NSAIDs, these are Aspirin derivative. The major side effects associated with all currently available, these are aspirin derivatives (compounds **2**,**3**).The main side effects associated with all currently available NSAIDs are gastric tract (GIT) hemorrhage and ulceration, due to inhibition of COX-1, which is responsible for biosynthesis of cytoprotective prostaglandins E2, While COX-2 is synthesized in response to pro-inflammatory stimuli such as, cytokines. Structural modification of available traditional NSAIDs might improve their specificity for COX-2 enzyme selectivity. These derivatives were prepared from Aspirin. The structures of synthesized compounds were confirmed by IR.

Reference

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