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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF INDOLE AND IT'S DERIVATIVES

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ABSTRACT

Objective: The objective of this study was to synthesize and evaluate the antimicrobial and antihelmintic activity of novel drug derivatives containing indole moieties. **Methods**: The present work involves the synthesis of indole derivative, where the starting material isatin is refluxed with Hydrazine hydrate in the presence of ethanol at 80° centigrade for 2-4 hours. Maintaining the same temperature samples was evaluated. The reaction was monitored by TLC. After completion of the reaction the product was filtered and recrystallized from hot ethanol, the product 1 was refluxed with chloro acetyl chloride in the presence of ethanol, the product 2 is treated with different anilines. Then anti- microbial studies were done for the synthesized derivatives. The synthesized compounds were evaluated for antihelmintic activity in Pheretima posthuma (earthworm) of nearly equal size (6≠1cm). Then the time taken for worms to become motionless was noted as paralysis time & to ascertain death each earthworm was frequently applied with external stimuli which stimulates and induce movements in the worms, if alive.

LIST OF ABBREVATIONS

IR	Infrared
µg	Microgram
Ml	Milli Liter
°C	Degree Centigrade
PSI	Pounds Per Square Inch
MIC	Minimum Inhibitory Concentration
TLC	Thin Layer Chromatography
ZOI	Zone Of Inhibition
API	Active Pharmaceutical Ingredient

Heterocyclic Chemistry
It is a branch of chemistry dealing exclusively with synthesis, properties and application of heterocyclic especially vital to drug design.

Heterocyclics
Heterocyclic compounds are occupying prime place in heterocyclic chemistry owing to their valuable properties has a therapeutic agents, drugs, dyestuffs.

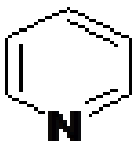
These compounds are reported to possess Antimicrobial, anti- inflammatory, anti-diabetic, haemo regulatory, blood platelet aggregation inhibiting property and also the pesticidal properties.

It is also reported in various journals that these compounds are showing very good antibiotic activities.

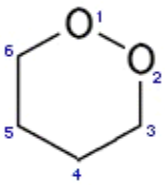
Incorporation of an oxygen and Nitrogen, sulphur an atom of a related element into an organic ring structure in place of a carbon atom gives rise to a heterocyclic compound.

Since the heterocyclic atom must form more than 1 bond in order to be incorporated into a ring structure, halogens do not form heterocyclic compounds although they may be substituents on a heterocyclic ring structure.

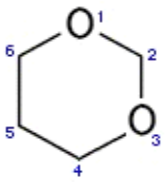
Heterocyclic compounds like polycyclic ring compounds are usually known by non- scientific names.



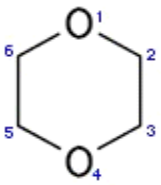
Pyridine



1,2-dioxane



1,3-dioxane

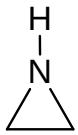


1,4-dioxane

- **Heterocyclic chemistry** is the branch of chemistry dealing exclusively with synthesis, properties and applications of heterocyclics especially vital to drug design.

3-Membered Rings

- Heterocyclic with three atoms in the ring are more reactive because of ring strain. Those containing one heteroatom are generally stable. Those with two hetero atoms are more likely to occur as reactive intermediates. Common 3-membered hetero cyclic are:

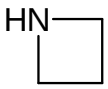


Aziridine

Ethylene Oxide

Ethylene Sulphide

4-Membered Rings
Azetidine, oxetane, and thietane—the four-membered rings containing, nitrogen, oxygen, and sulfur atoms respectively—are prepared by nucleophilic displacement reactions such as those used to prepare the corresponding three-membered rings.



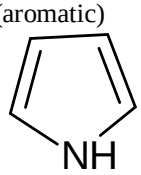
Azetidine

Oxetane

Thietane

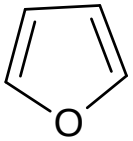
5-Membered Rings
With heterocyclics containing five atoms, the unsaturated compounds are frequently more stable because of aromaticity.

- With one heteroatom:
- With N: Pyrrole (aromatic)



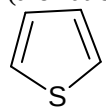
Pyrole

- With O: Furan (aromatic)



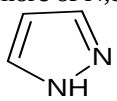
Furan

- With S: Thiophene (aromatic)

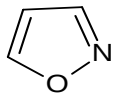


Thiophene

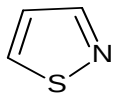
- With two heteroatoms:
- One N and one or more of N,S,O: the azoles



Pyrazole



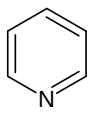
Oxazole



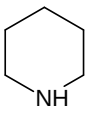
Thiazole

6-Membered Rings

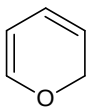
- With one heteroatom:
- With N: Pyridine, Pipperidine
- With O: Pyran
- With S: Thiane
- With two heteroatoms:
- Two N: Pyridazine, Pyrimidine, Pyrazine are the 1,2-, 1,3-, and 1,4- isomers, respectively.
- Two N: Piperazine
- One N and one O: Oxazine, Morpholine
- One N and one S: Thiazine
- Two S: Dithiane - 1,2-, 1,3- and 1,4- isomers, respectively
- Two O: Dioxane - 1,2-, 1,3- and 1,4- isomers, respectively.



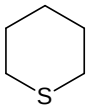
Pyridine



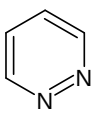
Piperidine



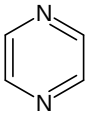
2*H*-pyran



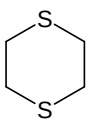
thiane



pyridazine



pyrazine



1,4-dithiane

The chemistry of phosphates, fully unsaturated five-membered heterocyclic rings containing a phosphorus element, has drawn much attention in terms of the development of synthetic methods and elucidation of its spectroscopic properties for applications in Organic Field-Effect Transistors (OFETs) and luminescent materials. The phosphorous atom of trivalent phosphorus compounds has a high chemical reactivity. Therefore, several reactions on the phosphorus atom such as oxidation, alkylation, and coordination to a Lewis acid can produce the corresponding phosphate derivatives with different electronic properties. Phosphate-based ladder-type π-conjugated heteroacenes were shown to exhibit high charge mobility and/or fluorescence quantum yields. For example, dibenzo-fused phospholo [3,2-b] phosphole dioxides and benzophosphole-fused tetracyclic heteroacenes, containing boron (B), silicon (Si), oxygen (O), and sulfur (S), were synthesized and their physical properties were studied. However, to the best of our knowledge, the synthesis of benzophosphole-fused indole derivatives as tetracyclic heteroacenes has not been reported. In 2015, Lu et al. reported the synthesis of only one phosphole and indole-fused pentacyclic heteroacene. Recently, we reported simple and efficient synthetic routes to benzothiophene-fused benzoheteroles containing the group 15 and 16 elements using the ring-closing reaction of dilithium compounds with electrophiles bearing heteroatoms. In continuation of our research, we were interested in the synthesis, molecular structure, and physicochemical properties of the parent benzophosphole-fused indole derivative and its various functionalized analogs such as the corresponding phosphine oxide, phosphonium salt, and borane–phosphine complex.

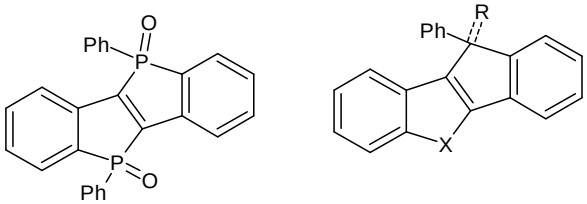


Figure 1: Phosphole-based tetracyclic heteroacenes.

LITERATURE REVIEW

Indole and several of their derivatives have been generally associated with various biological and pharmacological properties. The synthesis of a large number of Indole derivatives have been described to obtain biologically potent compounds. Many such compounds have been found to be promising. A few even have clinical application also.

This prominence aroused interest to several chemists and medicinal chemists to prepare day to day newer and newer potential Indole derivatives by molecular conjunction approach and evaluating them for possible pharmacological actions.

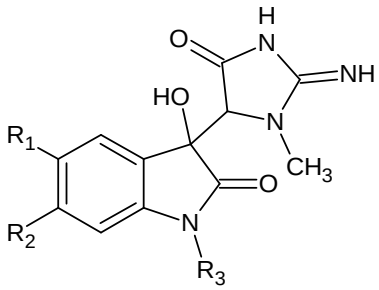
Since there have been numerous reports, it is highly impossible to cover all such reports in a single review, especially in the form of an introduction to a Master of Degree thesis like this. Hence, it is chosen to present some interesting reports of the recent two decades on the topic, mainly to indicate the recent trends in the progress of research on Indolederivatives. It is needless to quote that it is not at all an easy task to cover even these two decades, meticulously. However, efforts have been made to cover very broadly but briefly.

The present survey aims to synthesize of some new Indole derivatives of specific biological and pharmacological activity and looking for such activity (or) activities by their evaluation experimentally.

Since there have been numerous reports, some of the interesting reports are presented here according to their biological/pharmacological activity for orderly presentation.

Indole- As Anticancer (Antineoplastic) Agents

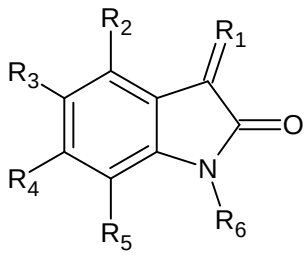
Narsimha Reddy Penthala et al.[1] reported the Synthesis and in vitro evaluation of N-alkyl-3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl) indolin-2-one analogs as potential anticancer agents.



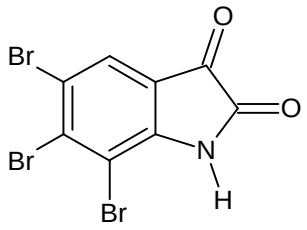
A series of novel substituted3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl) indolin-2-one derivatives (31a–w) were synthesized by condensation of the appropriate substituted N-alkyl isatins. The resulting analogs were then evaluated for their cytotoxic activity against a panel of 57 human tumor cell lines.

Compounds 31n and 31o were identified as molecules of interest from a single dose assay, and were then evaluated for dose-dependent growth inhibition and cytotoxicity in all 57 human cancer cell lines.

Kara L. Vine et al [2] reported the synthesis and In vitro cytotoxicity evaluation of some substituted isatin derivatives.

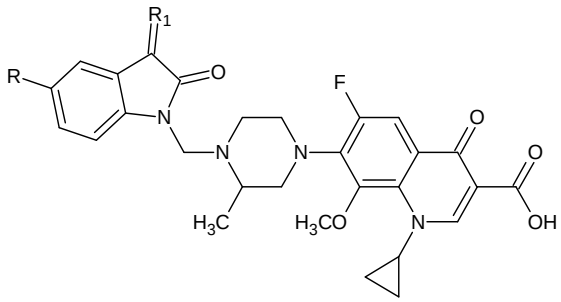


In summary, a range of isatin derivatives have been synthesized resulting in the generation of two new compounds (32o and 32p); Cytotoxicity screening revealed 32p to be the most active compound across all cell lines.



These results indicate that structural modification of di- and trisubstituted isatins may lead to new derivatives with enhanced and selective anticancer activity.

PerumalYogeeswari et al.[3] reported the Synthesis and in-vitro cytotoxicity evaluation of GatifloxacinMannich bases.

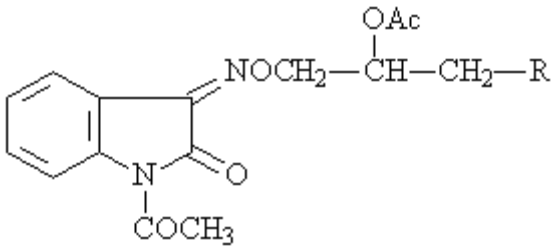


The compounds were tested in vitro against a panel of 58 human tumour cell lines derived from nine neoplastic diseases. In the leukemia cell lines compound 8 was found to be the most active compound. Compound 6was the most active compound and was more potent than standard etoposide against five cell lines (A549/ATCC, EKVX, HOP-92, NCI-H23 and NCI-H322 M). Compound 2, 6 and 8 were found to be the most potent compounds against colon cancer cell lines.

Indole as Antimicrobial Agents

Indole itself is inhibitory to the growth of Tubercule Bacillus Isatin-β-thiosemicarbazone was described as antibacterial by various workers. This compound also was found to be effective against tubercle bacillus to some extent.[124]

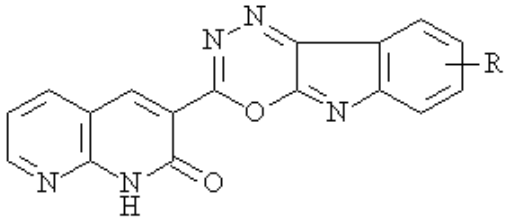
Padhy et al.[4] reported the synthesis of 1-acetyl-3-(2-acetoxy-3-substituted propyloximine) indol-2-(3H)-ones and evaluated for antimicrobial activity against B. subtilis, E. coli and C. albicans. Among them, compound with 2-acetoxy-3-substituted propyloximino chain at 3-position of indole-2, 3-dione exhibited high activity with MIC of 0.35 μg/ml



R-a: piperidino; b: pyrrolidino; c: dicyclohexylamino; d: diphenylamine; e: methylphenylamino.

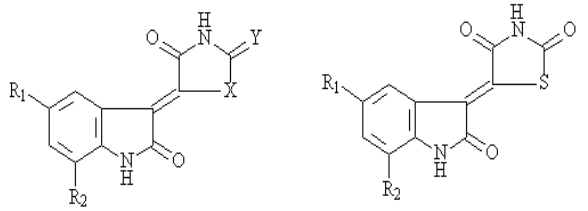
0.3to 12.5 μg/ml against B.subtilis, 0.16–3.12 μg/ml against E. coli and 6.2 – 100 μg/ml against C. albicans.

Mogilaiah et al.[6] reported the synthesis and antibacterial activity of some new[1,3,4]oxadiazino[6,5-b]indoles. Compound with R= 6-Cl & p-chlorophenyl showed significant inhibitory activity against E. coli and B. subtilis.



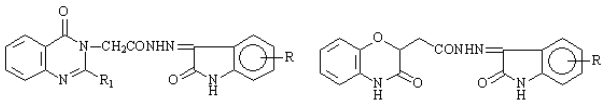
R = -H, 5-CH_3, 7-CH_3, 5-OCH_3, 5-Cl
5-Br, 6-CH_3, 8-CH_3, 6-OCH_3, 6-Cl

Pardasani e t al.[7] prepared some fused and Spiro imidazolidine derivatives and subjected for antimicrobial activity. The compounds and showed significant inhibitory activity against two bacteria.



$R_1 = R_2 = H$ $X = NH, Y = O$ $R_1 = R_2 = H$
$R_1 = R_2 = CH_3$ $X = NCH_3, Y = O$ $R_1 = R_2 = CH_3$
$R_1 = Br, R_2 = H$ $R_1 = Br, R_2 = H$

Sarangapani et al.[8] from our laboratory reported the synthesis of new isatinhydrazones and evaluated for antimicrobial activity. Compounds showed a noticeable degree of inhibition of gram-positive bacteria.

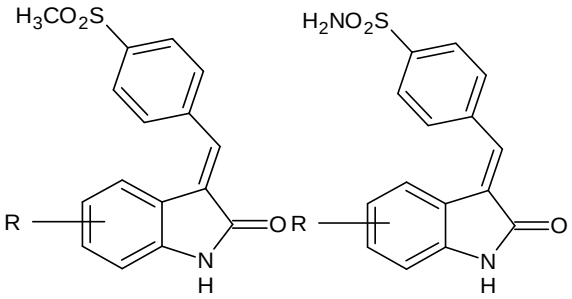


R = 5-CH_3, 7-CH_3, 5-Br, 7-Cl

R_1 = -CH_3, -C_6H_5

Indole As Anti Inflammatory Agents

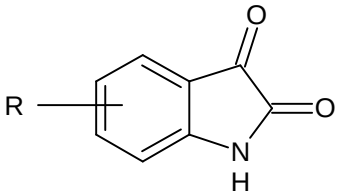
Yisheng Lai et al.[9] reported the Synthesis and biological evaluation of 3-[4-(amino/methylsulfonyl) phenyl] methyleneindolin-2-one derivatives as novel COX-1/2 and 5-LOX inhibitors.



a-g a-g

a: R = H, b:R = 5-F, c:R = 5-Cl, d: R = 5-CH_3, e: R = 6-Cl, f: R = 5-Br, g: R = 5-NO_2.

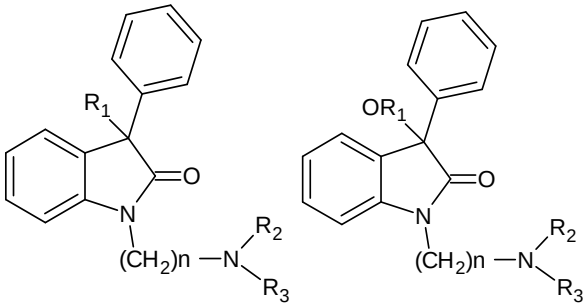
Maria Elaine Matheus et al.[10] reported the Isa tins inhibit cyclooxygenase-2 and inducible nitric oxide synthase in a mouse macrophage cell line



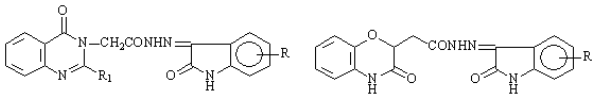
R = H, 5-F, 5-Cl, 6-Cl, 7-Cl, 5-CH_3, 4-Br, 5-I.

Isatin derivatives inhibited the activity of inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) enzymes in RAW264.7 activated cells, Except 4-Bromoisatin and 5-Iodoisatin suggesting isatins as a new synthetic iNOS and COX-2 inhibitors. These inhibitions were partly due to the inhibition at protein expression levels, because iNOS and COX-2-protein expression was significantly reduced by the treatment with isatins.

Hirose et al.[11] reported the synthesis of 3-alkyl-1-ω-(N'-substituted amino)alkyl-3-phenyl-indolin-2-ones (46) and 3-alkoxy-1-ω-(N-substituted amino) alkyl-3-phenylindolin-2-ones and related compounds, 3-alkyl analogues and 3-alkoxy (47) analogues shown similar inhibitory activity against acetic acid induced writhing syndrome and antiphlogistic analgesic activity against carrageenan or kaolin-induced edema.



Sarangapani et al.[12] reported the synthesis and pharmacological activities of isatin-3-[N^2-(2-methyl/phenyl-3,4-dihydro-4-oxoquinazolin)-3-methyl carbonyl] hydrazones and isatin-3-[N^2-(3, 4-dihydro-3-oxo-2H-1, 4-benzoxazin)-2-methyl carbonyl] hydrazones.



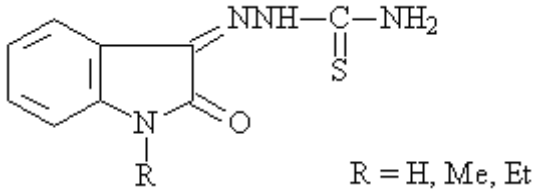
Indole As Antiviral Agents

Rough estimates that nearly fifty percent of the human ailments like small pox, poliomyelitis, influenza, measles etc. are caused by viruses. Besides these, the viruses are also responsible for mosaic diseases of tobacco, and sandal wood in plants, foot, mouth diseases, cow pox and rabies in animals. Viruses can grow and exist inside a host cell and they are dependent for their growth on the enzymatic activity of the host cell. Therefore, any interference with nucleic acid synthesis might help to inhibit viral synthesis, specifically without interfering with normal cell metabolism.

A number of isatin derivatives had been tested for their possible usefulness in the chemotherapy of viral infections. Isatins showing definite antiviral activity may be studied as under:

Indole -β-thiosemicarbazones and their derivatives

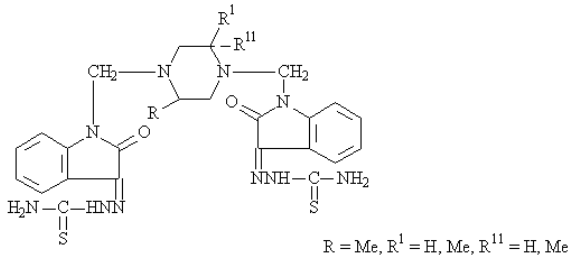
This has been reported by various workers. It had been found effective against rabbit pox, cowpox, alastrim and variola viruses.



R = H, Me, Et

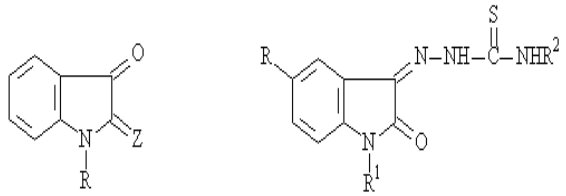
Bauer[13] was the first to investigate the antiviral chemotherapeutic activity of isatin-β-thiosemicarbazone in mice infected intracerebrally with vaccinia virus. It

was also known to have antiviral activity against certain other pox viruses.



Thiosemicarbazone derivatives inhibited the virus multiplication and thus showed their therapeutic importance. The compound (93, R = Me, R' = R" = H) had been found most effective in inhibiting vaccinia viruses.

Heinisch, Lothar et al.[14] reported the synthesis of N-alkyl, aryl or aralkyl substituted-isatins, 2-substituted isatins and 3-thiosemicarbazones etc were found to be virustats.

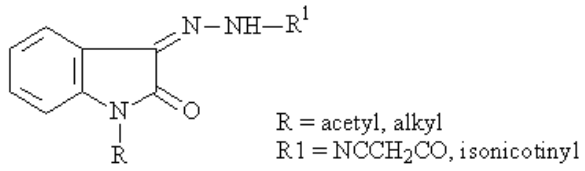


Miscellaneous Antiviral Indoles

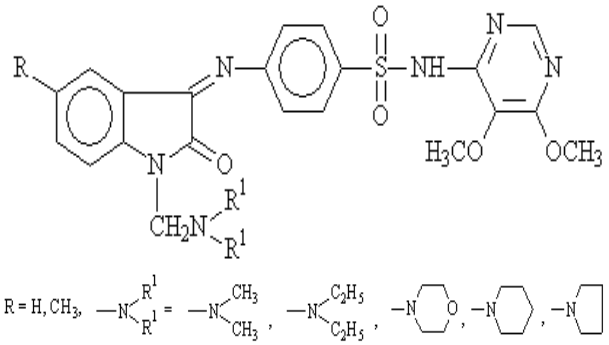
A number of different Indoleand its hydrazone derivatives had also been introduced as antiviral agents.

5-Nitroisatin showed its effectiveness for inhibition of vaccinia virus and naphthisatin formed by the ring fusion at 4, 5 or 5, 6 or 6; 7-position had also a little activity against poxvirus and entero virus infections.

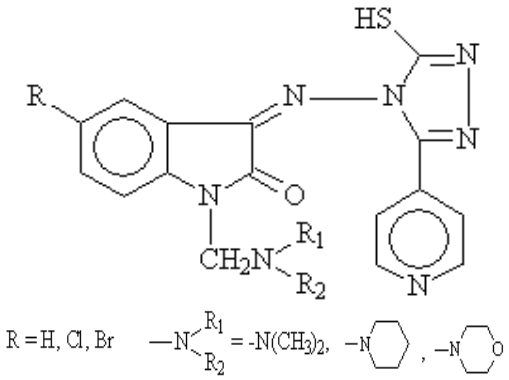
Following these reports Kontz et al.[13] prepared many hydrazone derivatives and studied them for their antiviral activity. Some of these derivatives (101) had been found responsible for their antiviral activity.



Pandeya et al.[15] reported Twelve new compounds of N-Mannich bases of isatin derivatives. They were synthesized and screened against HIV-1 (IIIB) and HIV-2 (ROD) strains in MT 4 cells. The compounds containingmorpholinomethyl group showed an appreciable activity up to 16% against HIV-2 (ROD) strain at an EC_{50} more than 2 µg/mol and about 12% against HIV-1 (IIIB) strain.

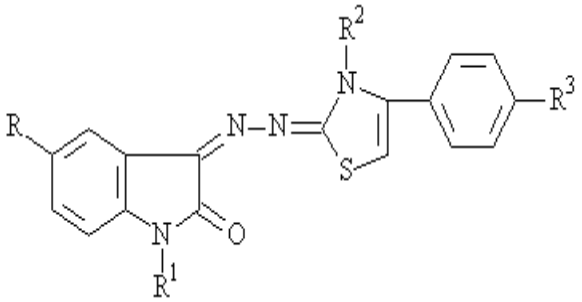


In the year 2000 the synthesis of 1-(substituted methyl)-5-bromo-3-(3'-(4''-pyridyl)-5'-mercapto-4'-(H)-1'2'4'-triazol-4'-yl] iminoisatins was reported by sriram.[16,7]



The anti-HIV and cytotoxicity results revealed that all the compounds were inactive against the replication of HIV-1 (IIIB) at subtonic concentration in MT-4 cells.

Omar et al.[16] reported the synthesis of isatinthiosemicarbazones in 98-100% yield by treating the corresponding isatin with appropriate thiosemicarbazides. These compounds were cyclized with substituted phenacyl bromide to give the thiazoline derivatives in 62-97% yields.



The study of structure activity relationships of isatinβ-thiosemi-carbazone derivatives may be summed-up briefly as follows.

The substitution at position-1 in the compound(R = H) by Cl, F, Br and I atoms resulted in diminished activity of the compound. The substitution by ethyl group at position 1 in had been reported which was responsible for the highest antiviral activity. The activity had been

shown to be reduced to one half by the removal of 1-methyl group and reduced still further by the substitution at the position-5.

The antiviral activity in the order of amyl <pr< -CH_2OH < iso-Pr< Ac < Me < 2-hydroxyethyl < Et- substituent at position-1 had been reported by Sadler

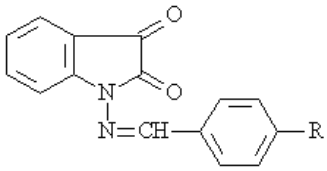
Introduction of substituent's into compound at positions 2 or 4 and replacement of S by O atom virtually abolished the original activity against vaccinia virus and substituents at position 4 and 7 have diminished the activity.

Substitution at positions 5 and 6 in compound 88 (R = H and R^1 = 5-OMe) and (R = H, R^1 = 6-Br) also decreased the virustatic activity. The cyanomethyl or carboxymethyl function at position-1 also abolished complete activity Thus the original activity abolished by the replacement of S by O atom indicated that =NNHC(S)-NH_2 group was essential for the antiviral activity and substitution of H-atom at position-1 by methyl or ethyl group resulted in the enhancement of activity but the activity seems to be decreased in the presence of the large size of any other function at position-1.

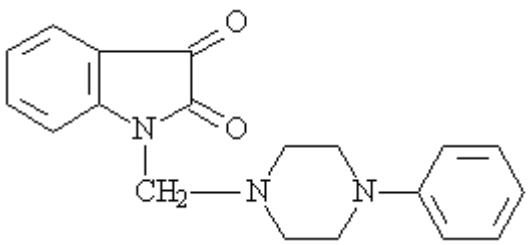
The addition of dialkyl group to the terminal N-atom of isatin-β-thiosemicarbazone moiety conferred a wide antiviral spectrum, embracing both DNA and RNA containing animal viruses. The basis for the extended range of activity appears to be the dialkyl substituted compounds which inhibited both cellular and viral DNA synthesis as well as viral RNA dependent synthesis.

Indole As Antihypertensive Agents

Behnisch et al.[17] Many derivatives of isatins were prepared and tested for their hypotensive activity. Compounds (R = Cl, OMe) were found to possess hypotensive activity.

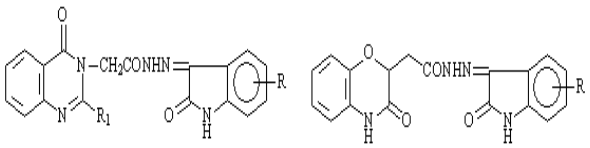


A number of N-substituted isatins were also prepared and tested. One of these compounds lowered the blood pressure in anaesthetized rats and thus indicated as a hypotensive agent.

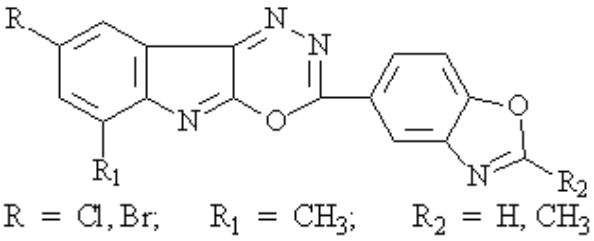


Isatins as Psychotropic Agents

From our laboratory Sarangapani et al.[18] reported the synthesis of new isatinhydrazones Some of them were moderately potent, while few of the compounds were found to have good potentiation of pentobarbitone induced narcosis.



Sarangapani et al.[19] reported the eight new 2-substituted-[1,3,4]oxadiazino-[6,5-b]indoles. All the compounds exhibited reduction in Locomotor activity and potentiation of pentobarbitone sodium induced sleeping time in experimental animals.



AIM

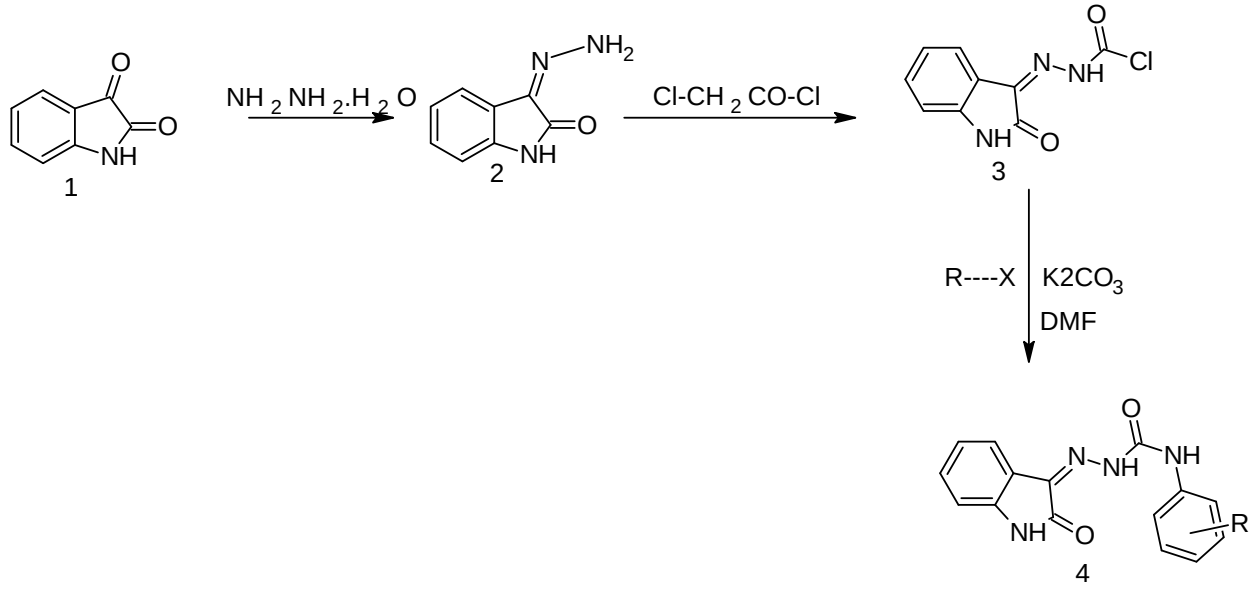
The aim of the present work is to synthesis and evaluate the biological activities of indole derivatives.
The objective is to ensure the satisfactory action of derivatives of indole and check for their better response to a stimuli when compared to a standard drug.

The derivatives were dissolved in ethanol and hexane for the test solution.
Effects of drug solution was observed and studied.

OBJECTIVE

Synthesis, purification and evaluation of drug.
Purification of drug using column chromatography.
Action of the drugs for antimicrobial, antihelmentic activities.

EXPERIMENTAL WORK

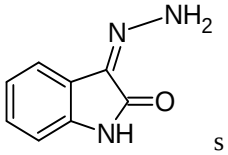


SCHEME

PROCEDURE FOR SYNTHESIS

Step 1: Synthesis of(3*Z*)-3-hydrazinylidene-1,3-dihydro-2*H*-indol-2-one:(2)

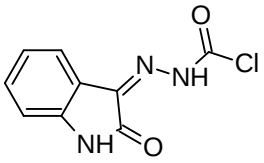
Isatin is refluxed with Hydrazine hydrate in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction was monitored by TLC. After completion of reaction, the product was filtered and recrystallized from hot ethanol



s

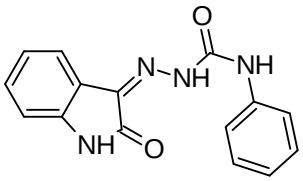
Step 2: Synthesis of(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carbonyl chloride:(3)

The product 3 was refluxed with Choloro acetyl choride in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction was monitored by TLC. After completion of the reaction the product was filtered and recrystallized from hot ethanol.



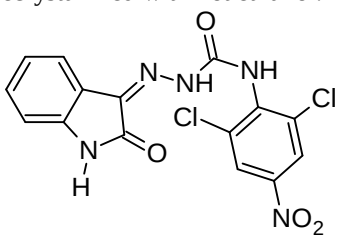
Step 3: Synthesis of(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenylhydrazine-1-carboxamide:(4)

The product 4 is treated with aniline, in basic condition in presence of ethanol at 80 degree centigrade for 2 to 4 hours**.** The reaction is monitored by TLC. After completion of reaction, the product was filtered and recrystallized with hot ethanol.



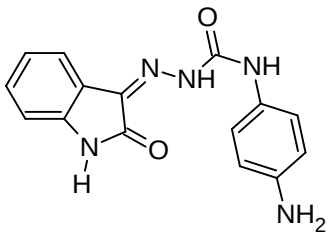
Synthesis of(2*Z*)-*N*-(2,6-dichloro-4-nitrophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide:(4a)

The product 4 is treated with 2, 6 –Dichloro-4-nitroaniline in the presence of ethanol at 80 degree centigrade for 2 to 4 hours**.** The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



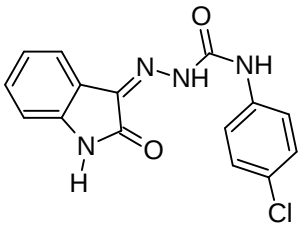
Synthesis of(2*Z*)-*N*-(4-aminophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide: (4b)

The product 4 is treated with *p*-phenylene diamine in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



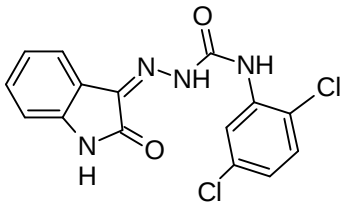
Synthesis of(2*Z*)-*N*-(4-chlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide: (4c)

The product 4 is treated with P- Chloro aniline in the presence of ethanol at 80 degree centigrade for 2 to 4 hours.The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



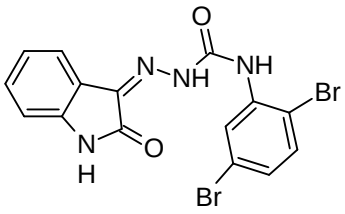
Synthesis of(2*Z*)-*N*-(2,5-dichlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide: (4d)

The product 4 is treated with 2,5 dichloroaniline in the presence of ethanol at 80 degree centigrade for 2 to 4 hoursThe reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



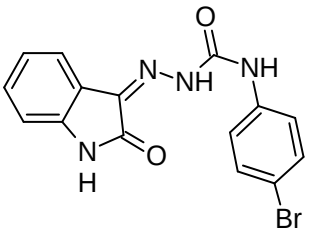
Synthesis of(2*Z*)-*N*-(2,5-dibromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide: (4e)

The product 4 is treated with 2,5 dibromo aniline in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



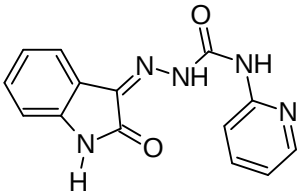
Synthesis of(2*Z*)-*N*-(4-bromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide: (4f)

The product 4 is treated with Bromoaniline) in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



Synthesis of(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-(pyridin-2-yl)hydrazine-1-carboxamide:(4g)

The product 4 is treated with Amino pyridine in the presence of ethanol at 80 degree centigrade for 2 to 4 hours. The reaction is monitored by TLC. After completion of reaction the product was filtered and recrystallized with hot ethanol.



Physical Data

S.No	Substituents	Molecular formula	Molecular weight	Melting point°(c)	Colour
1	Aniline	$C_{15}H_{12}N_4O_2$	280	169.1	Slight yellow
2	2,6- dichloro-4-nitro aniline	$C_{15}H_9CL_2N_5O_4$	394	166.6	Yellow
3	P-Phenylene diamine	$C_{15}H_{13}N_5O$	295	215.1	Crismon brown
4	P-Chloroaniline	$C_{12}H_8CL_2N_4O$	314	170.7	White
5	2,5 dichloro aniline	$C_{15}H_{10}Br_2N_4O$	349	235.3	Creamish white
6	2,5dibromo aniline	$C_{15}H_{11}BrN_4O$	438	215.3	Orangish yellow
7	p-Bromo aniline	$C_{15}H_{11}BrN_4O$	359	190.1	Brown liquid
8	Aminopyridine	$C_{14}H_{11}N_5O_2$	281	209.1	Slight grey

INTRODUCTION TO COLUMN CHROMATOGRAPHY

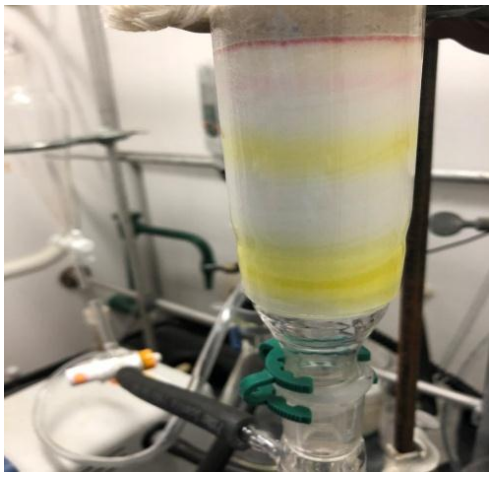
Matteucci first described the column use in the determination of the solutes in the solvents. Kuhn and Lederer explained the column chromatography theory by observing the separation of the polyene pigments.

PRINCIPLE: The main principle involved in column chromatography is adsorption of the solutes of a solution through a stationary phase and separates the mixture into individual components. This is based on the affinity towards the mobile phase and stationary phase. The molecules which are more affine towards the stationary phase elute later and which are less affine towards the stationary phase elutes first.

PREPARATION OF DRY COLUMN

Preparation of solvent: Using 10% of ethanol.
Stationary phase: Silica gel

- Place the column in a ring stand in a vertical position.
- A plug of glass wool is pushed down to the bottom of the column.
- Fill the column with solvent.
- Using a funnel, sprinkle dry stationary phase into the solvent, allow solvent to drain.
- Let the stationary phase settle and gently tap the column.
- Drain the excess solvent.
- The column is now packed.
- The resultant product was further taken for NMR spectroscopy
- The products purified from the column were further taken for TLC.
- Silica gel was stationary phase, mobile phase was n-ethylene/hexane.
- Further the RF values were calculated using UV radiation.



BIOLOGICAL ACTIVITY

Antimicrobial Activity

The microbiological assay performed with microorganisms like bacteria, yeast fungi etc. Microbiological assay is based upon accomparision of inhibition of growth of micro organisms by measured concentrations of compound to be examined with that produced by known concentrations of standard preparation of the of antibiotic having a known activity.

All the synthesized compounds were screened for their antimicrobial activity as for the reported methods on fallowing strains. The clinical samples of the fallowing strains are procured from national chemical laboratory, Pune are used for the evaluation of the compounds.

EVALUATION TECHNIQUES

Anti microbial studies can be evaluated by the fallowing techniques

1. Agar streak dilution method
2. Serial dilution Method
3. Agar diffution method

a. Cup plate method
b. Cylinder method
c. Paper disc method

In the present study serial tube dilution technique, cuppiate method was usead to evaluate anti bacterial, anti fungal activity of synthesisized compound in vitro.

ANTI BACTERIAL ACTIVITY

Anti bacterial activitywas tested by determining the minimum inhibitory concentration (MIC) for each compound using serial tube dilution technique. The following organisms were used in this technique.

Gram- positive bacteria
Staphlococus aureus
Bacillius subtilis

Gram –negative bacteria
Escherichia coli
Proteus vulgaris
The antibacterial activity of title compounds was assayed against different strains of bacteria by agar diffusion method.

Two Gram-positive bacteria: Bacillus subtilis and Staphylococcus aureus

Two Gram –negative bacteria: Escherichia coli and Proteus vulgaris

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar.

The bacterial inhibition can be measured by two methods:
1. Serial dilution method
2. Diffusion method
The serial dilution method is very useful for the determination of the microbial activity.

It is not much useful for the quantitative detection tests for the evaluation of large number of compounds.

The agar diffusion is of three types:
Cup-plate method (disc method)
Filter-paper strip method
Gradient-plate method

The method adopted in this investigation was cup plate method.in this method, cups are discs of standard diameter are made inth nutrient agar medium, containing standard inoculams.

The test compounds were introduced into the discs and diameter of zone of inhibition was measured.

CULTURED MEDIUM
Nutrient broth used for preparation of inoculums of the bacteria and the nutrient agar used for the screening method.

Composition of medium, nutrient agar

Peptone	5.0 gm
Sodium chloride	5.0 gm
Beef extract	1.5 gm
Yeast extracts	1.5 gm
Agar	1.5 gm
Distilled water	1000 ml
PH	7.42±0.2

The test organism was sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 ±1°C for 24 hours, they were stored in refrigerator. The stock culture to nutrient broth.

The flasks were incubated at 37 ± 1°C for 48 hours before the experimentation. Solution of test compounds was prepared by dissolving 10 mg each in dimethylsulfoxide (DMSO, 10ml).

A reference standard for Gram- positive and Gram – negative bacterfoia was made by dissolving accurately weighed quantities of Streptomycin in DMSO (10µg/ml).

The nutrient agar medium was sterilized by autoclaving at 121°C(15lb/sq. Inch) for 15 minutes. Petri-plates tubes and flasks plugged in cotton were sterilized in hot air oven at 160°C for an hour.

Into each sterilized petri plate (10cm diameter), about 27ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of inoculum into each plate) was transferred aseptically.

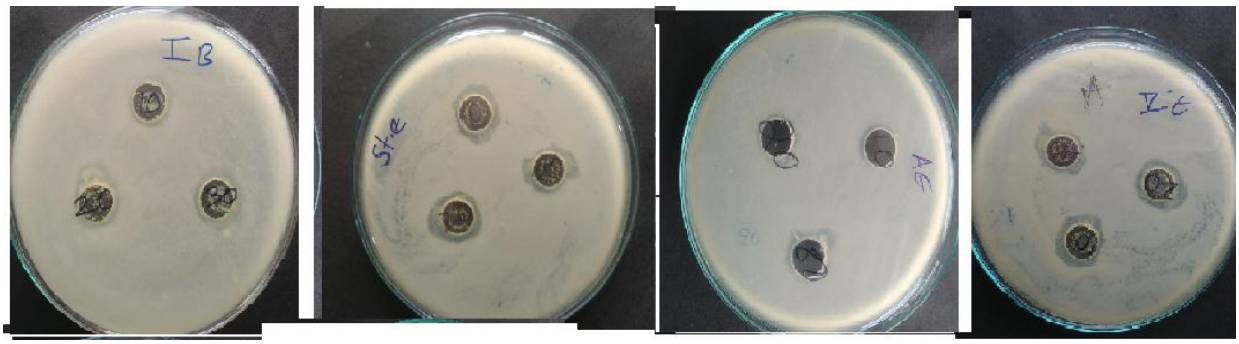
The plates were left at room temperature to allow solidification in each plate, 6mm diameter were made with a sterile borer.

These solutions at concentrations (150µg/ml 100µg/ml and 50µg/ml) was added to respective disc aseptically and labelled accordingly.

The plates were kept in undisturbed for 1 hr at room temperature to allow the diffusion of the solution properly in the nutrient agar medium.

After incubation of the plates at 37±1°C for 24 hours, the diameter of the zone inhibition surrounding each of discs was measured with of an antibiotic zone reader.

All the experiments were carried out in triplicate. simultaneously, controls were maintained employing 0.1 ml of DMSO to observe solvent effects. The results were represented in table.



Samples	Cultures	50µg/ml	100µg/ml	150µg/ml
Compound 4	Bacillus subtilis	0.1	0.2	0.1
	Escherichia coli	0.5	0.4	0.2
Compound 4a	Bacillus subtilis	0.1	0.4	0.1
	Escherichia coli	0.5	0.1	0.1
Compound 4b	Bacillus subtilis	0.2	0.1	0.2
	Escherichia coli	0.3	0.2	0.5
Compound 4c	Bacillus subtilis	0.1	0.2	0.1

	Escherichia coli	0.1	0.1	0.5
Compound 4d	Bacillus subtilis	0.4	0.2	0.2
	Escherichia coli	0.7	0.3	0.2
Compound 4e	Bacillus subtilis	0.3	0.1	0.2
	Escherichia coli	0.2	0.1	0.4
Compound 4f	Bacillus subtilis	0.1	0.2	0.6
	Escherichia coli	0.1	0.2	0.6
Compound 4g	Bacillus subtilis	0.2	0.1	0.1
	Escherichia coli	0.1	0.1	0.5
Standard (streptomycin)	Bacillus subtilis	4.1	5.0	4.6
	Escherichia coli	4.2	3.8	4.5

ANTI HELMENTIC ACTIVITY

Take earthworm into 3 groups
Each group consisting of 6 earthworms
Note the normal activity of these putting them in perti plate
Petriplate consist of standard, and test samples each
The earthworms are observed for paralysis time and the death time, compared to standard.

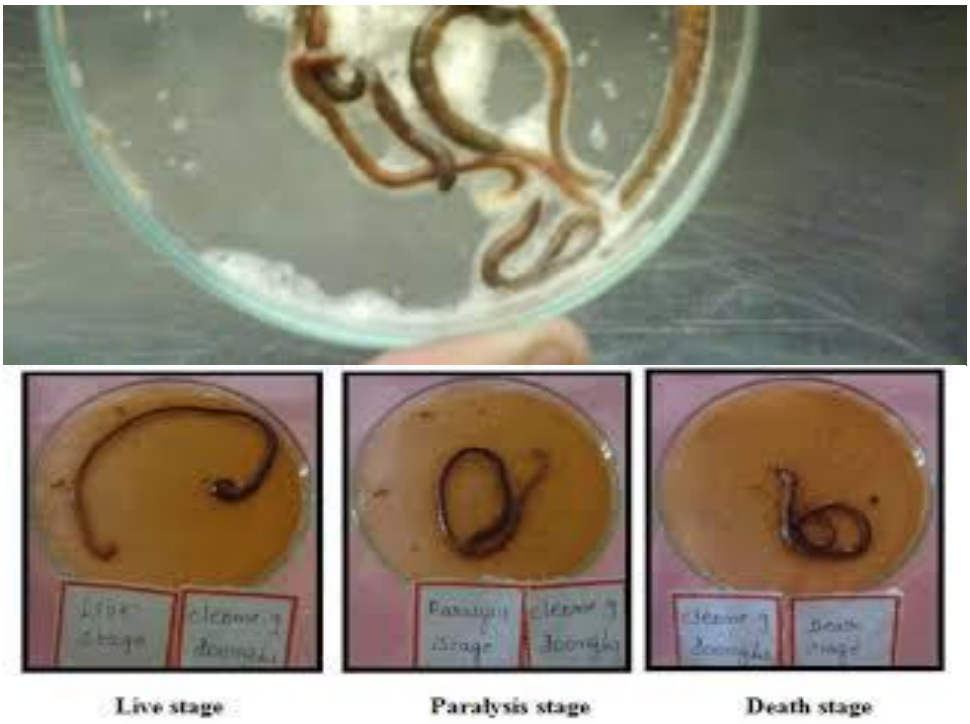
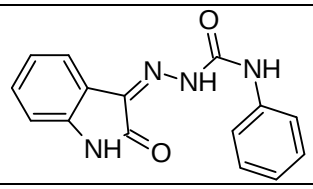
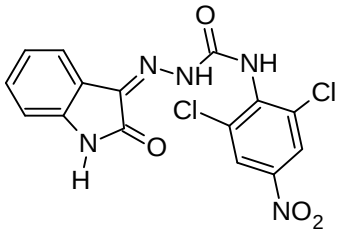
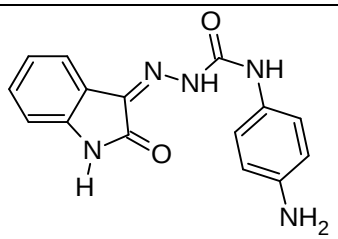
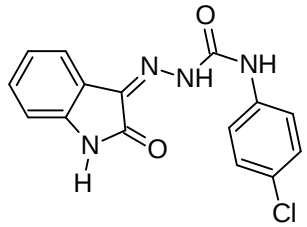
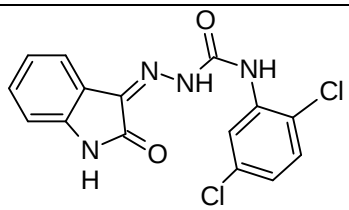
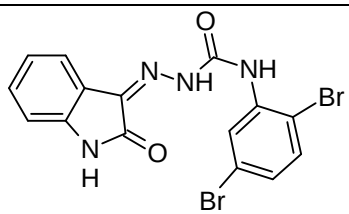
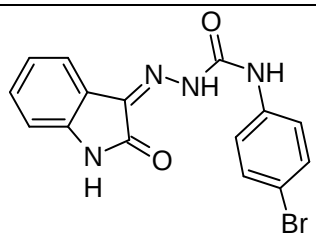
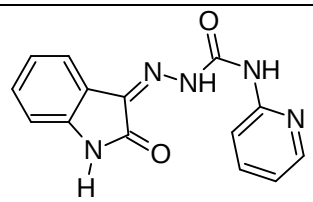


Figure 9: Anthelmintic activity of *Cleome gynandra* aqueous leaves extract (300 mg/ml)

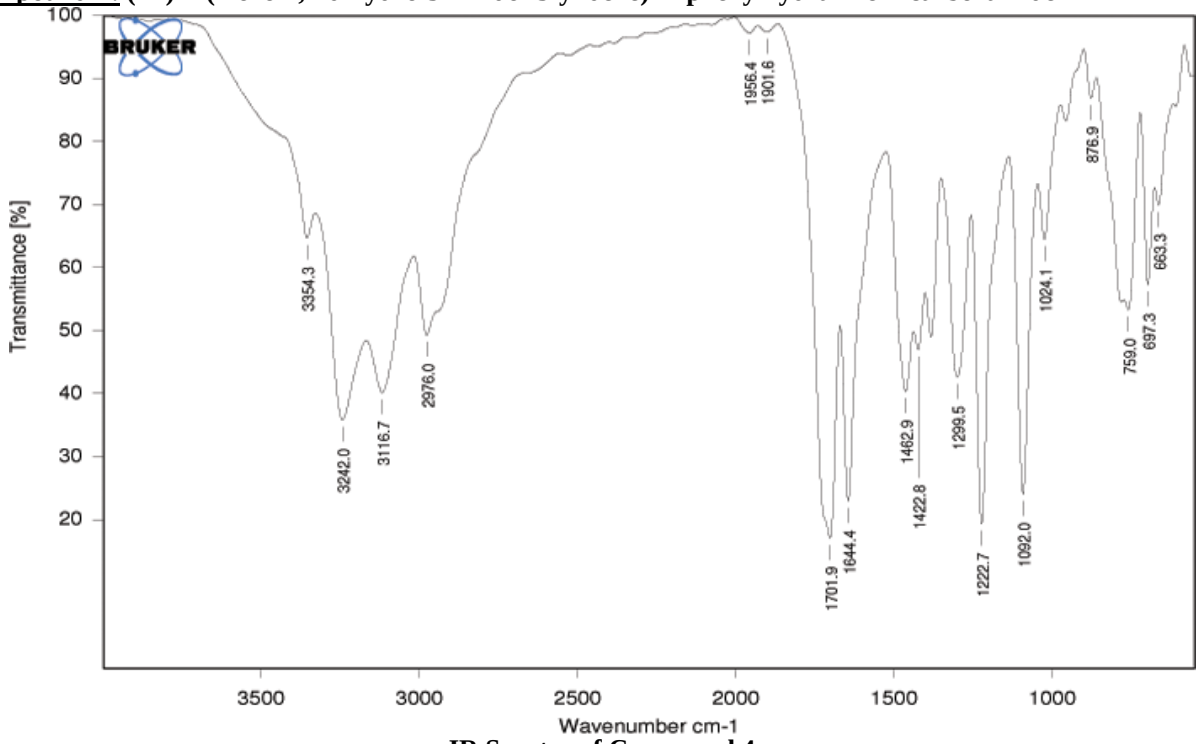
ANTI HELMENTIC ACTIVITY

Compound	Concentration	Time of paralysis(mins)	Time of death(mins)
Standard	In normal saline	3.28+_o.200	3.40+_0.338
Compound 4	in 10%ethanol	4.20+-0.346	431+_0.256
Compound 4a	In 10% ethanol	3.12+_0.098	5.56+_0.164
Compound 4b	In 10% ethanol	4.50+_0.240	4.75+_0.294
Compound 4c	In 10% ethanol	3.92+_0.092	4.16+_0.167
Compound 4d	In 10% ethanol	4.68+_0.356	4.85+_0.256
Compound 4e	In 10% ethanol	3.72+_0.254	3.96+_0.122
Compound 4f	In 10% ethanol	4.35+_0.147	4.77+_0.186
Compound 4g	In 10% ethanol	3.15+_0.351	3.36+_0.224

RESULTS AND DISCUSSIONS

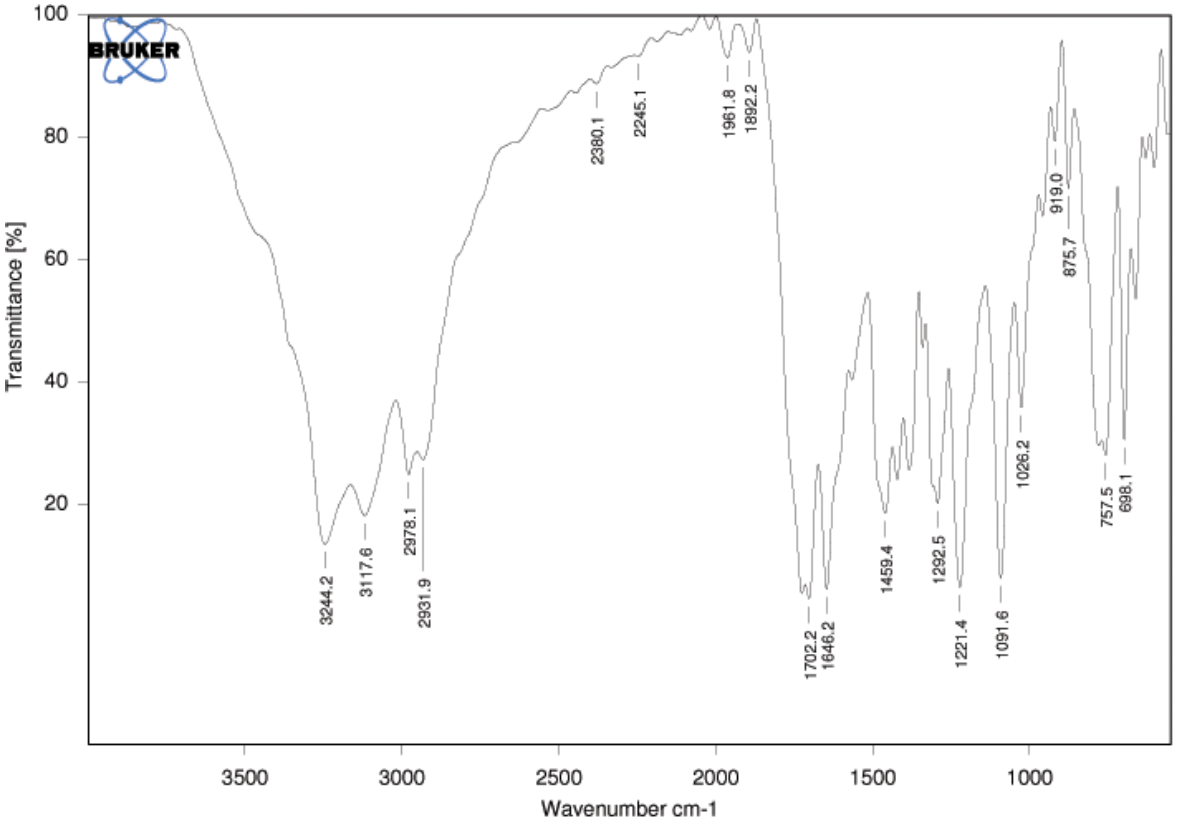
S.No	Compound	Structure	IUPAC Name
1	Compound 4		(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenylhydrazine-1-carboxamide
2	Compound 4a		(2*Z*)-*N*-(2,6-dichloro-4-nitrophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
3	Compound 4b		(2*Z*)-*N*-(4-aminophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
4	Compound 4c		(2*Z*)-*N*-(4-chlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
5	Compound 4d		(2*Z*)-*N*-(2,5-dichlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
6	Compound 4e		(2*Z*)-*N*-(2,5-dibromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
7	Compound 4f		(2*Z*)-*N*-(4-bromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide
8	Compound 4g		(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-(pyridin-2-yl)hydrazine-1-carboxamide

Compound-4: **(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenylhydrazine-1-carboxamide**



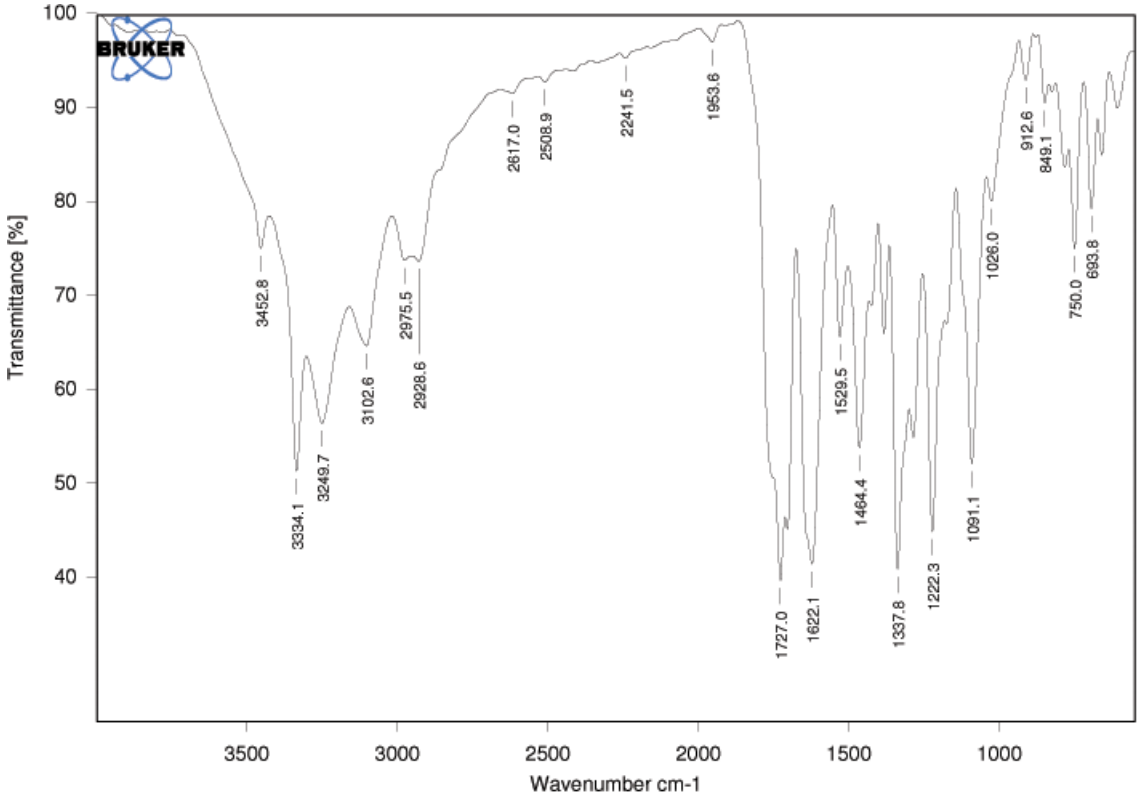
IR Spectra of Compound 4

Compound-4a: **(2*Z*)-*N*-(2,6-dichloro-4-nitrophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide**



IR Spectra of Compound 4a

Compound-4b: **(2*Z*)-*N*-(4-aminophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide 4b**



IR Spectra of Compound 4b

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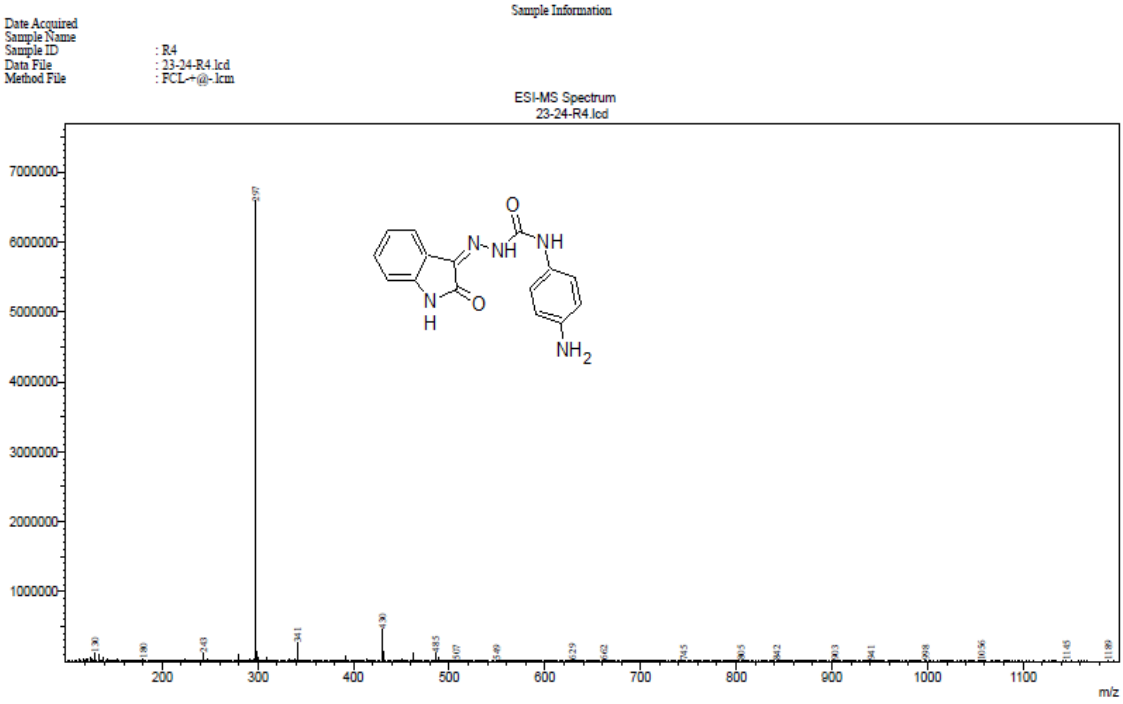
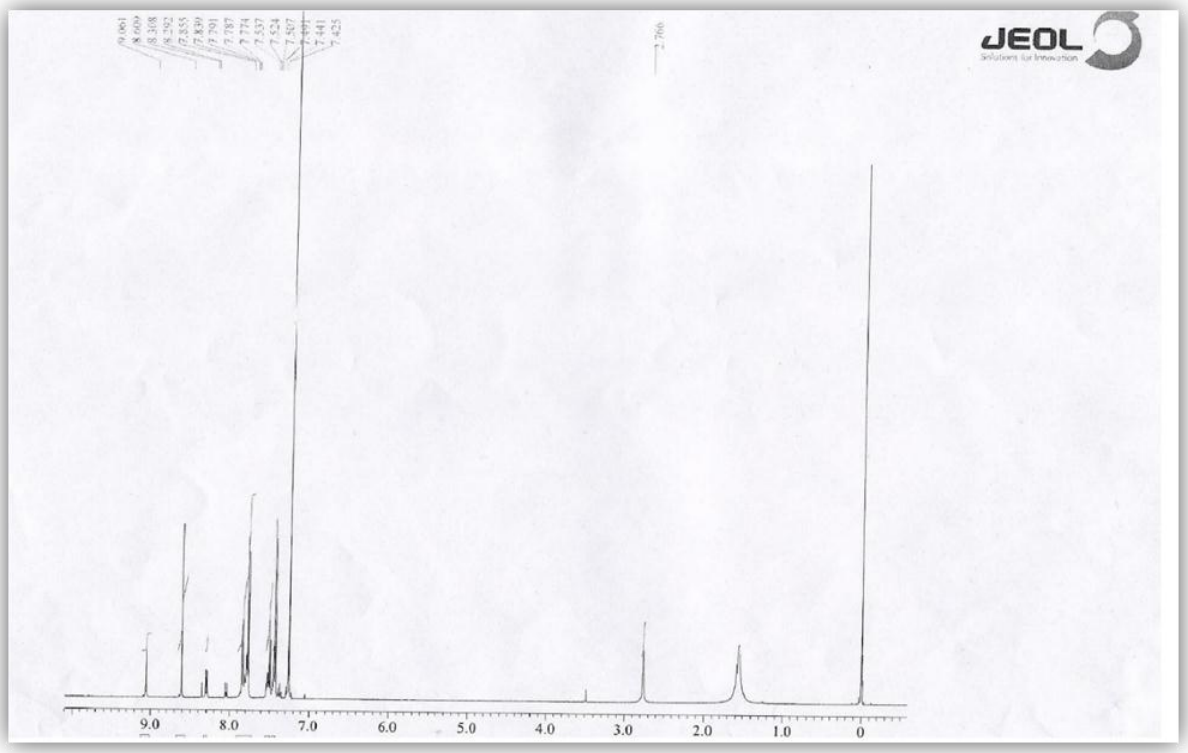
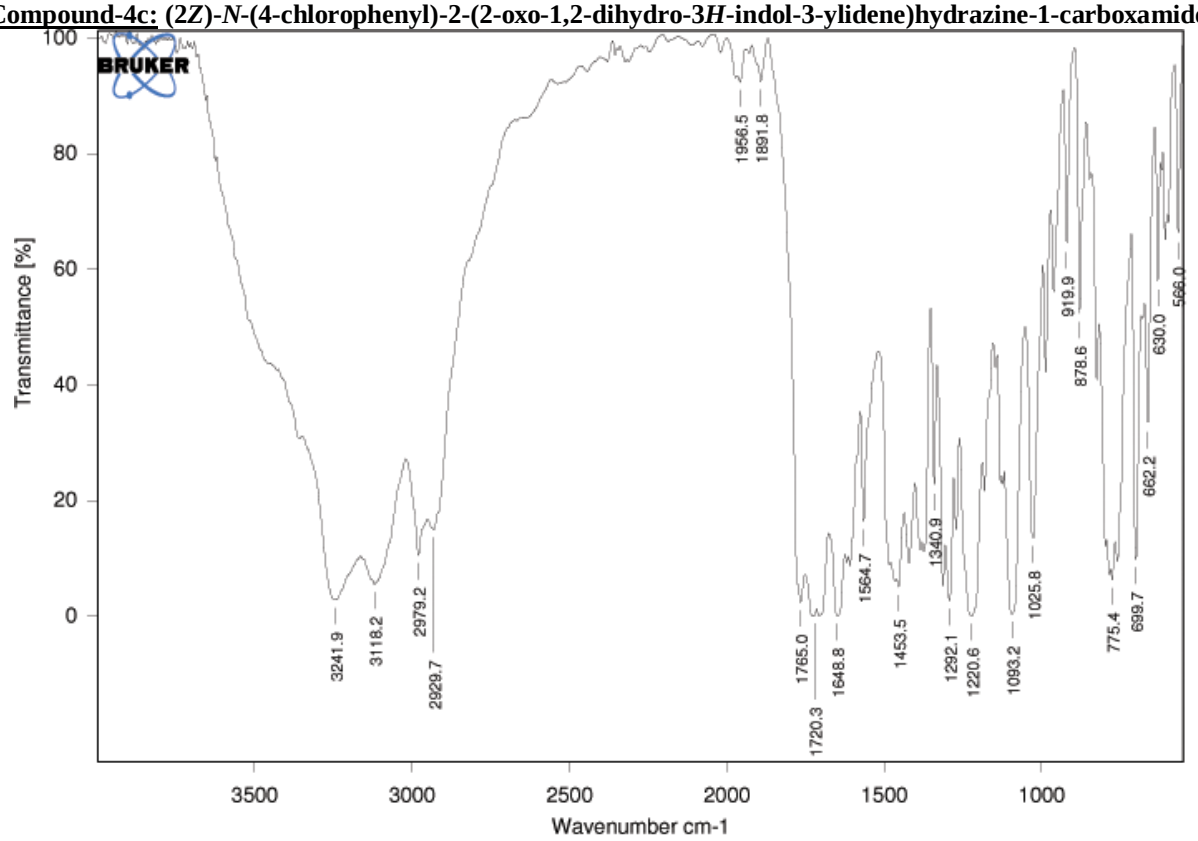


Mass Spectra of 4b



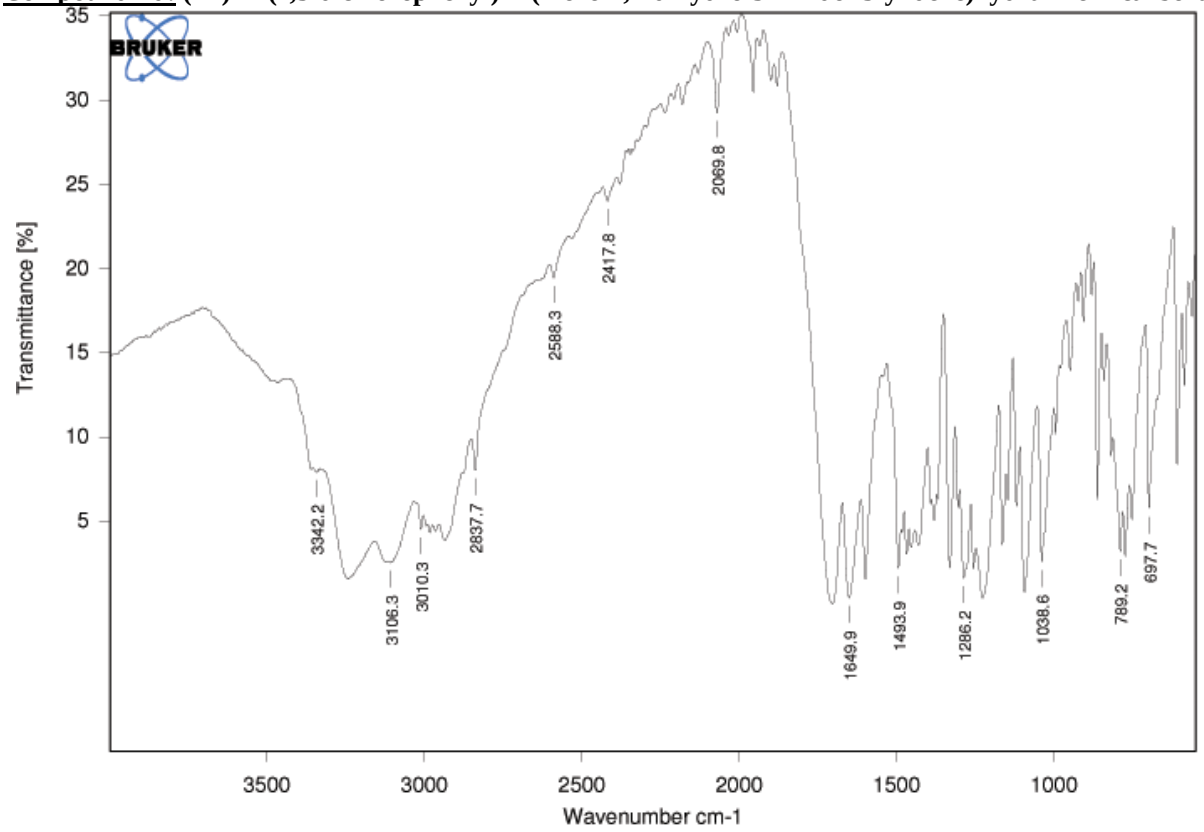
NMR Spectra of 4b

Compound-4c: (2*Z*)-*N*-(4-chlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide



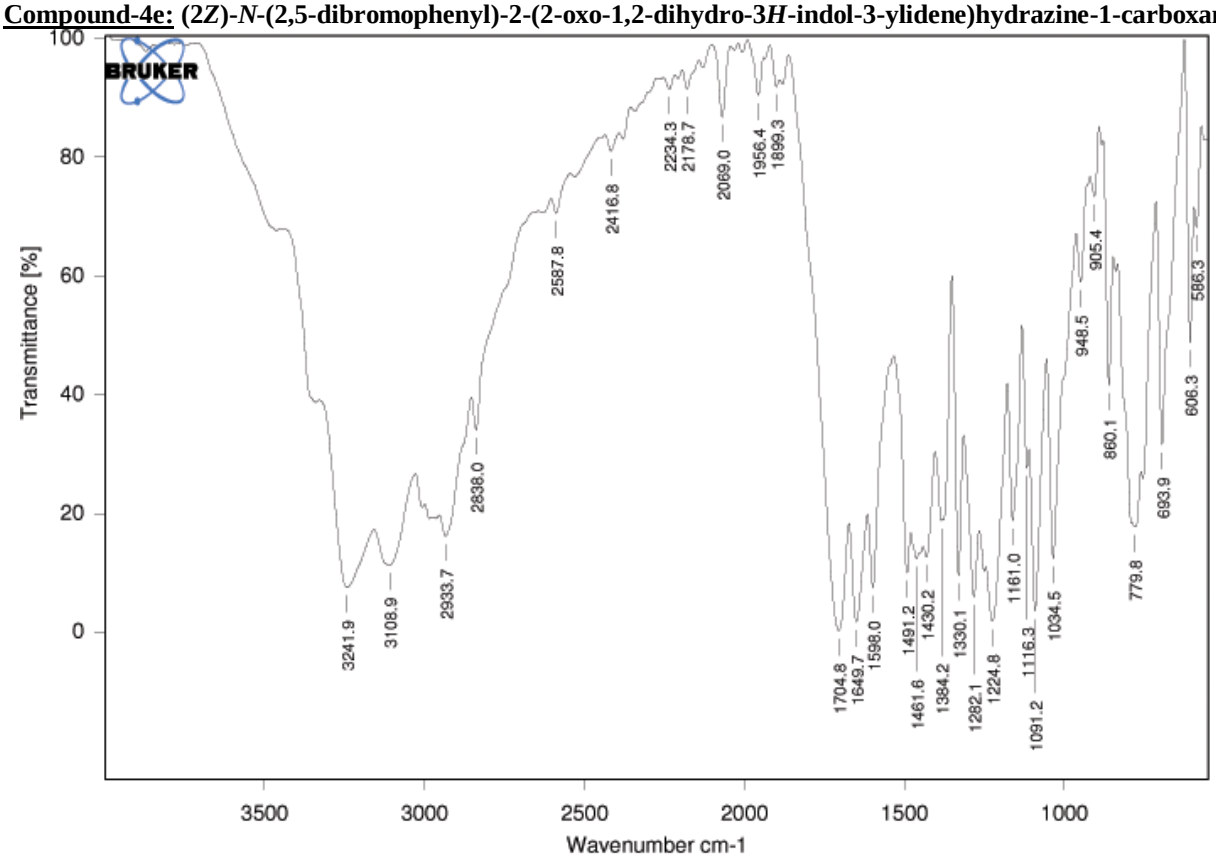
IR Spectra of Compound 4c

Compound-4d: (2*Z*)-*N*-(2,5-dichlorophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide



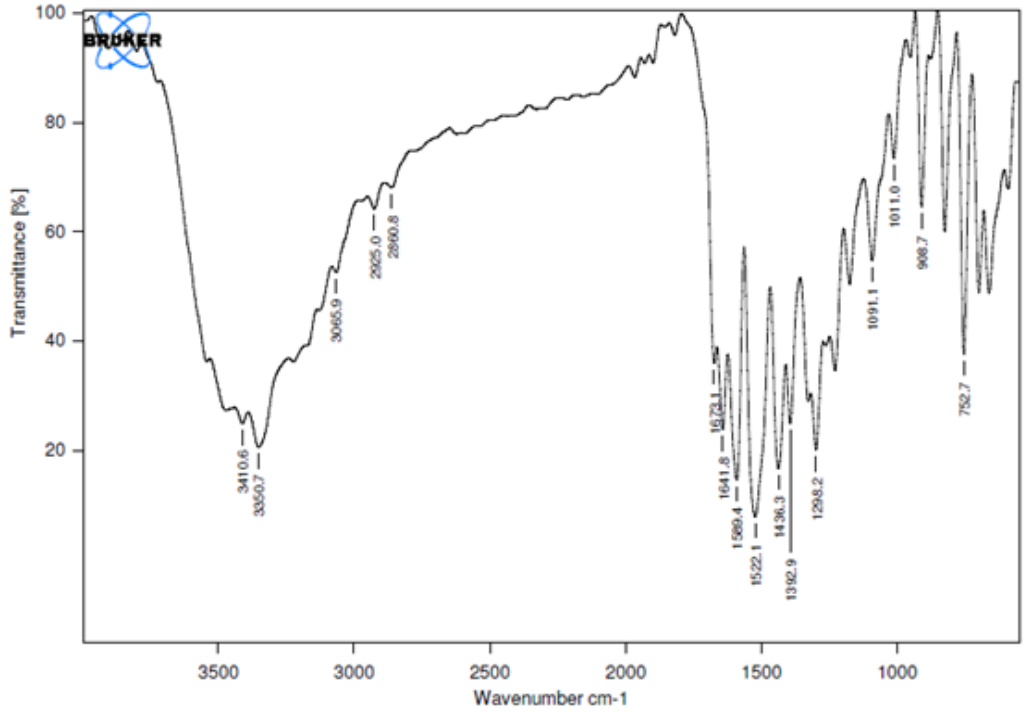
IR Spectra of Compound 4d

Compound-4e: (2*Z*)-*N*-(2,5-dibromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide



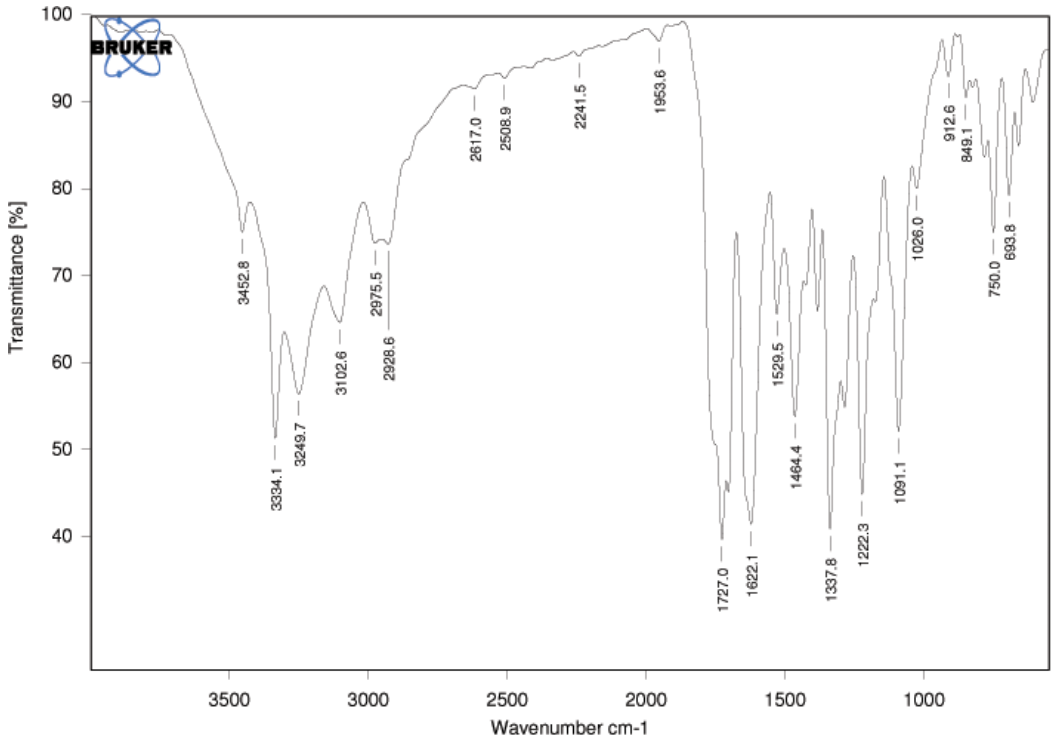
IR Spectra of Compound 4e

Compound-4f: **(2*Z*)-*N*-(4-bromophenyl)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazine-1-carboxamide**



IR Spectra of Compound 4f

Compound-4g: **(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-(pyridin-2-yl)hydrazine-1-carboxamide**



IR Spectra of Compound 4g

CONCLUSION

- A great deal of work has been done in order to synthesize indole derivative and evaluate anti helminthic activity.
- Among the synthesized derivative, compound 4 had shown good activity against E. Coli and Staphylococcus, compound 4b had shown good activity against bacillus subtilis and staphylococcus when compared with standard drug Albendazole.

- The indole derivative synthesized and tested in the study were shown to be reassuring importance for the development of the new drugs.

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