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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL INDOLE DERIVATIVES

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ABSTRACT

Heterocyclic compounds have occupied a prominent place among various classes of organic compounds by virtue of their diverse biological activities and chemistry. The aim is to synthesize a drug with better efficacy, less toxicity, and fewer side effects. In view of the important biological activities in the present work, an attempt has been made to synthesize some novel indole derivatives and evaluate the biological profile of these compounds.The present work isfour novel indole (1-4) derivatives are synthesized, characterized by the IR spectra and screened for *In-vitro* anti-inflammatory activity by protein denaturation method and *In-vitro* antioxidant activity by Hydrogen peroxide scavenger assay. The synthetic scheme of indole involves the reaction between substituted indole with chloro acetyl chloride results into 2-chloro-1-(3aH-indol-1(7aH)-yl) ethanone. The formed 2-chloro-1-(3aHindol-1(7aH)-yl)ethanone is treated with heterocyclic compound to form indole derivative, remaining compounds fused with various aryl amines to give three novel indole derivatives. Results of the activities revealed that some of the derivatives showed excellent to good anti- inflammatory, anti-oxidant activities.

INTRODUCTION

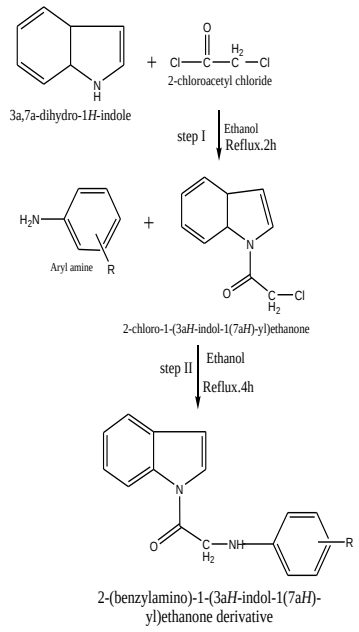
Hetero substituted rings are those in which one are more carbon atoms in a purely carbon containing ring (known as carboxylic ring) is replaced by some other atom is known as hetero atom. Most commonly found in hetero atom is Nitrogen, Oxygen, Sulphur. Many other atoms can form the stable covalent bonds for ring construction which leads to structures of considerable importance known as heterocyclic chemistry. The Plant kingdom contains loads of nitrogen heterocyclic compounds most of which are weakly basic and called alkaloids .Complex heterocyclic compounds used as antibiotics in medicine, Most of the compounds such as drugs, vitamins and natural products contains heterocyclic nucleus. The most important heterocyclic systems are mainly of five and six membered rings. Indole[1] is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a sixmembered benzene ring fused to a five-membered nitrogencontaining pyrrole ring. Indole alkaloids have been proved to be

medicinally important natural compounds. Indole or benzo [b] pyrrole is a planar heteroaromatic molecule in which the benzene ring fused to position -2 and -3 of the pyrrole ring. This nucleus has ten π electrons which are free to circulate throughout the molecule. Two of these electrons originate from nitrogen atom and each of the eight carbon atoms contributes one electron to π-cloud. Since these ten electrons are distributed over nine ring atoms, indole is an electron rich or π – excessive system. Since the ring nitrogen atom contributes two electrons to the overall π system,it is a very weak base. The chemistry of indole began in the mid of the 19th century with extensive research on the natural dye indigo, a violet-blue dye,imported to Europe mainly from India since the 16th century. This research resulted in the early development of the German chemical industry, culminating in the development of a viable industrial process for indigo, as well as the first preparation of indole in 1866 by zinc dust distillation of oxindole.

Recent literatures suggest that several indole-3-substituted derivatives were synthesized using acid catalysed Michael addition,29 Mannich reaction,30 multi component coupling reaction,31 etc. Number of alkaloids, agrochemicals, pharmaceuticals and perfumes had been synthesized using several 3substituited indoles as starting materials. In the present work, we have developed indole-3-yl derivatives using Knoevenagel reaction, which get acetylated with simple acetyl chloride to give 1acetyl-1H-indol-3-yl derivatives. All the synthesized compounds were characterized by 1H-NMR, LCMS, FT-IR and UV-Visible spectroscopy. A large number of heterocyclic compounds containing the indole ring are associated with diverse pharmacological properties such as Analgesic, Anti-allergic, Antibacterial, Anticonvulsant, Antifungal, Antihistaminic, Antiinflammatory, Anticancer, Antiviral, Antioxidant, Anthelminthic, Anti-hypertensive, Cardiovascular etc,.

MATERIALS AND METHODS:

All starting materials, reagents and solvents were purchased from commercial suppliers like merck and aldrich companies. The purity of the prepared compounds were proved by thin layered chromatography(TLC).the IR spectra were obtained on a schimadzu 470 spectrophotometer(potassium bromide discs).melting points were determined by using electrothermal melting point analyser apparatus. Activity screening by *invitro*anti-inflammatory (protein denaturation method) and *invitro*anti-oxidant method(hydrogen peroxide scavenging activity method).



R1-Aniline, R2-Para Amino Benzoic Acid, R3-Pyridine, R4-4-amino phenol
Figure 1: scheme

STEP-1*:* Synthesis of 2-chloro-1-(3ah-indol-1(7aH)-yl)ethanone[2,3] : Equimolar concentrations of Indole and chloro acetyl chloride were taken in round bottomed flask. Ethanol was added as solvent. This entire mixture was refluxed for 2 to 3 hour. After reflux hot solution was transferred to ice cold water to get precipitate of chloro acetyl derivative of indole by liberating hydrochloric acid as byproduct.

STEP-2: Synthesis of aromatic amine of acetyl indole derivatives [4,5]: To the above chloro acetyl derivative of indole and arylamine derivatives are taken in equi-molar concentrations. Ethanol was added and refluxed for 4-5hours. After reflux hot solution was transferred to ice cold water to get product of arylamine derivatives of indole.

SPECTRAL DATA OF THE COMPOUNDS;

1-(1H-indol-1-yl)-2-(phenylamino)ethenone(R1) : Rf value 0.63, Melting point (oC)150-152 ,(Amide(c=o)Stretch) 1799,(N-H Bend) 1619.52,(N-H Stretch)3400.25,(Amine C-N Stretch) 1156.71,(Aromatic C-H Stretch)3056.62.

4-(2-(1H-indol-1-yl)-2-oxoethylamino)benzoic acid(R2);Melting point (oC)179, Rf value0.88,(Amide(c=o)Stretch)3392,(N-H Bend) 1525.57,(Amine C-N Stretch)1249,(C-H Stretch)3392,(O-HStretch)2928.19.

1-(1H-indol-1-yl)-2-(pyridin-1(2H)-yl)ethenone(R3); Rf value 0.78, Melting point (oC)140-143, (Amide(c=o)Stretch)1676,(N-H Bend)1606.78,(N-HStretch)3355.90,(Amine C-N Stretch) 1170.03,(C-H bend)748.83.

2-(4-hydroxyphenylamino)-1-(1H-indol-1-yl)ethenone(R4); Rf value 0.78, Melting point (oC) 182-183,(Amide(C=0)Stretch)1684.54,(N-H Bend) 1620,(N-HStretch)3397.33,(Phenol C-N Stretch)1293.11,(Phenol-O-Hbend)3397.33.

In-vitro* Anti-inflammatory Assays: *(Albumin denaturation Assay)

REAGENTS: Bovine serum albumin (1%) solution was prepared by taking one gm of bovine serum in volumetric flask and the remaining area filled with water to make 100ml.

PROCEDURE [6,7,8]: Prepare the test extracts of different concentrations.1ml of sample and 1ml of aqueous solution of bovine albumin fraction. Adjust the pH(6.8) by using glacial acetic acid. The sample were incubated 72^{o}C for 5minutes and then cooling for 10minutes. After the turbidity was measure the absorbance spectrophotometrically at 660nm. The experiment was performed triplicate.

CALCULATION

% inhibition = Absorbance control – Absorbance sample ×100 / Absorbance control

IC 50 = Concentration × 50 % of population % inhibition

ANTIOXIDANT ACTIVITY: (Hydrogen peroxide scavenging activity) [9,10]The hydrogen peroxide scavenging activitywere determined according to the method of the ruch (ruch et. 1989). A solution of H_2O_2 (40mM) was prepared buffer (pH 7.4). 1ml of different concentration of sample was added to a H_2O_2 solution (0.6ml, 40mM). the absorbance value of the reaction was recorded at 230nm. Blank solution was containing the phosphate buffer without H_2O_2. The % of H_2O_2 scavenging of the samples and standard compounds was calculated as

$$\% \text{ scavenging } H_2O_2 = [A_0 - A_1]/A_0 \times 100$$

Where A_0 is the absorbance of the control, A_1 is the absorbance in the presence of the sample or standard [Gu" lc in et al ., 2003a]

Table no.1; *In-vitro* anti-inflammatory activity results:

Concentration (µg/ml)	R3	R2	R1	R4
	% inh	% inh	% inh	% inh
100	12%	14%	24%	21%
200	14%	17%	40%	40%
300	22%	20%	46%	53%
400	34%	21%	50%	62%
500	44%	40%	56%	69%

Table no.2; *In-vitro* anti-inflammatory activity compared to that standard

S.NO	COMPOUND	IC_{50}VALUES
1	STD	208
2	R3	681
3	R2	750
4	R1	326
5	R4	283

Table No.3; *In-Vitro* Anti-Oxidant Activity Compared To That Standard

S.NO	COMPOUND	IC_{50}VALUES
1	STD	210
2	R3	306
3	R2	300
4	R1	238
5	R4	217

Table no.4; *In-vitro* anti-oxidant activity results:

Con(µg/ml)	**R3**	**R2**	**R1**	**R4**
	% inh	**% inh**	**% inh**	**% inh**
100	25	30	24	25
200	32	33	32	58
300	49	50	63	69
400	50	52	66	64
500	62	53	70	62

RESULTS AND DISCUSSION:

In this present research work, based on the wide literature survey, novel Indole derivatives were synthesized in two-step facile procedure and four in number. All the reactions were monitored by TLC and purification was done by recrystallization process. All the derivatives were characterized using spectral studies like FT-IR spectroscopy. All the synthesized derivatives were screened for their *in-vitro* anti-inflammatory activity by protein denaturation method &*in-vitro* anti-oxidant activity by using hydrogen peroxide scavenging activity (HPSA).

In-vitro anti-inflammatory activity

The anti-inflammatory activity of the synthesized derivatives,(R1,R2,R3,R4) was carried out using protein denaturation assay method, aspirin as reference standard. All the titled compounds were evaluated for *in-vitro* anti-inflammatory activity. The effect of all the synthesized compounds were tested with various concentrations (100, 200, 300, 400 and 500 µg /ml).All derivatives were able to inhibit inflammation production .the most potent compound was R4.The results were tabulated. The order of anti-inflammatory activity of the synthesized compounds at various concentrations (µg) based on their inhibition is as follows: (R4>R1>R3>R2)

In-vitro Anti-oxidant activity:

Antioxidant activity was performed by Hydrogen Peroxide scavenging activity(HPSA) method using ascorbic acid as reference standard. All the title compounds (R1,R2,R3,R4) were evaluated for In-vitro anti-oxidant activity. The effect of the synthesized titled compounds were tested with different concentrations (100,200,300,400 and 500µg/ml) against free radicals produced by Hydrogen peroxide. All derivatives were able to inhibit free radical production. . The most effective one was R4. The results were tabulated. The order of anti-oxidant activity of all synthesized compounds against free radicals as follows. (R4>R1>R3>R2).

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