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Synthesis, docking and biological evaluation of some NSAID derivatives of amino acids

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Abstract : Non-steroidal anti-inflammatory drugs exert analgesic, antipyretic, and anti-inflammatory effects. But the use of these drugs has several side and these side effects may be reduced by coupling them with amino acids. The coupling blocks the carboxyl group and reduces acidity. A series of NSAID derivatives of amino acids were synthesized and characterized by IR, NMR and Mass spectroscopy. Docking studies of the compounds were carried out using Hex software and the receptor taken was 3LAF (colon cancer receptor). The compounds were tested for anticancer and antioxidant activities. Anticancer activity was tested on HT-29 Cells. Antioxidant activity was tested using DPPH. Ascorbic acid was taken as standard and the absorbance was measured at 517 nm. Majority of the compounds showed good antioxidant activity.

Keywords : NSAID, amino acids, Hex software, anticancer, antioxidant.

Introduction

In recent times NSAIDs are also studied for their anticancer activities. Many COX-inhibiting NSAIDs inhibit proliferation of cells in colon cancer and cancer tumour cells *in vitro*. The cell quinescence induced by NSAIDs is due to reduction in the levels of D-type cyclins and cyclin dependent kinases.

Amino acids have good solubility in body fluids and can penetrate well into the body cells. NSAIDs can be coupled to amino acids using different coupling reagents such as DCC, EDC etc. the coupling can minimize the side effects of NSAIDs.

In the present work ligands were designed to target the cancer cell protein. Molecular docking was carried out by HEX software using colon cancer receptor (PDB ID : 3LAF).

Experimental

Anhydrous conditions were maintained using dry apparatus. All reactions were magnetically stirred unless otherwise stated. Anhydrous sodium sulphate was used to dry the organic extracts. Melting points were determined by capillary method. Amino acids, coupling reagents and solvents were obtained from Spectrochem Ltd., Mumbai. DPPH was obtained from AVRA. FTIR spectrometer was used to record IR spectra. The values were reported as υ_{max} (cm^1). ^1H NMR spectra was obtained using $CDCl_3$ solvent by ^1H NMR Brucker JEOL (400 MHz) NMR spectrometer. The chemical shift values are reported in ppm relative to TMS ($\delta = 0$) as internal standard.

Protection of carboxylic group of amino acids :

Thionyl chloride (20 mmol, 1.45 ml) was dissolved dropwise in methanol (50 ml) at 0 °C. To this amino acid (20 mmol) was added. The reaction mixture was refluxed for 8–10 h in a mantle. The solvent was evaporated obtained ester was triturated with ether at 0 °C to remove excess dimethyl sulphite. Recrystallization was done using methanol and diethyl ether.

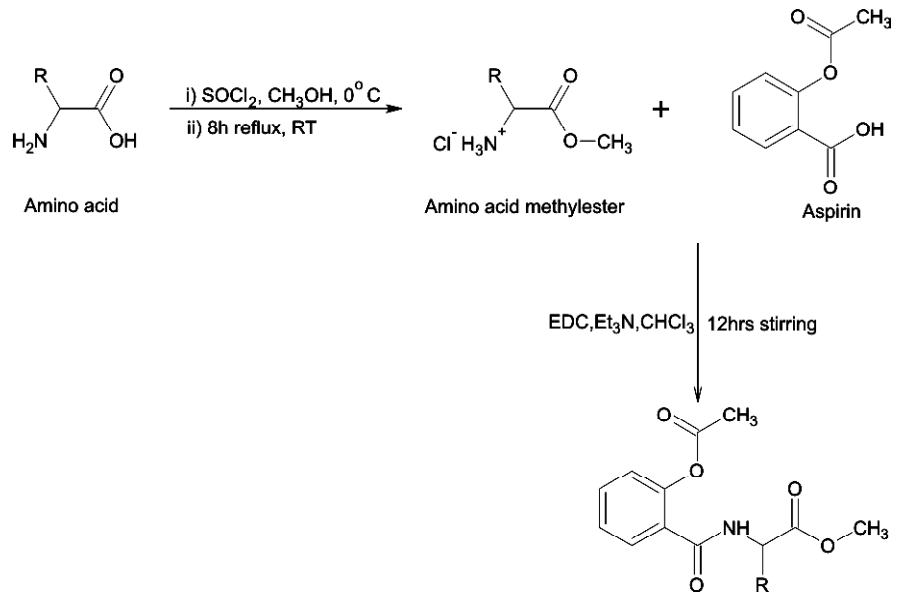
Coupling of NSAIDs with amino acids :

The carboxyl protected ester (5 mmol) was dissolved in chloroform (10 ml). Triethylamine (3 ml) was added at 0 °C. NSAID (5 mmol) and EDC (5 mmol) were dissolved in chloroform (10 ml) and added to the above mixture. The combined reaction mixture was stirred for 12 h at room temperature. EDU was filtered and the filterate was washed with 5% $NaHCO_3$ (20 ml), 5% HCl

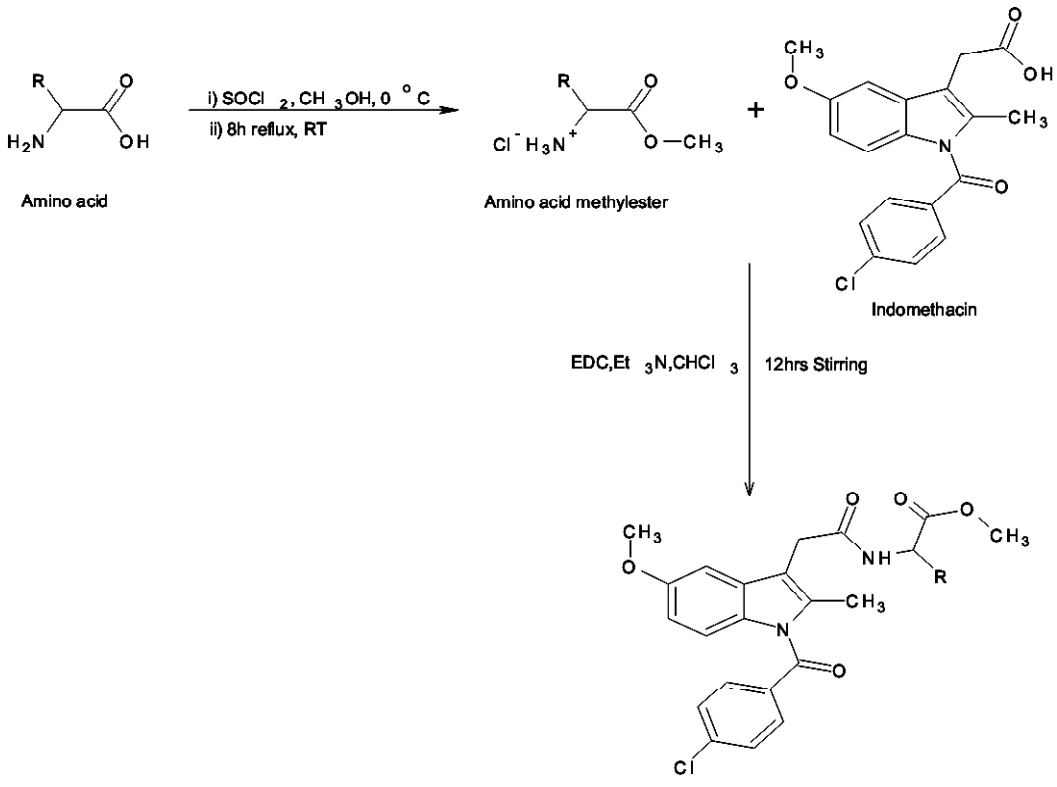
(20 ml) and water (20 ml) respectively. Anhydrous Na_2SO_4 was used to dry the resulting product. Chloroform was evaporated. Chloroform and petroleum ether was used for recrystallization.

Antioxidant activity :

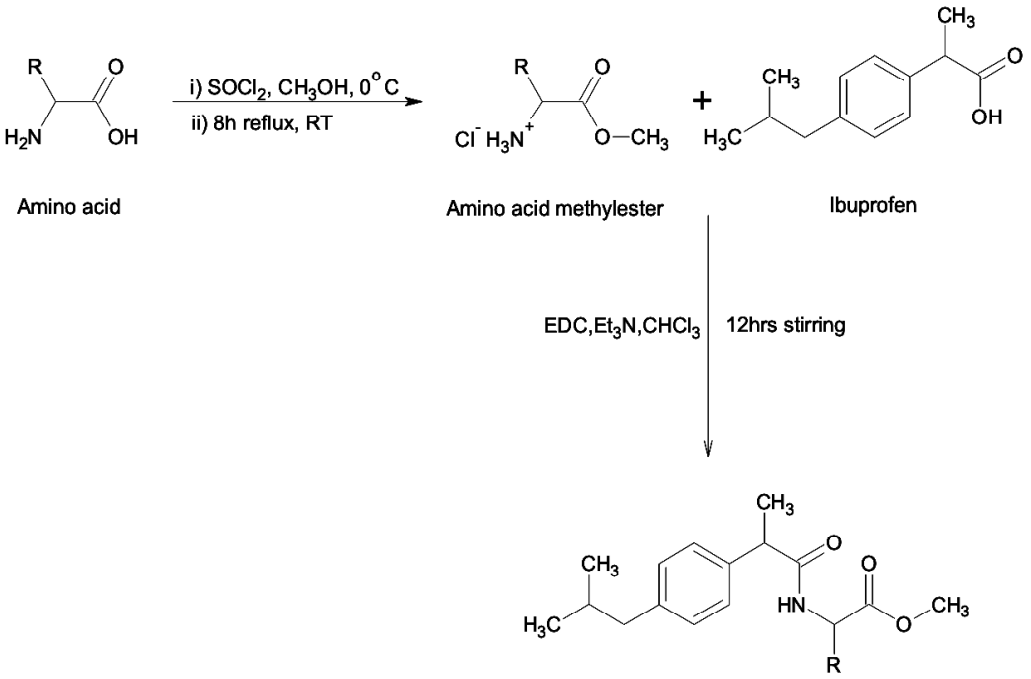
DPPH method was used to determine the free radical scavenging activity of the compounds. A standard solution of DPPH is prepared by dissolving 2 mg of DPPH in



Scheme 1



Scheme 2



Scheme 3

50 ml methanol. The stock solutions of 100 ppm concentration of ascorbic acid and the synthesized compounds were prepared by dissolving 1 mg of the compound in 10 ml methanol. Dilutions of 50 ppm and 25 ppm concentration were prepared from the stock solutions. From each compound and each concentration 3 ml solution was taken in test tubes and to this 1 ml DPPH solution was added. 3 ml of methanol and 1 ml of DPPH solution in a test tube was taken as blank. The test tubes were incubated in dark (room temperature) for 30 min. The absorbance was measured at 517 nm in a UV spectrophotometer. A higher free radical scavenging activity was indicated by a lower absorbance. The standard used was ascorbic acid.

DPPH scavenging effect (%) =

$$[(A_{control} - A_{sample})/A_{control}] \times 100$$

Results and discussion

Docking : Docking studies were carried out using Hex software. Docking of the compounds with colon cancer receptor (PDB ID : 3LAF) revealed that Ind-Trp-OMe had highest docking score and hence a strong binding affinity towards the protein 3LAF (Table 1)

Synthesis :

Table 2 shows the physical data of the synthesized compounds.

Table 1. Docking results

Sl. no.	Compounds	Dock score
1.	Aspirin	–73.99
2.	Asp-Val-OMe (**1**)	–82.30
3.	Asp-Pro-OMe (**2**)	–82.30
4.	Indomethacin	–89.02
5.	Ind-Pro-OMe (**3**)	–95.25
6.	Ind-Trp-OMe (**4**)	–104.33
7.	Ibuprofen	–76.29
8.	Ibu-Pro-OMe (**5**)	–83.09

Table 2. Physical data of compounds synthesized

Sl. no.	Compounds	State	Colour	% yield
1.	Asp-Val-OMe (**1**)	Semi-solid	Colourless	45.11
2.	Asp-Pro-OMe (**2**)	Semi-solid	Colourless	38.80
3.	Ind-Pro-OMe (**3**)	Semi-solid	Blackish brown	58.73
4.	Ind-Trp-OMe (**4**)	Semi-solid	Yellow	96.19
5.	Ibu-Pro-OMe (**5**)	Semi-solid	Dark brown	34.06

Spectral analysis :

FT-IR, ^{1}H NMR and FAB-Mass were used to characterize the compounds.

^{1}H NMR spectrum of **(2)** : 7.1–7.6 (Ar-H), 1.4–2.4 (CH_3, d), 3.7 (CH, m), 3.75 (CH, m), 3.51–1.87 (pyrrolidine CH), 5.3 (OH, s).

IR spectrum (ν/cm^{-1}) of **(1)** cm^{-1} : 2968 (Alph C-H), 1743 (C=O), 1651 (Amide C=O), 1541 (Ar C=C), 1373, 1205 (C-O).

FAB-Mass of **(2)** : The molecular ion peak was obtained at 340 (M+23).

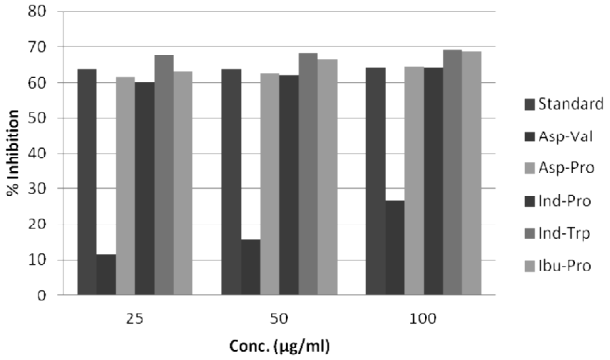


Fig. 1. Antioxidant activity of NSAID derivatives of amino acids.

Antioxidant activity :

The antioxidant results are illustrated in Fig. 1. Ind-Trp-OMe showed best antioxidant activity compared to other synthesized compounds.

Table 3. Results for anticancer activity

Sr. no.	Compound	Concentration (µG)	% of cell lysis	IC_{50}
1.	Control	–	No lysis	–
2.	Ind-Trp-OMe (**4**)	10	No lysis	> 30 µG
3.	Ind-Trp-OMe	20	No lysis	
4.	Ind-Trp-OMe	30	No lysis	

Anticancer activity :

Anticancer activity of the NSAID derivatives of amino acids were carried out using the cell line, HT-29 – Human Colorectal Adenocarcinoma cell and the results were tabulated in Table 3.

Conclusion

NSAID derivatives of amino acids were synthesized by conventional method using EDC as the coupling reagent. Characterization of the synthesized compounds were carried out by NMR, IR and Mass spectroscopy. Docking studies were carried out using a colon cancer receptor with PDB ID: 3LAF. Ind-Trp-OMe showed the best dock score. However, the compound did not show *in vitro* anticancer activity on HT-29 cell lines. All the synthesized compounds showed moderate to good antioxidant property. Ind-Trp-OMe showed the best antioxidant activity which can be attributed to the labile hydrogen present in the indole ring in tryptophan.

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