Research Paper

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Study and Characterization of Indole Carboxylate Derivative Synthesized Via Condensation

Nikita Mishra[1], Tejal Gavit[2], Ajay Katariya[3], Dilip Vasava[4*]

*Department of Chemistry, Gujarat University, Ahmedabad, India
E-mail:dilipvasava20@gmail.com

*Corresponding Author

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Abstract

High potency can be obtained when one biologically active molecule linked to another for resultant molecule. Here, cyclization is done by fused indole moiety to obtain highly potent, more specific and less toxic agent. In the present study, the reaction of phenyl hydrazine with a mixture of ethyl aceto acetate and glacial acetic acid by condensation reaction to synthesize desired moiety. The derivatives of indole carboxylate were obtained and characterized by FTIR and UV. The newly prepared compounds were screened for their antibacterial activity. The obtained molecule can be further applied for potential application and as intermediate for antibacterial active compounds.

Keywords: Phenyl hydrazine, Ethyl acetoacetate, Hydrazine hydrate, Indole, antibacterial activity.

Introduction

Heterocyclic compounds are of very much interest in daily life. Heterocyclic compounds have one or more hetero atoms in their structure. They may be cyclic or non-cyclic in nature. Heterocyclic compounds have a wide range of application. They also find applications as sanitizers, developers,

antioxidants, as corrosion inhibitors, as copolymers, dye stuff. They are used as vehicles in the synthesis of other organic compounds. Heterocyclic compounds are also finding an increasing use as intermediate in organic synthesis. Very often this is because a relatively stable ring system can be carried through a number of synthetic steps and then cleaved at the required stage in a synthesis to reveal other functional groups. In the family of heterocyclic compounds nitrogen containing heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes [1-2].Indole, a privileged heterocyclic nucleus has diverse biological activities and inspired chemists to utilize this skeleton as bioactive moiety to synthesize new compounds possessing pharmacological and biological properties. The beauty of indole moiety is that it provides a privilege scaffold for the discovery of different kinds of more active, less toxic novel drugs with different modes of action. [3- 5].Amongst the wide range of methods available, the Fischer indole synthesis was employed to synthesis the indole compounds. This method involves the preliminary condensation of a carbonyl compound (usually a ketone, but occasionally an aldehyde) with an aryl hydrazine to form an aryl hydrazone, which is then rearranged to give the desired indole in a step that is often described as "indolisation". So the Fischer indole synthesis can be regarded as the elimination of ammonia from the aryl hydrazone of an aldehyde or ketone, by treatment with an acid or various metal and anhydrous metal salt catalysts, with formation of an indole nucleus [6-8].Phenyl-hydrazine is used to prepare indoles via the Fischer Indole Synthesis, which are intermediates in the synthesis of various dyes and pharmaceuticals. The indole ring system represents one of the most abundant and important heterocycles in nature. Indole and its derivatives have occupied a unique place in the chemistry of nitrogen heterocyclic compounds because of their varied biodynamic properties. Although indole motif is very small, it is an attention grabbing molecule and captured the attention of scientists and researchers over the years [9, 10]. Thus indole derivatives occupy a unique place in medicinal chemistry due to their wide range of pharmacological activities such as anti-inflammatory, antifungal, antimicrobial, antioxidant, antiviral, anti-tubercular, anticancer, anticonvulsant, antihistaminic and antagonistic, etc. [11-13].The reaction mechanism of fischer indole synthesis have been extensively studied and results has been summarized[14].In a typical fischer procedure, the aryl hydrazone is obtained by treating the requisite ketone (or aldehyde) with an equimolar quantity of the appropriate

aryl hydrazine. The key indolisation sequence may then effected by moderate heat under thermal conditions, normally with acid catalysis [15-17]. Many different catalysts may be used, including mineral acids (sulphuric acid or hydrogen chloride), organic acids (trifluoroacetic acid and various sulphonic acids) and Lewis acids [18, 19]. A particularly convenient method involves the use of an acidic organic solvent, such as formic or acetic acid (or a homologue if a higher temperature than the boiling point of acetic acid is needed). In many instances, isolation of the intermediate aryl hydrazone is unnecessary; the reaction can be conducted as a "one-pot" procedure starting from equimolar quantities of the ketone or aldehyde and the aryl hydrazine, so that indolisation of the aryl hydrazone occurs in situ [20-23]. Preliminary studies showed that the direction of indolisation and the ratio of the product are greatly affected by the reaction conditions (i.e. the solvents and the catalysts); under weakly acidic conditions, such as with glacial acetic acid as the solvent without the addition of stronger acids, the more substituted ene-aryl hydrazines, predominated. The choice of catalysis is subjective and more a matter of task rather than based on critical experimentation [24- 26].Glacial acetic acid as a protic acid was employed as a catalyst in a solvent free condition for facile preparation of di(indolyl)methanes via one-pot condensation of indole with aryl or hetero aryl aldehydes[27-29]. AcOH as a mild and efficient catalyst for the promotion of the condensation reaction of indoles with aromatic aldehydes. So the choice of acid catalyst is very important. Bronsted acids such as HCl, H2SO4, polyphosphoric acid and p-toluenesulfonic acid have been used successfully while, lewis acids such as boron trifluoride, zinc chloride, iron chloride, and aluminium chloride are also useful catalysts [30-32]. Kalaskar et al. synthesized indole-3-acetic acids and evaluated them for their in vivo anti-inflammatory activity. The compound 1, 2-disubstituted-5-methoxyindole/ benz(g)indole-3-acetic acid showed significant activity[33-35]. The significant contribution of many derivatives of indole in the development of medicinal chemistry should be recognized.

The aim of present work is to synthesize Indole carboxylate derivatives with potential application and as intermediate for antibacterial active compounds.

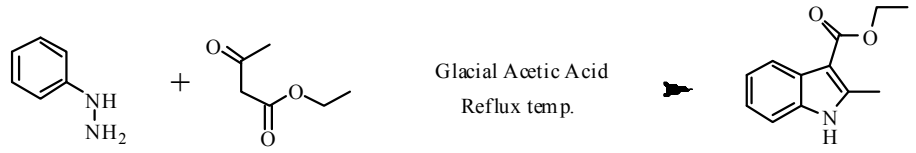
2 Experimental

2.1 Material and Chemicals

Phenyl hydrazine, Ethyl Aceto Acetate, Glacial Acetic acid, 40% bromine solution, HNO3, H2SO4 All chemicals used were of synthetic grade. The purity of compounds was ascertained by TLC on pre coated silica F254 plates (Merck, Mumbai, India) using iodine vapors and UV light as detecting agents. The melting points of the synthesized compounds were determined by open capillary method and are uncorrected. Melting points were determined in open capillaries in electrical apparatus and are uncorrected. IR spectra were recorded on a FT-IR spectrometer using KBR pellet.

Synthesis of Indole Derivatives

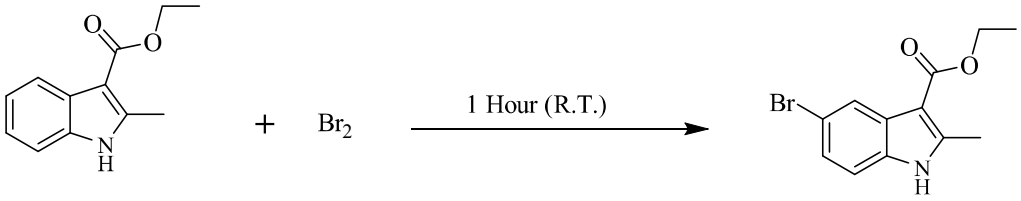
STEP-1: Synthesis of Ethyl-2-methyl-1H-indole-3-carboxylate[36] [C1]



1-Phenyl Hydrazine ethyl 3 - oxobutanoate ethyl 2-methyl-1H-indole-3-carboxylate

In a flat bottom flask (250 ml), a sealed stirrer unit and reflux condenser, a mixture of ethylacetoacetate (2.4 ml; 0.018 mole) and glacial acetic acid (1.0 ml,0.018mole)was placed in the flat bottom flask and heated under reflux with stirring. Add phenyl hydrazine (1.8 ml; 0.018mole) slowly during first 1hr. Continue the stirring for further 1hr. Reaction progress is checked by TLC method. Pour the reaction mixture into a 50ml beaker with cooled water and stir vigorously while it solidifies. Then add sufficient water and filter. Dry the crude product, the crude product thus obtained was recrystallized from methanol.

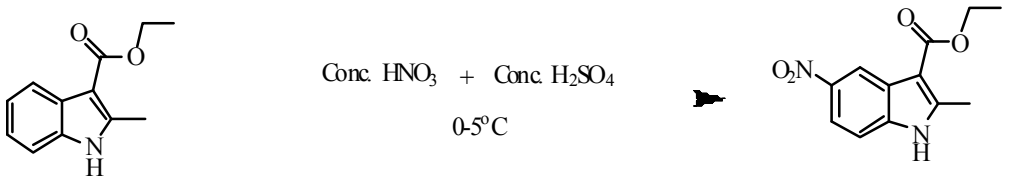
STEP 2: Synthesis of Ethyl5-bromo-2-methyl-1H-indole-3-carboxylate [B1]



ethyl 5-bromo-2-methyl-1*H*-indole-3-carboxylate

In 250 ml conical flask take Ethyl-2-methyl-1-H-Indole-3-Carboxylate (2 gm, 0.0098mole) dissolve in suitable solvent. i.e. Methanol. Put this solution in ice bath to maintain temperature 0-10°C. Take 40% Br solution (2.5 ml) and transfer it into separating funnel. Then add this Br solution dropwise to above solution at temperature maintaining 0-10°C. After addition put the mixture at R.T for 20 min. then dump this into ice water, stir vigorously so the product is obtained.

Step 3: Synthesis of Ethyl 6-nitro-2-methyl-1H-indole-3-carboxylate [N1]



ethyl 2-methyl-1H-indole-3-carboxylate

ethyl 2-methyl-5-nitro-1*H*-indole-3-carboxylate

Take HNO3 (1 ml, 0.019 mole) in one test tune and add, in portions with shaking, another test tube containing concentrated H2SO4 (2 ml, 0.039 mole). Keep the mixture cool during the addition by immersing the test tube in cold water. Then add this mixture dropwise in RBF containing dissolved Ethyl-2-methyl-1-H-Indole-3-Carboxylate (2 gm, 0.0098 mole) in methanol. Try to maintain temperature by immersing RBF in cold water. When all nitrating reagent mixture added, fix the reflux condenser to RBF; Heat in water bath to maintained temperature 60°-70°C for 30 minutes. Then pour this into cold water to get the product.

2.3 Properties and Characterization Techniques

2.3.1 SOLUBILITY [37]

Solubility in the different solvents depends upon the solvent type and on the functional groups if present in the solution. Neutral organic compounds tend to be hydrophobic; that is, they are less soluble in water than in organic solvents. Organic compounds tend to dissolve in organic solvents. The solubility of compounds can be determined by placing approximately 0.1gm of finely grind polymer in test tube with 5ml of particular solvent for each. The mixtures were stored at 25°C for some time with continues shaking. While shaking, formation of streaks indicated dissolution. Samples that swelled without dissolving at 25°C were heated to 50°C in order to affect the process of dissolution.

2.3.2 UV-VIS SPECTROSCOPY[38]

Ultraviolet-visible spectroscopy involves the absorption of ultraviolet/visible light by a molecule causing the promotion of an electron from a ground electronic state to an excited electronic state. A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device.

Ultraviolet/Visible light: wavelengths between 190 and 800 nm

Ultraviolet range between **190-380 nm&**

Visible region falls between **380-750 nm**

UV-Vis Spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range.

UV-Vis spectroscopy is routinely used in the chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Solvent polarity and pH can affect the absorption spectrum of an organic compound.

2.3.3 FT-IR SPECTRA [39]

It is a very popular method infrared spectroscopy is being utilize for characterizing samples. Vibration of atoms of the molecules is main concept based on this technique. Through a sample IR radiation are passed in which IR spectrum is obtained with an energy. IR spectrum explains characteristic of the entire molecule, in which certain groups of atoms give rise to bands at or near the same frequency regardless of the structure of the rest of the molecule.

2.3.4 ANTIMICROBIALACTIVITY [40]

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Several bioassays such as

disk-diffusion, well diffusion and broth or agar dilution are well known and commonly used. Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome.

Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macro dilution or micro dilution). Either broth or agar dilution method may be used to quantitatively measure the in vitro antimicrobial activity against bacteria and fungi. MIC value recorded is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested, and it is usually expressed in µg/mL or mg/L.

3 Results and Discussion

3.1 Physical Properties

Name of Compound	Mol. formula	M.W. (gm/mole)	M.P.	Color
Ethyl-2-methyl-1H-indole-3-carboxylate	$C_{12}H_{13}NO_2$	203.24	135°C	Cream yellow
Ethyl 5-bromo-2-methyl-1H-indole-3-carboxylate	$C_{12}H_{12}BrNO_2$	282.13	127°C	Yellowish Orange
Ethyl 6-nitro-2-methyl-1H-indole-3-carboxylate	$C_{12}H_{12}N_2O_4$	248.23	194°C	Yellowish Brown

3.2 Solubility Chart

Sr. No.	Name of Compound	Solubility
1	Methanol	++
2	DMF	++
3	THF	++
4	Hexane	+-
5	Toluene	+-
6	$CHCl_3$	++
7	MDC	++
8	Ethyl Aceto acetate	++
9	Acetone	++
10	DMSO	++
11	Acetonitrile	++
12	CCl_4	--
13	Water	--

3.3 IR Analysis

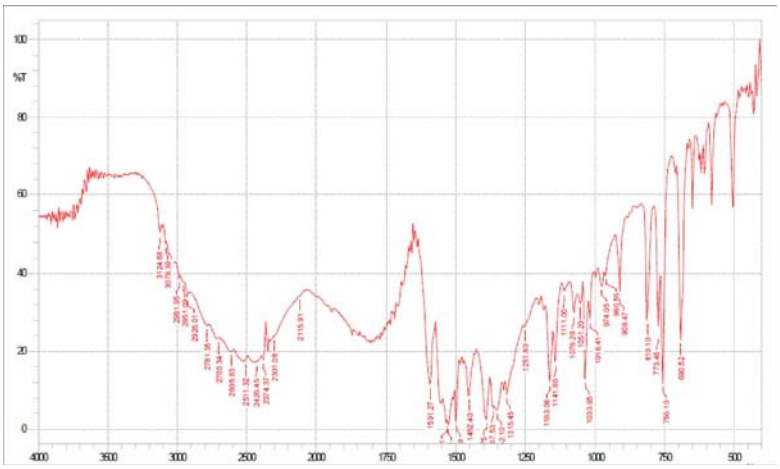


Figure 1: IR analysis of C1

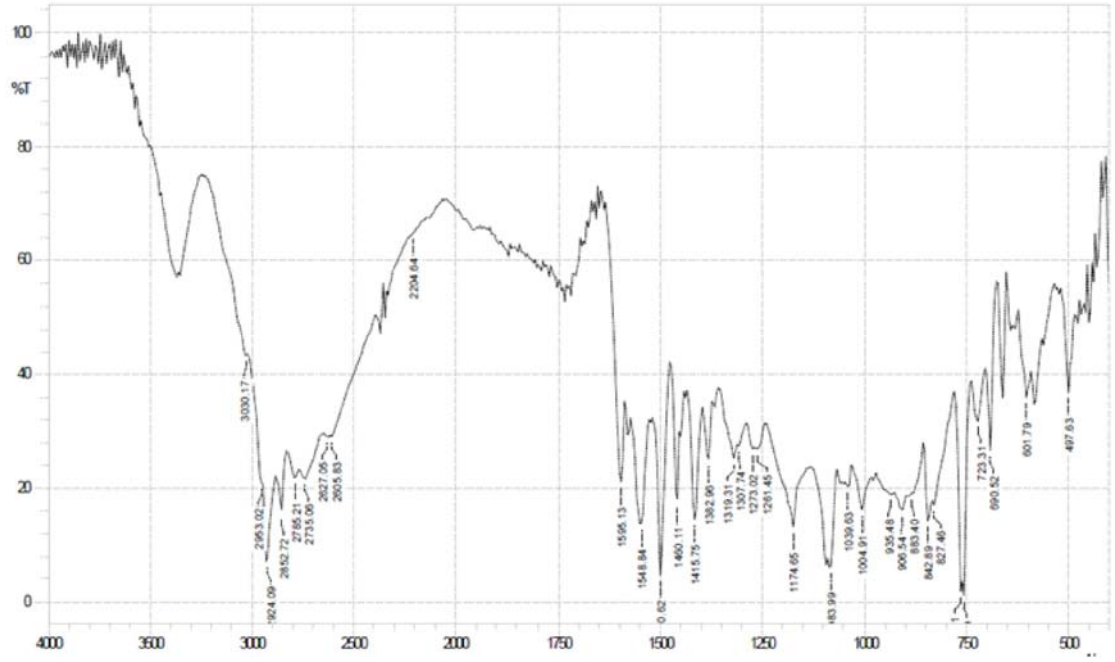


Figure 2: IR analvsis of N1

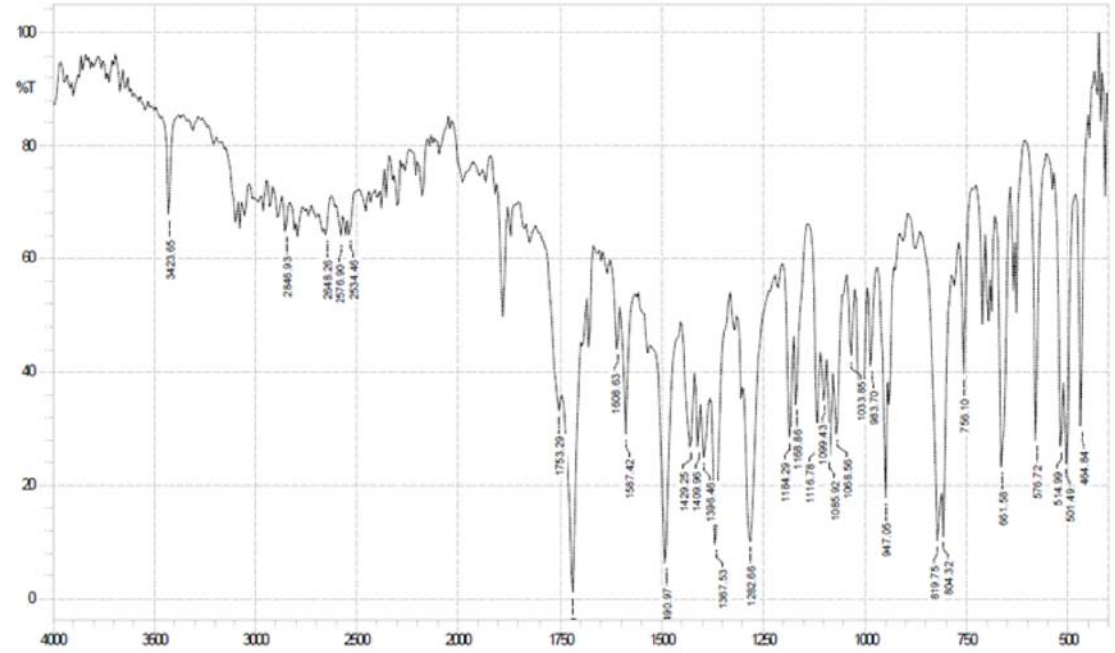


Figure 3: IR analysis of B1

Interpretation of IR

Compound	C-H (cm^{-1})	C-N (cm^{-1})	N-H (cm^{-1})	C=C (cm^{-1})	C-X (X=Br) (cm^{-1})	C=O (cm^{-1})	N-O (cm^{-1})	C-Mono Substituted(CH_3)(cm^{-1})
C-1	3124	1163	3109	1533	-	1591	-	908
B-1	3124	1163	3109	1533	661	1591	-	908
N-1	3124	1163	3109	1533	-	1591	1548	908

3.4 UV Analysis

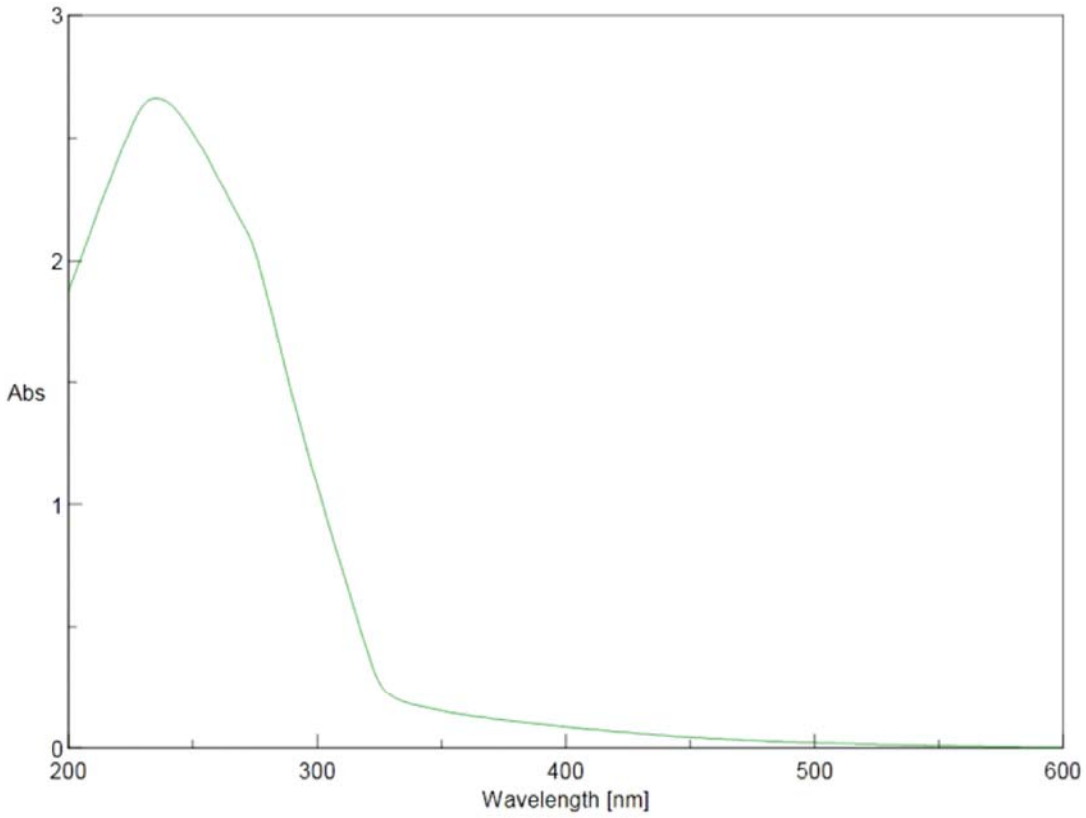


Figure 4: UV analysis of C1

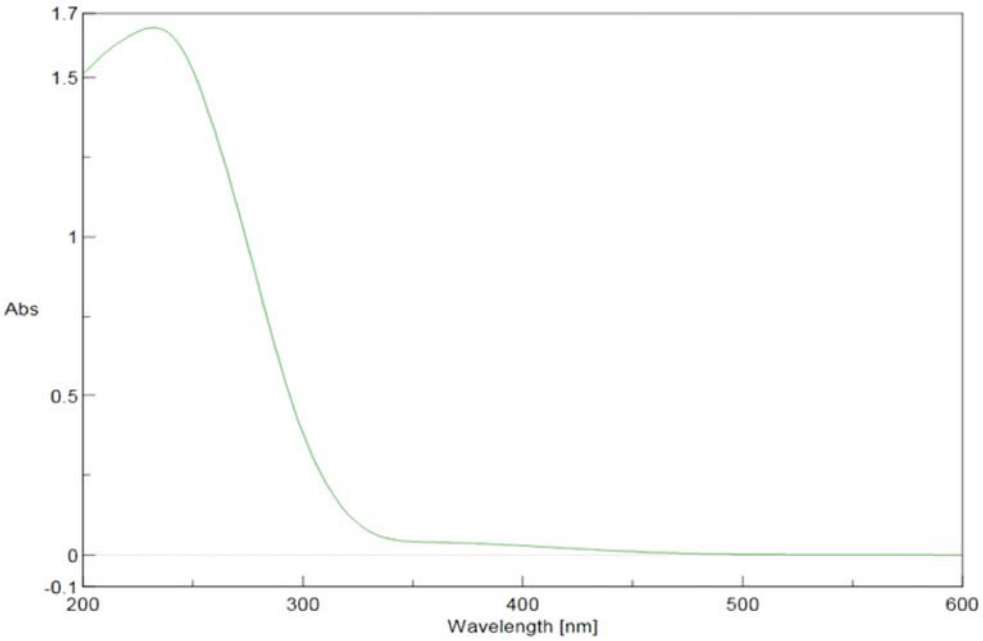


Figure 5: UV analysis of N1

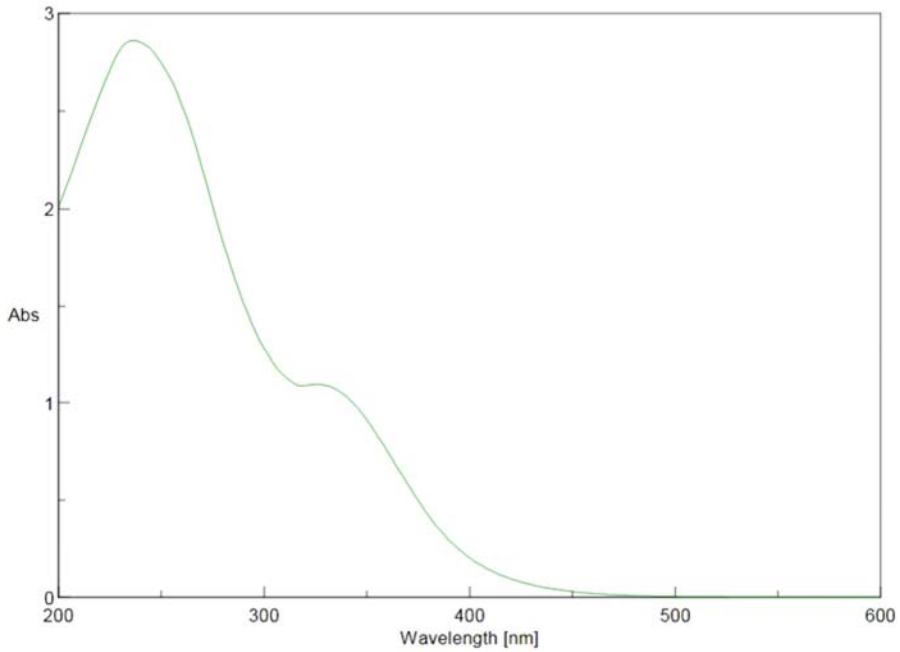


Figure 6: UV analysis of B1

Interpretation of UV

Molecule	Wavelength of Maximum Absorption (nm)
C1	250nm
N1	217nm
B1	248nm

A jump from a pi bonding orbital to a pi anti-bonding orbital ought to have a fixed energy and therefore absorb a fixed wavelength.

All of the molecules give similar UV-visible absorption spectra - the only difference being that the absorptions move to longer and longer wavelengths as the amount of delocalization in the molecule increases.

3.5 Antibacterial Activity Analysis

The newly prepared compounds were screened for their antibacterial activity two gram- positive bacteria S. aureus and two gram-negative bacteria E.coli using Nutrient agar medium.

Antibacterial activity was carried out by Disc-diffusion method [zone of inhibition] and serial dilution method [MIC].Antibacterial activities were studied by subjecting the compounds to pharmacological screening by standard procedures.

All the compounds synthesized in the present investigation were tested for their antibacterial activity. The antibacterial activities were tested on nutrient medium against Staphylococcus aureus and Escherichia coli.

- Investigation: Antimicrobial activity of B2 sample:

Method: Agar Diffusion

Sr. No.	Test organism	Sample Name- bromo-B2		
		100 µg	50 µg	25 µg
1	E. coli ATCC 25922	100 mm	NO ZONE OBSERVED	NO ZONE OBSERVED
2	S. Aureus ATCC 25923	20 mm	15 mm	14 mm

• Investigation: Antimicrobial activity of C2 sample:

Sr. No.	Test organism	Sample Name- C-2		
		100 µg	50 µg	25 µg
1	E. coli ATCC 25922	10 mm	NO ZONE OBSERVED	NO ZONE OBSERVED
2	S. Aureus ATCC 25923	10 mm	NO ZONE OBSERVED	NO ZONE OBSERVED

Conclusion

The indole derivatives are abundantly available in wide variety of natural sources and new indole derivatives are being synthesize on regular basis. The synthesized Ethyl 5- bromo 2-methyl-1-H-indole-3-carboxylate showed enhanced biological activities. This synthesized indole carboxylate derivatives are analyzed by IR and UV analysis. They showed a broad spectrum of antibacterial activity against G(+ve) and G(-ve) bacteria. It may be worthwhile to explore the possibility in this area by fusing other heterocyclic moieties and increase the potency of synthesized compounds.

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