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SYNTHESIS, DOCKING AND ANTIOXIDANT ACTIVITY OF SOME NSAID DERIVATIVES OF AMINES

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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 effects and these side effects may be reduced by coupling them with amines. The coupling blocks the carboxyl group and reduces acidity. A series of NSAID derivatives of amines 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 antioxidant activity using DPPH method. Ascorbic acid was taken as standard and the absorbance was measured at 517 nm. Majority of the compounds showed good antioxidant activity.
Keywords: NSAID, amines, Hex software, antioxidant, DPPH.

INTRODUCTION

NSAIDs are non-steroidal anti-inflammatory drugs. They exert analgesic, antipyretic, and anti-inflammatory effects. There are two major categories for non-steroidal anti-inflammatory drugs:

- The first is non-selective anti-inflammatory drugs.
- The second is selective anti-inflammatory drugs, COX-2 inhibitors.

In recent times NSAIDs are also studied for their anticancer activities[1,2]. There is a growing interest in unravelling the mechanisms mediating the anti-proliferative effects of NSAIDs and in determining if such effects are due to their COX- inhibiting activities or are independent of them. Evidence to date indicates that majority of NSAIDs attenuate tumor growth in vivo by either induction of apoptosis or inhibition of angiogenesis or both. Many COX-inhibiting NSAIDs, including sulindac sulfide, indomethacin, piroxicam, naproxen, the ``profen''-type compounds, and aspirin, inhibit cell proliferation of colon cancer and other tumor cells in vitro[3-6]. The cell quiescence induced by NSAIDs is due to reduction in the levels of cyclin-dependent kinases and increased D-type cyclins that participate in the transitions through the different phases of the cell division cycle[7,8].
But in spite of showing such wide spectrum of activities, NSAIDs show different side effects like –

- Gastro intestinal irritation
- Acidity
- Ulcer formation

NSAIDs can be coupled to amines using different coupling reagents such as DCC, EDC etc. The coupling can minimize the side effects of NSAIDs. Such coupled products may also show the following advantages.

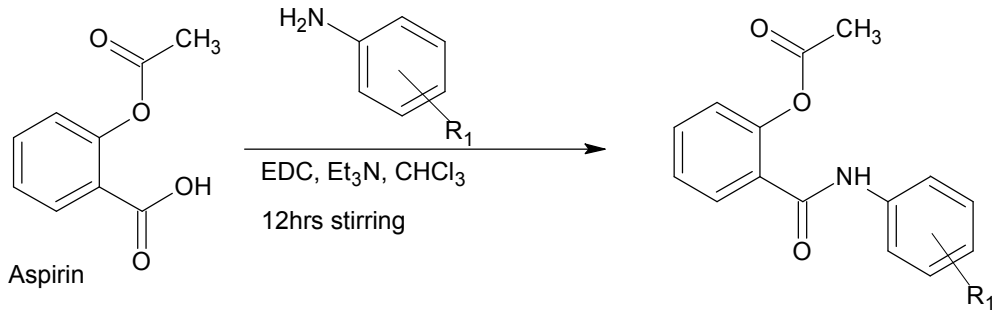
- The carboxyl group is blocked which may reduce the adverse effects like GIT irritation, ulcer formation etc.
- It can act as a prodrug and release the drug in acidic pH.
- Moreover NSAIDs can also be used to treat cancer.

Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and a receptor, fit together and docks to each other well. The molecules binding to a receptor inhibit its function and thus act as drug. The collection of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations. In the present work the designed ligands were targeted to the cancer cell protein, Colon cancer receptor with the PDB ID: 3LAF using Hex software[10-12].

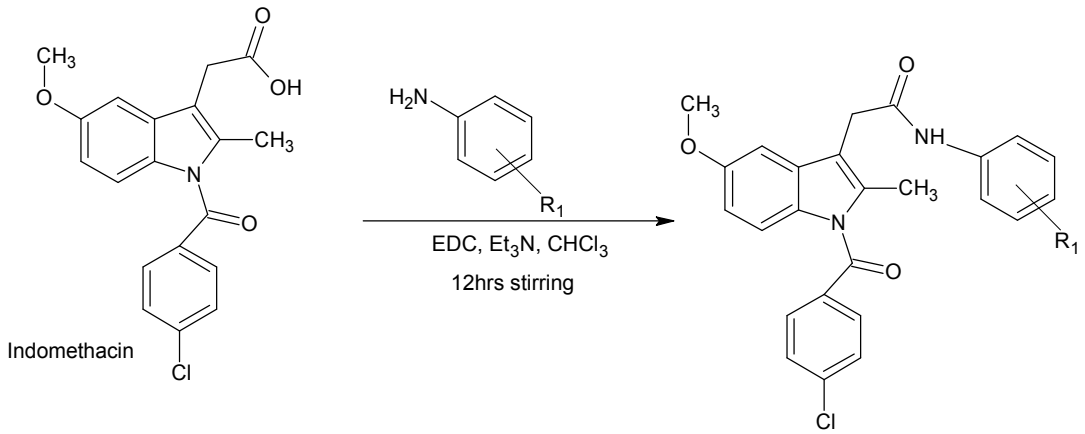
MATERIALS AND METHODS

Commercially available reagents and analytical grade solvents were used without further purification. Anhydrous conditions for all the reactions were conducted in dried apparatus. All the reactions were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method. Amines, di-tertbutylpyrocarbonate, trifluoroacetic acid, EDC, Diethyl ether, Methanol and Chloroform were obtained from and Spectrochem Ltd, Mumbai. DPPH was obtained from AVRA. IR spectra were recorded on FTIR spectrometer using a thin film support on KBr pellets. The values are reported as υ_{max} (cm^{-1}). ^1H NMR spectra was recorded on ^1H NMR Brucker JOEL (400MHz) NMR spectrometer. The spectra was obtained in $CDCl_3$ and the chemical shift values are reported as values in ppm relative to TMS (d=0) as internal standard. FAB Mass spectra were recorded.

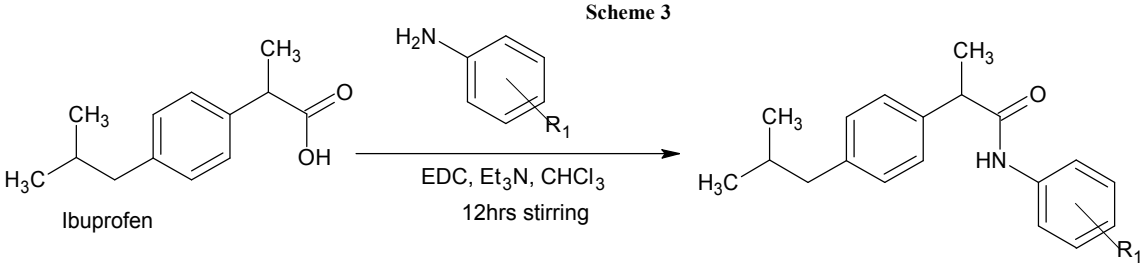
Scheme 1



Scheme 2



Scheme 3



R_1 = 4-fluoroaniline, p-nitroaniline

Coupling of NSAIDs with Amines

Amine (4-fluoroaniline/p-nitroaniline) (5mmol) was dissolved in chloroform (10ml). To this triethylamine (3ml) was added at 0ºC. NSAID (Aspirin/Ibuprofen/Indomethacin) (5mmol) and EDC (5mmol) was dissolved in chloroform (10ml) and was added to the reaction mixture containing amine. The combined reaction mixture was stirred for 12hrs at RT. EDU was filtered and the filtrate was washed with 5% $NaHCO_3$ (20ml), 5% HCl (20ml) and water (20ml) respectively. The resultant product was dried over anhydrous Na_2SO_4. Chloroform was evaporated. The product was recrystallized from chloroform and petroleum ether[9].

Antioxidant Activity

The free radical scavenging activity of the synthesized compounds was measured by DPPH[13]. A standard solution of DPPH is prepared by dissolving 2mg of DPPH in 50ml distilled water. The stock solutions of 100ppm concentration of Ascorbic acid and the synthesized compounds were prepared by dissolving 1mg of the compound in 10ml distilled water. From these stock solutions, solution of 50ppm and 25ppm concentration were prepared. From each compound and each concentration 3ml solution was taken in test tubes and to this 1ml DPPH solution was added. A blank was also prepared by taking 3ml of methanol and 1ml of DPPH solution in a test tube. Then the test tubes were kept in dark for 30mins. Then the absorbance was measured at 517 nm in a UV spectrophotometer. A low absorbance indicated higher free radical scavenging activity. Ascorbic acid was taken as a standard in this study.

DPPH Scavenging Effect (%) = $[(A_{control} - A_{sample})/A_{control}]$ x 100

RESULTS AND DISCUSSION

Docking: A Preliminary study was carried out on the ligands using Hex software where the ligands were docked with Colon cancer receptor with the PDB ID: 3LAF (listed in Table 1). The docking score revealed that Ind-4-fluoroaniline showed highest docking score and hence a strong binding affinity towards the protein 3LAF effectively.

Table 1: Docking Results

Sl. No	Compounds	Dock Score
1	ASPIRIN	-73.99
2	Asp-4-fluoroaniline (1)	-81.78
3	Asp-(p-nitro)aniline (2)	-84.63
4	INDOMETHACIN	-89.02
5	Ind-4-fluoroaniline (3)	-94.69
6	Ind-(p-nitro)aniline (4)	-94.65
7	IBUPROFEN	-76.29
8	Ibu-4-fluoroaniline (5)	-82.02

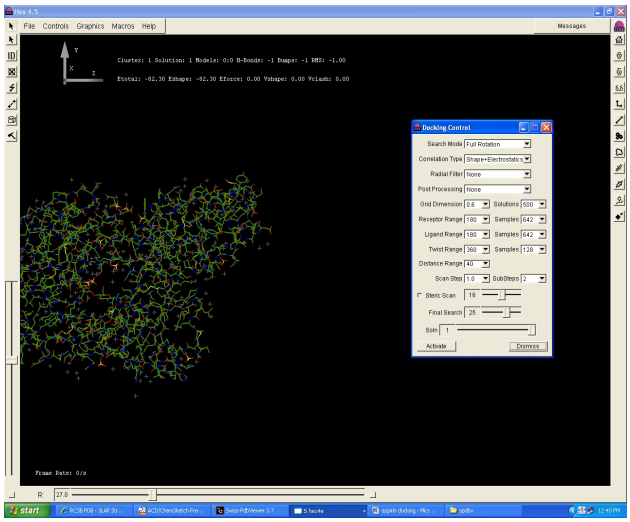


Figure 1: Print screen of Hex workspace

Synthesis: The derivatives were synthesized using solution phase synthesis and the physical data are given in Table 2

Table 2: Physical data of compounds synthesized

SN	Compound	State	Colour	% yield
1	Asp-4-fluoroaniline (1)	Semi-solid	Reddish brown	46.25%
2	Asp-(p-nitro)aniline (2)	Semi-solid	Greenish yellow	44.44%
3	Ind-4-fluoroaniline (3)	Semi-solid	Reddish brown	53.0%
4	Ind-(p-nitro)aniline (4)	Semi-solid	Orange yellow	91.73%
5	Ibu-4-fluoroaniline (5)	Semi-solid	Reddish brown	78.99%

Spectral Analysis

The structure of the synthesized compound was characterized by FT-IR, ^{1}H NMR and FAB-MASS. ^{1}H NMR spectrum of (5) : 7.07-7.62 (Ar-H), 1.01-1.60 (CH_3,d), 2.31 (CH_2,t), 2.02-3.9 (CH,m), 8.2 (NH,d). IR spectrum (v/cm^{-1}) of (1) : 3305 cm^{-1} - N-H ; 3064 cm^{-1} – Ar C-H; 2976 cm^{-1} -Aliph C-H; 1757 cm^{-1} -C=O; 1641 cm^{-1} -Amide C=O; 1548, 1508 cm^{-1} -Ar C=C; 1228 cm^{-1}-Ar C-F. FAB-MASS of (3) : The molecular ion peak was obtained at 473 (M+23).

Antioxidant activity

The result of the sample was compared with the standard (ascorbic acid). With this method it was possible to determine the antiradical power of an antioxidant compound by measuring the decrease in the absorbance of DPPH at 517 nm. A color change from purple to yellow indicated that the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form stable DPPH molecule. Table 3 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of prepared sample and standards. Ind-4-fluoroaniline showed best antioxidant activity compared to other synthesized compounds.

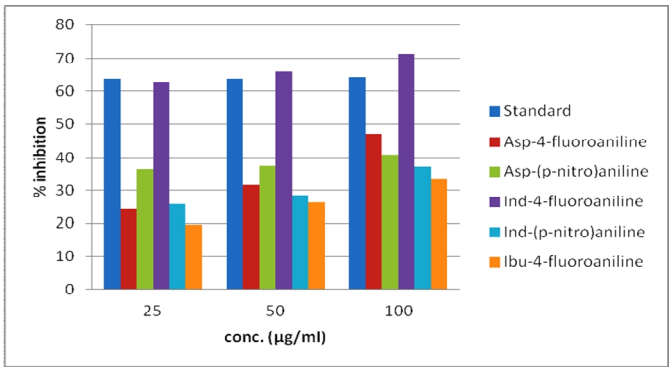


Figure 2: Antioxidant activity of NSAID derivatives of amines

Table 3: % Inhibition of NSAID derivatives of amines

SN	Sample	Conc. (µg)	Absorbance	% Inhibition
1	Blank		0.422	
2	Ascorbic Acid (std.)	25	0.153	63.74%
		50	0.152	63.9%
		100	0.151	64.2%
3	Asp-4-fluoroaniline (13)	25	0.319	24.4%
		50	0.288	31.7%
		100	0.223	47.1%
4	Asp-(p-nitro)aniline (14)	25	0.268	36.4%
		50	0.264	37.4%
		100	0.249	40.9%
5	Ind-(p-nitro)aniline (17)	25	0.325	25.9%
		50	0.302	28.4%
		100	0.265	37.2%
6	Ind-4-fluoroaniline (18)	25	0.157	62.7%
		50	0.143	66.1%
		100	0.121	71.3%
7	Ibu-4-fluoroaniline (20)	25	0.340	19.4%
		50	0.311	26.3%
		100	0.281	33.4%

CONCLUSION

NSAID derivatives of amines were synthesized by conventional method using EDC as the coupling reagent. The synthesized compounds were characterized by NMR, IR and MASS spectroscopy. Docking studies of the synthesized compounds were carried out using a colon cancer receptor with PDB ID: 3LAF. Ind-4-fluoroaniline showed the best dock score. All the synthesized compounds showed moderate to good antioxidant property.

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