

# SYNTHESIS & EVALUATION OF PYRROLE DERIVATIVE CONJUGATES OF ASPIRIN AS ANTIPLATELET AGENTS

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## ABSTRACT

A new series of NSAID'S in which aspirin is joined by ester linkage to pyrrole-2-carboxylic acid were synthesized and tested for anti-platelet activity. Related propyl esters of aspirin and aspirin as such were taken as references. All the compounds described here show anti-platelet activity but they do not behave as prodrugs of aspirin in blood serum.

**KEYWORDS:** NSAID'S, Aspirin, Anti-Platelet Agents, Pyrrole.

## INTRODUCTION:

Now a day's wide interest has been developed in synthesis of heterocyclic molecules which possess wide range of therapeutic applications. The anti-inflammatory, analgesic, & antipyretic drugs are a heterogeneous group of compounds, often chemically unrelated (although most of them are organic acids), which nevertheless share certain therapeutic actions & side effects<sup>(4)</sup>. The prototype is aspirin; hence these compounds are also referred to as aspirin-like drugs; they are also frequently called as *Non Steroidal anti-inflammatory drugs, or NSAID's*<sup>(2)</sup>. Aspirin apart from its anti-inflammatory and analgesic activity is used in long term prophylaxis against myocardial infarction and shock due to its anti-thrombotic properties. On the basis of this fact we have reported the synthesis and anti-platelet activity of three pyrrole derivative conjugates of aspirin. Aspirin was taken as reference drug for testing the anti-platelet activity of the synthesized compounds.

## MATERIALS & METHODOLOGY:

All the chemicals were of laboratory grade & were purified by the established methods. Melting points were determined in open capillary tubes & are uncorrected. Purity & homogeneity of the synthesized compounds was routinely ascertained by TLC on glass plates using silica gel G as adsorbent and solvent system benzene: methanol (1:1). Spots were visualized by iodine vapor or by irradiation with UV light (254 nm). IR spectra were recorded using KBr disc on FTIR 8010 Shimadzu model. Mass spectra of the synthesized

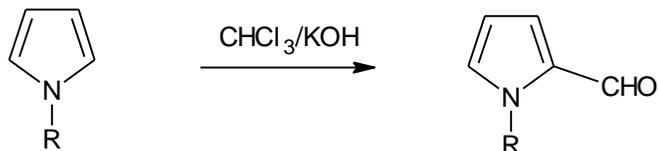
compounds were recorded using double focusing mass spectrometer. Anti-platelet aggregatory activity was performed on aggregated albino rat platelet rich plasma (PRP) using aspirin as reference drug.

## EXPERIMENTAL:

### 1. Synthesis of Pyrrole-2-Carboxylic acid:

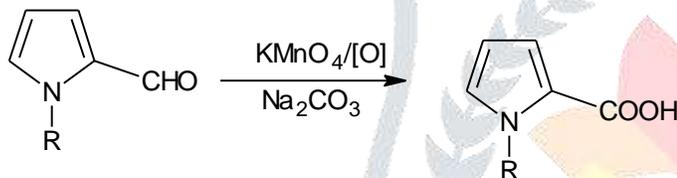
The synthesis of pyrrole-2-carboxylic acid was achieved in two steps:

#### a) Synthesis of pyrrole-2-carbaldehyde:



50 ml of chloroform was taken in a 250 ml conical flask and to it slight excess KOH solution (55ml) was added. The mixture was stirred on a magnetic stirrer without external warming. 25ml pyrrole was then added drop wise & mixture was allowed to stir on a magnetic stirrer for about 2.5 hrs. After the completion of stirring the mixture was extracted thrice with ether (10ml). The organic layer was separated and the ether was evaporated. Finally the residue was distilled off to get pyrrole-2-carbaldehyde<sup>(5, 6)</sup>. Yield=66%.

#### b) Synthesis of Pyrrole-2-carboxylic acid:

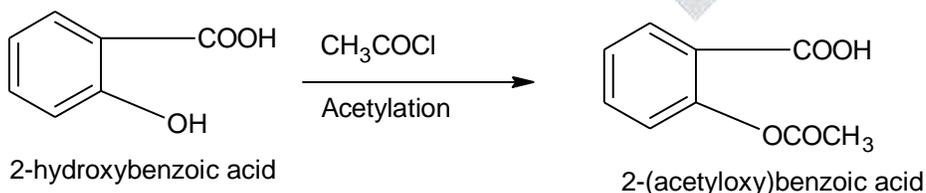


About 20ml of pyrrole-2-carbaldehyde was taken in a 250ml conical flask and to it 15ml of saturated  $\text{KMnO}_4$  solution and 0.5 gm of  $\text{Na}_2\text{CO}_3$  were added. The mixture was then warmed on a water bath for 20 minutes. Then the resulting mixture was acidified using conc. HCl, and then 25% solution of sodium sulphite was added until the precipitated manganese dioxide got re-dissolved. The mixture was then cooled to give the precipitate of pyrrole-2-carboxylic acid<sup>(6, 12)</sup>. Yield=63%.

### 2. Synthesis of Pyrrole derivative conjugates of Aspirin:

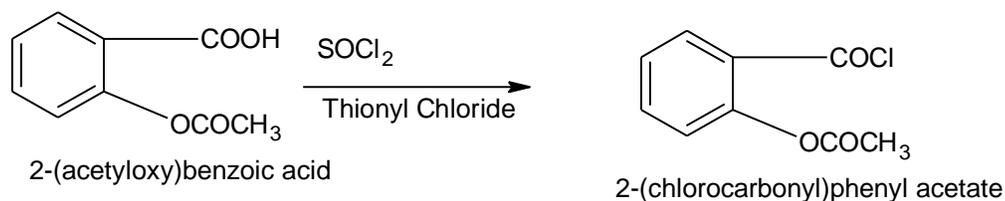
#### a) [5(2-acetyloxy) phenyl keto pyrrole-2-carboxylic acid]. [Compound-I]:

i.) Protection of the phenolic “-OH” group of salicylic acid by acetylation:



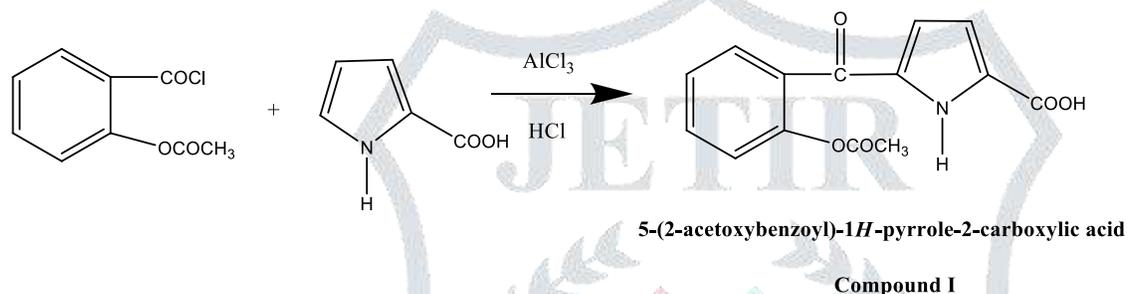
10 gm of salicylic acid was dissolved in 7ml of dry pyridine in a 250ml conical flask. After that 7.5ml of acetyl chloride was added (adding 1ml each time) and the mixture was shaken continuously. The mixture was then heated on a water bath for 5 minutes and then after cooling it was poured into 300ml of cold water. The crude acetyl salicylic acid separated as a white precipitate, which was then filtered off & then recrystallized from equimolar mixture of water & acetic acid. The acetyl salicylic acid was obtained as white crystals<sup>(12)</sup>. Yield=70%.

ii) Chlorination of acetyl salicylic acid to its acid chloride:



An equimolar quantity of acetyl salicylic acid was taken in a 250ml conical flask & to it about 20ml of chloroform was added. Than to it 1gm  $\text{K}_2\text{CO}_3$  was added and the mixture was stirred at room temperature. Than to it 5ml of thionyl chloride was added drop wise and the mixture was allowed to stir for 30 min. After that the excess of thionyl chloride and chloroform was evaporated to get the acid chloride <sup>(6, 11)</sup>. Yield=59.5%.

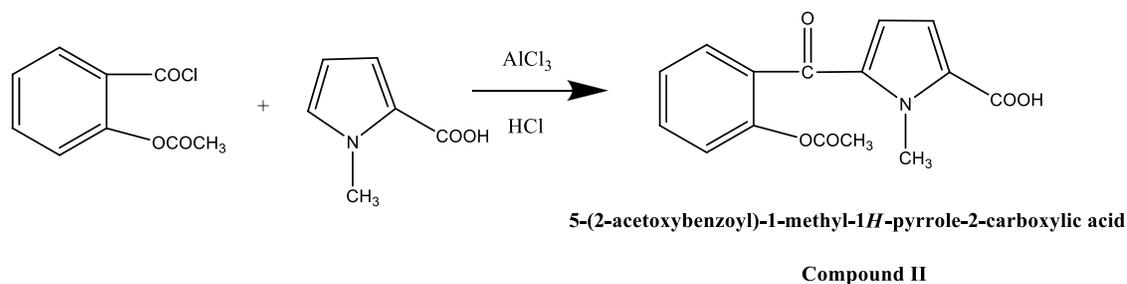
iii) Treatment of the acid chloride with pyrrole-2-carboxylic acid to get Compound I:



6gm of finely powdered aluminum chloride was taken in a 250ml RBF fitted with a reflux condenser. Equimolar quantities of the acid chloride and pyrrole-2-carboxylic acid were dissolved in about 20ml chloroform and this mixture was added in the RBF. The reaction mixture was then refluxed on the water bath for 30 minutes at  $50^\circ\text{C}$ . Steady evolution of hydrogen chloride gas occurred. When the evolution of hydrogen chloride gas ceased the mixture was then poured into about 50ml of cold water. Immediately the yellow colored protected pyrrole derivative separated off. It was then washed with dilute NaOH to remove the traces of hydrogen chloride. The product was then filtered off and was recrystallized from ethanol <sup>(7)</sup>. Yield=56.2%.

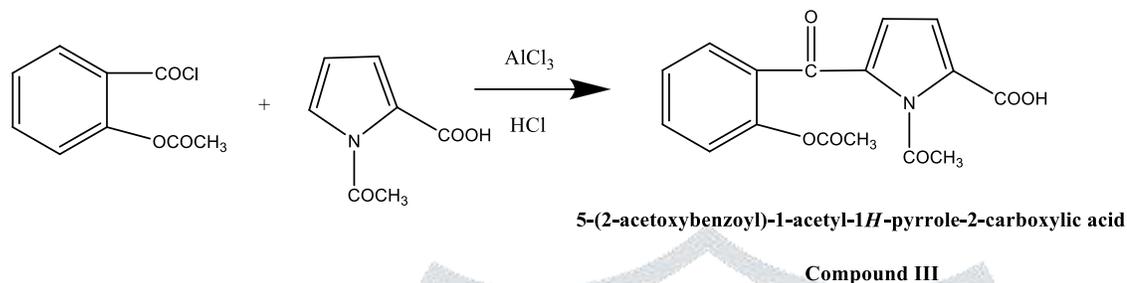
b) [5(2-acetyloxy) phenyl keto – N - methyl pyrrole-2-carboxylic acid]. [Compound-II]:

6gm of compound (I) was taken in a conical flask and to it 25ml of 10% NaOH solution was added. The solution was diluted with about 30ml of water and then 10ml of dimethyl sulphate was added. The flask was then stoppered and was shaken vigorously for 30 minutes. The mixture became warm and then compound (II) started to separate as a yellowish solid. When the separation was complete the solid was filtered through suction. The solid was then washed with little 10% NaOH solution to remove excess dimethyl sulphate and traces of compound (I). The product was then filtered off and was recrystallized from ethanol <sup>(7)</sup>. Yield=60.8%.



**c) [5(2-acetyloxy) phenyl keto – N - acetyl pyrrole-2-carboxylic acid]. [Compound-III]:**

7 gm of compound (I) was taken in a conical flask and to it 13.89 ml of triethyl amine was added. The resulting solution was stirred on ice bath followed by the drop wise addition of slightly excess acetyl chloride (about 12ml). The resulting mixture was allowed to stir on an ice bath for about 45 minutes when pale brown colored compound (III) separated as a fine solid. It was filtered through suction and was washed with water to remove excess of acetyl chloride. The product was then filtered off and was recrystallized from chloroform <sup>(7)</sup>. Yield=62.3%.

**SPECTRAL CHARACTERIZATION** <sup>(8, 13)</sup>:**Table 1: Melting points and I.R. spectral interpretation of compounds:**

S.No	Structure of the compound	Melting point (°C)	I.R. Spectra (cm <sup>-1</sup> )
1		150-162	N-H str. (pyrrole ring)=3267.19 C=O str. (COOH gp)=1670.24 O-H str. (COOH gp)=2977.89 C-H str. (benzene ring)=3136.04
2		175-190	C-N str. (pyrrole ring)=1296.08 C=O str. (COOH gp)=1658.67 O-H str. (COOH gp)=2854.45 C-H str. (benzene ring)=2360.71
3		210-220	C-N str. (pyrrole ring)=1087.78 C=O str. (COOH gp)=1631.09 O-H str. (COOH gp)=2358.78 C-H str. (benzene ring)=2989.4

**Table 2: Mass spectral interpretation of compounds** <sup>(8, 13)</sup>:

S.No.	Compound	Molecular mass	m/z value (M <sup>+</sup> +1 peak)
1.	I	232	233
2.	II	272	273
3.	III	298	299.1

**PHARMACOLOGICAL SCREENING** <sup>(14, 15)</sup>:

Blood samples were taken from the caudal vein of rats that had not taken any food or received any drug for the past 2 weeks. Platelet rich Plasma (PRP) was prepared by centrifugation of citrated blood for 20 minutes at 1000rpm. Aliquots (300µL) of PRP was added into the aggregometer (Elvis) cuvettes and aggregation was recorded as increased light transmission under continuous stirring at 1000rpm at 37°C for 5 minutes after the addition of the stimulus. Collagen (2.5 mg/ml) was used as platelet activator in PRP. The inhibitory activity of the compounds was tested by the addition of compounds to PRP 10 minutes before the addition of the stimulus (collagen). Drug vehicle (<0.5% DMSO) added to PRP served as control and did not affect platelet function.

The anti-platelet activity of the compounds was evaluated as % inhibition (pIC<sub>50</sub>) of platelet aggregation compared to control samples. When inhibition of aggregation at maximal inhibitory concentration exceeded 50%, pIC<sub>50</sub> values were calculated by non linear regression analysis.

**Table-3: Anti-platelet activity of tested compounds on Collagen-Induced PRP Aggregation:**

S.No.	No. of animals used	Drugs Used	pIC <sub>50</sub> (µM) ± SEM	% Inhibition ± SEM (50 µM)
1	4	Control	-----	-----
2	4	Aspirin	4.38±0.10	25.9±17.3
3	4	Compound I	4.25±0.04	11.2±4.5
4	4	Compound II	5.5±0.23	13.4±6
5	4	Compound III	7.32±0.15	19.3±18.5

**CONCLUSION:**

From anti-platelet screening of the synthesized compounds it is clear that platelet inhibition increases in case of conjugates with N-methyl pyrrole or N-acetyl pyrrole residues when compared with that of Aspirin as the standard. Thus there are tremendous possibilities that such conjugates may be useful in the treatment of various thrombotic disorders, with lower incidence of GI ulceration. This may be due to the presence of pyrrole ring which acts as the basic template of COX-2 inhibition and not COX-1 which protects the GI tract.

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