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Biological studies of syntheised azo compounds of indole: A comparative study

S. Jasmin Sugantha Malar[1] and T.F. Abbs Fen Reji[2]

[1]Department of Chemistry, Women's Christian College, Nagercoil, Tamilnadu, India

[2]Department of Chemistry and Research Centre, Nesamony Memorial Christian College, Marthandam, Tamilnadu, India

ABSTRACT: In the present investigation, azo compounds were synthesized in good yields *via* the diazotization of different aromatic amines followed by coupling with indole. The synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques and have been tested *in vitro* against a number of microorganisms in order to assess their antimicrobial properties using disk diffusion method and the minimum inhibitory concentrations (MIC) by the broth micro dilution technique. Their anticancer activity against human breast cancer cell line (MCF7) was determined by MTT assay. All exhibit comparable biological activity.

KEYWORDS: Benzo pyrrole; Antimicrobial; Anticancer; Disc diffusion; Broth dilution.

1 INTRODUCTION

Indole is an aromatic heterocyclic organic compound, having a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen containing pyrrole ring. Hence it is known as benzopyrrole. The indole structure can be found in many organic compounds like the amino acid tryptophan and in tryptophan-containing protein, in alkaloids, and in pigments.

Indole along with their several derivatives finds a prominent place in synthetic organic chemistry, as they found to be potent pharmacophores. The indole skeleton is one of the most attractive frameworks with a wide range of biological and pharmacological activities. This physiologically important nucleus is abundantly found in therapeutic agents as well as in natural products. The indole ring system represents a privileged structure in drug discovery. Indole derivatives are used as analgesic[1], antiallergic [2], antibacterial[3], antifungal[3], anticonvulsant[4], anti-inflammatory[5], antiviral[6], antidiabetic[7], anticancer[8], anti hypertensive[9] and antioxidant[10], *etc*. The discovery of many indole alkaloids with varied biological properties has attracted the organic chemists to find new routes for the synthesis of derivatives of indole.

Thus the important role displayed by indole and its derivatives for various therapeutic and biological activities prompted us to synthesize some azo compounds bearing indole moiety in order to achieve compounds having better therapeutic activities. In the present work, we have synthesized and characterized five azo compounds namely 3-[phenylazo]-indole (PAI), 3-[4-methylphenylazo]-indole(MPAI), 3-[4-nitro phenylazo]-indole (NPAI), 3-[4-chloro phenylazo]-indole (CPAI) and 3-[4-methoxy phenylazo]-indole (MyPAI).

2 EXPERIMENTAL

All chemicals were of analytical grade and were obtained from Merck, Nice and CDH. Melting points were determined in open capillary tubes and are uncorrected. The purities of the compounds were checked by thin layer chromatography (TLC) using glass plates coated with silica gel (Merck) and chloroform: ethanol as a solvent system. The spots were developed in an iodine chamber and visualized under ultraviolet (UV) lamp. In the present investigation the IR spectra of azo compounds were recorded on Schimadzu FTIR spectrophotometer model 8400 S in KBr wafer, the NMR spectra were obtained on 400

MHz FT NMR spectrometer using $CDCl_3$ as solvent and reported relative to TMS as internal standard and the mass spectrum was taken using mass spectrometer by LCQ technique.

2.1 SYNTHESIS OF AZO COMPOUNDS

Azo compounds were synthesized according to the method reported in literature [11]. There are two steps in the synthesis of azo compounds:

2.2 DIAZOTISATION OF AMINES

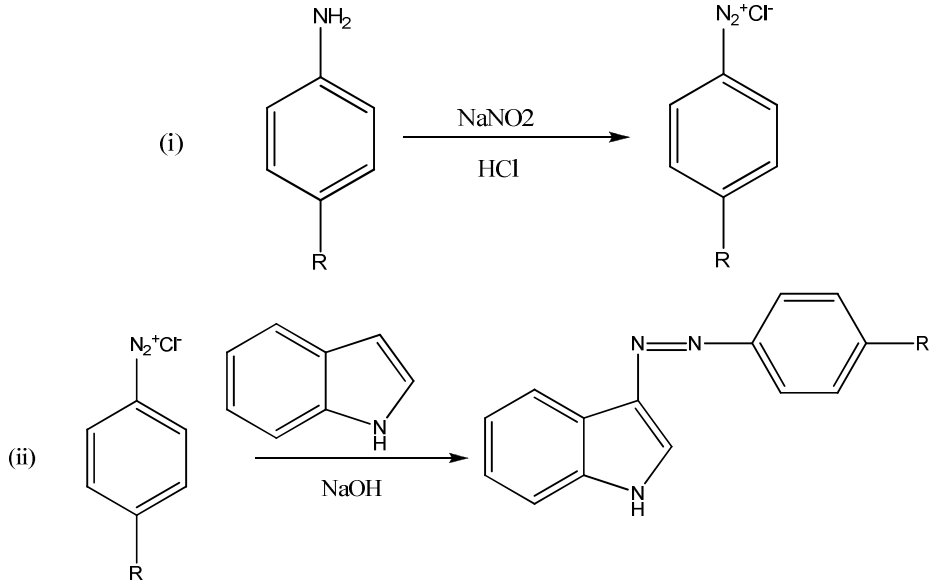
A solution aromatic amine (10 mmol) and 8 mL of 3 M HCl was heated gently, then water (10 mL) was added in order to dissolve the solid. The mixture was cooled to 0^o C in an ice bath with stirring. This solution was cooled to 0–5 ºC, and a freshly prepared solution of 1 M sodium nitrite (10 mL) was then added drop wise, maintaining the temperature below 5 º C. The solution was kept in an ice bath and used immediately in the next step.

2.3 COUPLING WITH INDOLE

Alcoholic solution of indole (10 mmol) was dissolved in 10 mL of 2 M sodium hydroxide, and cooled to 0–5 ºC in an ice bath. This solution was then gradually added to the cooled benzene (or substituted) diazonium chloride solution. The resulting mixture was stirred at 0–5°C for at least 15 minutes until the crystallization is complete (giving a coloured solid). The pH of the solution was adjusted with dilute HCl or NaOH solutions (0.1 M) in order to induce precipitation. The resulting coloured precipitate was filtered, washed several times with cold water and was recrystallized from hot chloroform to yield azo compound. Azo compounds were synthesized according to the following scheme 1. The physical and analytical data obtained for these compounds are shown in table 1.

Table 1: Physical and Analytical data of substituted azo compounds of indole

Compounds	R	Molecular Formula	Molecular Weight (Calculated)	Colour	% Yield	Melting Point ^oC
1	H	$C_{14}H_{11}N_3$	221.21	Orange red	83	93
2	CH_3	$C_{15}H_{13}N_3$	235.28	Violet red	85	109
3	NO_2	$C_{15}H_{10}N_4O_2$	278.25	Scarlet red	72	85
4	Cl	$C_{14}H_{10}N_3Cl$	256.70	Rosy red	86	75
5	OCH_3	$C_{16}H_{13}N_3O$	251.28	Blood red	94	83



Scheme 1 : Preparative route of substituted azo compounds of indole

2.4 ANTIMICROBIAL ACTIVITY

The synthesized azo compounds were screened for the presence of antibacterial activity against four strains of bacteria *i.e, Staphylococcus aureus*, *Klebsiellapneumoniae*, *E.Coli, Streptococcus,* and two species of fungi *i.e.,* against *Candida albicans and Candida glabrata* by disc diffusion method.[12,13,14] The bacterial inhibition zone values were summarized in table 2. All the newly synthesized azo compounds showed good activity against *Klebsiella pneumonia.*

Table 2. Antimicrobial screening data (zone of inhibition in mm) of the synthesized azo Compounds of indole

Compds	*Klebsiella pneumoniae (-)*	*E.coli (-bacilli)*	*Staphylococcus aureus(+cocci)*	*Streptococcus (+cocci)*	*Candida albicans*	*Candida glabrata*
I	12	8	10	9	10	10
II	8	9	9	9	9	9
III	9	9	9	8	10	10
IV	10	8	8	9	9	9
V	10	9	10	9	9	9
Std	20	22	23	20	16	15

2.5 MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimal inhibitory concentration (MIC) was determined by broth dilution method [15]. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculums). The MIC values are reported at table 3. The MIC range was between 1200 - 1600 □g/mL.

Table 3: Minimum inhibitory concentrations (MIC) in μg/mL of azo compounds of indole

Compds	*Klebsiella pneumoniae (-)*	*E.coli (-bacilli)*	*Staphylococcus aureus(+cocci)*	*Streptococcus (+cocci)*	*Candida albicans*	*Candida glabrata*
I	1400	1600	1200	1200	1400	1600
II	1200	1600	1600	1600	1400	1600
III	1400	1600	1400	1400	1600	1600
IV	1400	1600	1400	1600	1600	1600
V	1600	1600	1600	1600	1600	1600

2.6 ANTICANCER ACTIVITY

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. [16,17]

The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software. The values are given in table 5.

Table 5: % cell inhibition of the compound I in different concentration

Sl. No.	Conc. (μM)	% Cell Inhibition	IC50 μM
1	0.1	0	47.33
2	1	4.059652	
3	10	8.367854	
4	50	61.30903	
5	100	99.8343	

3 RESULTS AND DISCUSSION

Azo compounds of indole were synthesized by coupling of different aromatic amines. They were characterized by UV, IR, ^{1}H NMR, C^{13} NMR and mass spectrum.

3.1 SPECTROSCOPIC CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

UV SPECTRA

All the synthesized compounds showed max in the region 350 – 370 nm, confirms the presence of N=N bond in these compounds.

IR SPECTRA

In azo compounds, one may expect the absorption bands due to N=N, -N-H, C-H=C-H and C-N vibrations in IR region. All the synthesized compounds showed absorption bands for different types of vibrations which were shown by azo compounds. This confirms the success of the synthesis**.** Table 4 shows important IR peak values of the newly synthesized azo compounds.

Table 4: Infrared spectral data of substituted azo compounds of indole

Compounds	R	ν_{N-H}	$\nu_{N=N}$	$\nu_{C=N}$	$\nu_{CH=CH}$
I	H	3307	1672	1519	2925
II	CH_3	3317	1666	1454	2972
III	NO_2	3313	1666	1452	2972
IV	Cl	3309	1664	1595	2972
V	OCH_3	3319	1666	1600	2927

NUCLEAR MAGNETIC RESONANCE SPECTRA

^{1}H NMR SPECTRA

The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum for the compound I showed a singlet at δ 6.411 has been attributed to H-2 of indole ring. The multiplet at δ 7.19 – 7.3 is assigned to two hydrogen, H-3 and H-5 of aryl ring. The multiplet at δ 7.35 – 7.45 shows H-4 of indole ring. The multiplet at δ 7.46 – 7.58 is assigned to three hydrogen, H-5, H-6 and H-7 of indole ring. The multiplet at δ 7.77– 7.86 is assigned to one hydrogen, H-4 of aryl ring and δ at 8.35 – 8.44 is assigned to two hydrogen, H-2 and H-6 of aryl ring. The singlet at δ 11.1 is assigned to NH hydrogen of the indole ring and thus accounting for the number of hydrogen atoms in the compound I.

^{13}C NMR SPECTRA

The C^{13} NMR spectrum of the compound I showed fourteen peaks, thus accounting for all the fourteen carbons.

MASS SPECTRA

The FAB MS shows a strong MH^{+} peak at m/z 252 for compound V confirmed the molecular weight of the compound.

3.2 ANTIMICROBIAL ACTIVITY

The synthesized azo compounds of indole shows bactericidal and fungal activity From the antimicrobial screening it was observed that all the compounds exhibited activity against all the organisms employed. As we consider all results obtained from antibacterial and antifungal tests together we can say that entire compounds tested were active towards bacteria and fungi.

3.3 MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC is the lowest amount of drug at which, it is able to inhibit the growth of specified microorganism. MIC value of the synthesized azo compounds were calculated against four strains of bacteria *i.e, Staphylococcus aureus*, *Klebsiellapneumoniae*, *E. Coli, Streptococcus,* and two species of fungi *i.e.,* against *Candida albicans and Candida glabrata* using broth micro dilution method. The MIC value for all the synthesized compounds were found to be between 1200-1600 μg/mL.

3.4 ANTICANCER ACTIVITY

Anticancer activity of the compound I was found out using human breast cancer cell line (MCF 7) by MTT assay and found to be cancer active. IC_{50} value is 47.33 μM.

4 CONCLUSION

The synthesized azo compounds were characterised by elemental and spectral datas and the antimicrobial activity of these compounds against pathogenic microorganism shows that these compounds have the capacity of inhibiting the growth of different microorganisms to some extent. The compounds discovered in this study may provide valuable therapeutic intervention for the treatment of cancer disease. Hence in future the azo compounds with indole moiety can be used in medicinal fields for better recoverment.

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