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Synthesis, characterization and biological evaluation of isatin based Schiff base derivatives and Thiazolidine -4-one derivatives

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ABSTRACT

Isatin (1H-indole-2,3-dione) and its derivatives belong to an important class of indole-fused heterocyclic compounds. Isatin serves as a versatile intermediate for the synthesis of a wide range of biologically active heterocycles. The present work provides a detailed account of the synthesis of isatin-based Schiff bases and their corresponding thiazolidine-4-one derivatives, which can be further utilized for the development of novel heterocyclic molecules. The isatin moiety exhibits a broad spectrum of biological and pharmacological activities, including antihypertensive, analgesic, anthelmintic, antitumor, antiviral, antifungal, anticonvulsant, anti-Parkinson, anti-HIV, anti tubercular, and antioxidant activities. In the present study, a series of isatin and Schiff base derivatives were synthesized. Initially, isatin was reacted with acetaldehyde in the presence of sodium hydroxide and ethanol to yield an aldol-condensed product at the C-2 position. This intermediate was further reacted with nitro-substituted benzene diamines in ethanol under basic conditions to form Schiff base derivatives. The synthesized Schiff bases were subsequently cyclized with thioglycolic acid to obtain thiazolidine-4-one derivatives. The progress and completion of the reactions were monitored by thin-layer chromatography (TLC). The synthesized compounds were evaluated for anthelmintic activity using earthworms as the experimental model. Structural characterization of the compounds was carried out using ^{1}H-NMR spectroscopy and mass spectrometry, which confirmed the proposed structures.

Keywords: Isatine derivatives, Thiazolidine dione, anti-helmentic, anti-tubercular

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INTRODUCTION

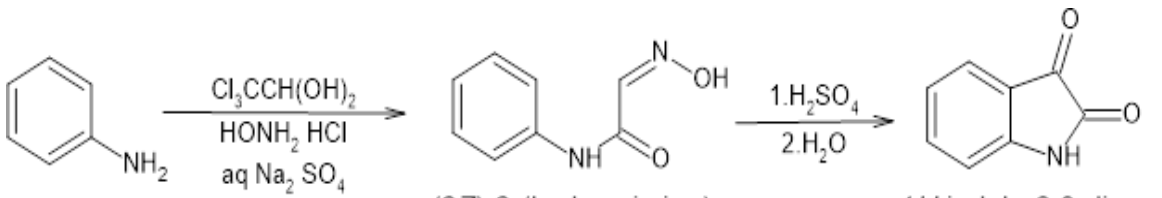
Isatin was first synthesized in 1840 by Erdmann and Laurent as an oxidation product of the indigo dye by nitric acid and chromic acid, which resulted in isatin's bright orange-colored monoclinic crystals product [1].Tribulin was the initial name given to Isatin [2]. Some plants, such as Corupita guianensis, Genus Isatin, Isatis tinctoria, and Calanthe discolor, contain this compound. It's also a part of the secretion of the parotid gland in Bufo frogs, and it's a metabolic derivative of adrenaline in humans. Melosatin alkaloids (methoxy phenyl pentyl isatins) derived from the Caribbean tumorigenic plant Melochia tomentosa are examples of substituted isatins present in plants [2]. Isatin derivatives are very effective against micro-organism especially, S aureus, S epidermis, Micrococcus luteus and B cereus. The Schiff base of isatin derivatives 5-substituted and N-acetyl isatin with different substituted aromatic aldehydes was assumed to be very operative against micro-organisms [3]. Isatin, also known as indole quinine and indenedione, is a biologically active compound with a wide range of properties. Isatin has two cyclic rings in its structure, one of which is six-membered (aromatic property) and the other is five-membered (antiaromatic character). Both rings lie in the same plane, a five-membered ring contains a nitrogen atom and two carbonyl groups [4-5]. Due to their immense biological profile, these heterocyclic moieties either alone or the fused form are continuously exploited by researchers for the development of the new drugs [5]. The isatin (1*H*-indole-2,3-dione) motif is ubiquitous in nature, and its derivatives also possess a variety of pharmacological properties [11-12]. Moreover, some isatin-containing compounds such as semaxanib, sunitinib and nintedanib have already used in clinical practice or under clinical trials for the treatment various diseases [13]. Thus, isatin derivatives represent important classes of bioactive heterocyclic compounds in the field of pharmaceuticals. Isatin and its analogs are synthetically useful substances where they may be utilized for the production of a broad range of heterocyclic molecules, which are depicting a wide reach of biological and pharmacological activities, as well as anticancer, anti-inflammatory, antiviral, anticonvulsant, anti-TB, antidiabetic, anti-microbial, antitumor, antimalarial, anti-HIV, antibacterial, anti-analgesic, and antiplasmodial activities. Isatin is a precursor for many synthesized therapeutic molecules that are amenable to pharmacological action and have excellent biological potential. Isatin has a magnificent scaffold for both the natural and synthetic construction of molecules. These molecules are being used in drug therapy such as anticancer, antibiotic, and antidepressant drugs and have many more clinical applications [11-13]. Furthermore, successfully synthesized Schiff bases of N-methyl and N-ace-tyl isatin derivatives in conjunction with various aryl amines. Their findings highlight the

heightened efficacy of N-methyl-5-bromo-3-(p-chloro-phenylimino) isatin compared to standard drugs such as phenytoin, carbamazepine, Isatin-3-phenylhydrazone Schiff base is an excellent chelating agent due to their ease of synthesis, flexibility for synthesis and characteristic effect of –C=N (azomethine) moiety, the ability to form chelates with various metal ions and also can attune the ligation aspects by varying denticity and basicity [13]. Moreover, isatin was chosen as a biologically active frame for coupling because it is thought to be a sophisticated spectrum of heterocyclic moieties with various pleasing activities and superior acceptance in humans. The major proteinase of SARS-CoV-1 and SARS-CoV2, 3C-like protease (3CLpro), has also been reported to be inhibited by isatin derivatives. The multi component reaction based on isatin is used to develop bioactive and drug like compounds [14]

MATERIALS AND METHOD

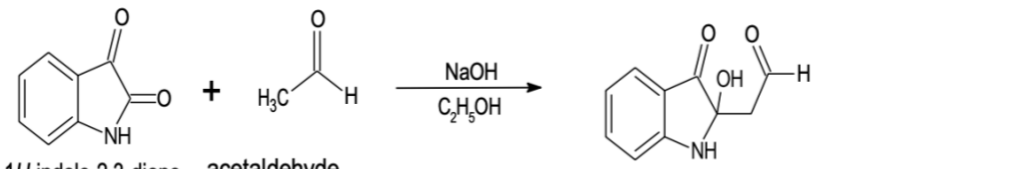
It involves the condensation of reaction of aniline with chloral hydrate and hydroxyl amine hydrochloride in aqueous sodium sulfide leads to formation of an isonitroacetimide and subsequent electrophilic cyclization of the later in the presence of strong acid such as concentration sulphuric acid to produce an isatin derivative. The Sandmeyer isatin synthesis is the name given to this reaction quinolones, acridines, indophenazines have all been made from the isatin derivatives produced by this reaction. In the field of heterocyclic and pharmaceutical chemistry isatin are useful intermediates. Isatin is a well-known pharmacophore that can be found in wide range of synthetic and natural products and it has number of significant pharmacological and material-like properties. Isatin have a long been regarded as important synthetic intermediates in the manufactures of pharmaceuticals and dyes

The synthesis of acetyl heterocyclic isatin derivatives was carried out in one step reaction.



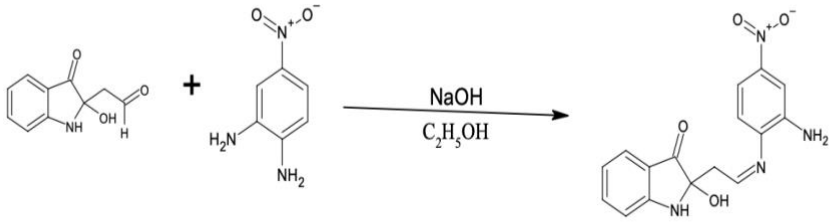
aniline (2Z)-2-(hydroxyimino)-*N*-phenylacetamide 1*H*-indole-2,3-dione

In first step acetaldehyde 5moles (0.05) 2ml was mixed with isatin 1mole (0.01) 1.4713g in ethanol as solvent in the presence sodium carbonate as a catalyst and refluxed for 22-24 hours. The reaction completion checked by using thin layer chromatography. After completion of reaction the crude mixture was filtered and then washed with ethanol. The solid precipitate of pure product was obtained



1*H*-indole-2,3-dione acetaldehyde (2-hydroxy-3-oxo-2,3-dihydro-1*H*-indol-2-yl)acetaldehyde

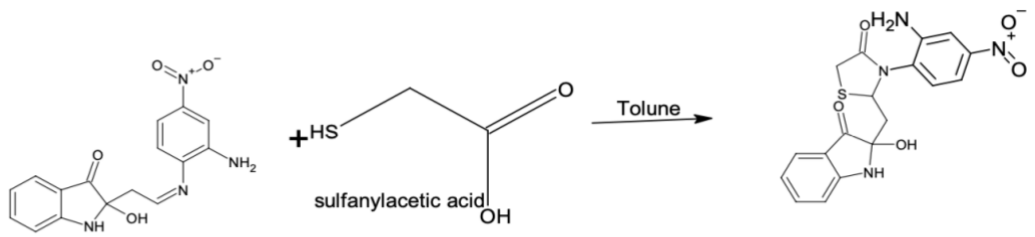
To a solution of 1.53g 4-nitro benzene, 1,2diamine (0.1mole) in 30ml of ethanol, a solution of1.77 grams of aldol condensation product (0.1 mole) in 20 ml of ethanol was added. A few drops of 10% NaOH were added to adjust the pH and the reaction mixture then refluxed with stirring for three hours and the obtained precipitate was collected by filtration through Buchner funnel, recrystallized from methanol, and dried at room temperature to afford yellow needles (yield 65%, melting point 192-194℃)



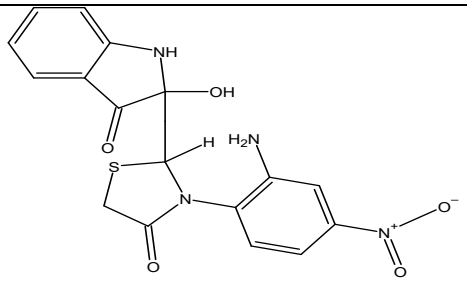
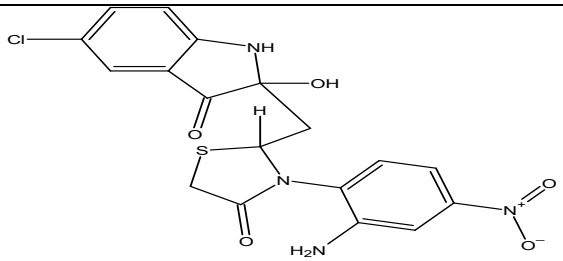
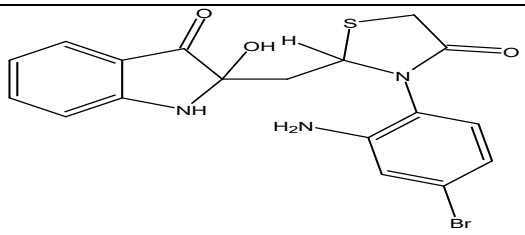
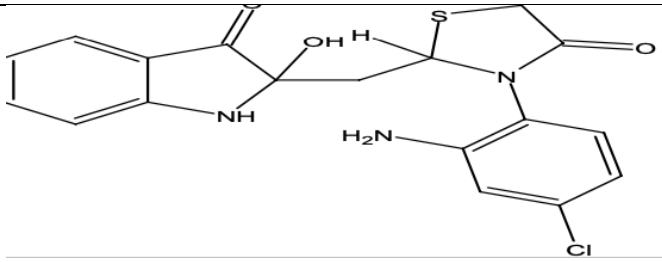
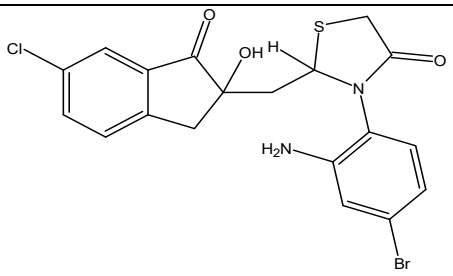
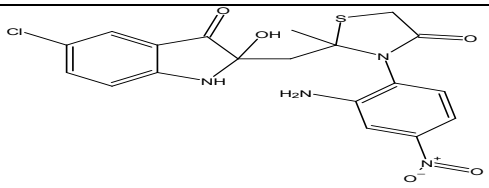
(2-hydroxy-3-oxo-2,3-dihydro-1*H*-indol-2-yl)acetaldehyde

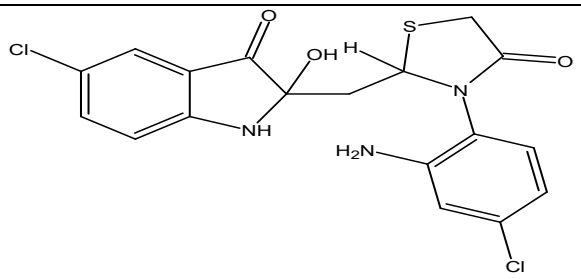
2-{(2Z)-2-[(2-amino-4-nitrophenyl)imino]ethyl}-2-hydroxy-1,2-dihydro-3*H*-indol-3-one

A solution of Schiff base (0.1 mole) in toluene(7ml) the resulting solution was refluxed for 12 hours. The progress of the reaction was monitored by TLC (eluent: n-hexane/ethyl acetate 1/1 v/v)). The mixture was washed with a 3% NaHCO3 solution (3*10 ml) and brine. The organic layer was dried over Na2SO4 then concentrated in vacuo. The product was re-crystallized in ethanol.



2-{(2Z)-2-[(2-amino-4-nitrophenyl)imino]ethyl}-2-hydroxy-1,2-dihydro-3*H*-indol-3-one

S.no	Compound	Activity
1		Anti-bacterial
2		Anti-Inflammatory
3		Anti-Cancer
4		Anti-Helmentic
5		Anti-Viral
6		Anti-Inflammatory

7		Anti-Inflammatory

Elemental Analysis

Molecular Formula	M.W	C	N	O	S	
$C_{18}H_{16}N_4O_5S$	400.408	53.99%	13.99%	19.98%	8.01%	
$C_{18}H_{15}clN_4O_5$ S	334.82	49.72%	12.88%	18.40%	7.37%	
$C_{18}H_{15}BrN3O_3$ S	434.45	49.78%	9.68%	11.05%	7.38%	
C18H16ClN3O3S	389.85	55.45%	10.78%	12.31%	8.2%	9.09% (Cl)
C19H16BrClN2O3S	467.76	48.79%	5.99%	10.26%	.85%	7.58% (Cl)
C19H17ClN4O5S	448.88	50.84%	12.48%	17.82%	7.14%	7.90% (Cl)
$C18H15Cl_2N3O3S$	421.81	50.95%	9.90%	11.31%	7.56%	16.71 % (Cl)

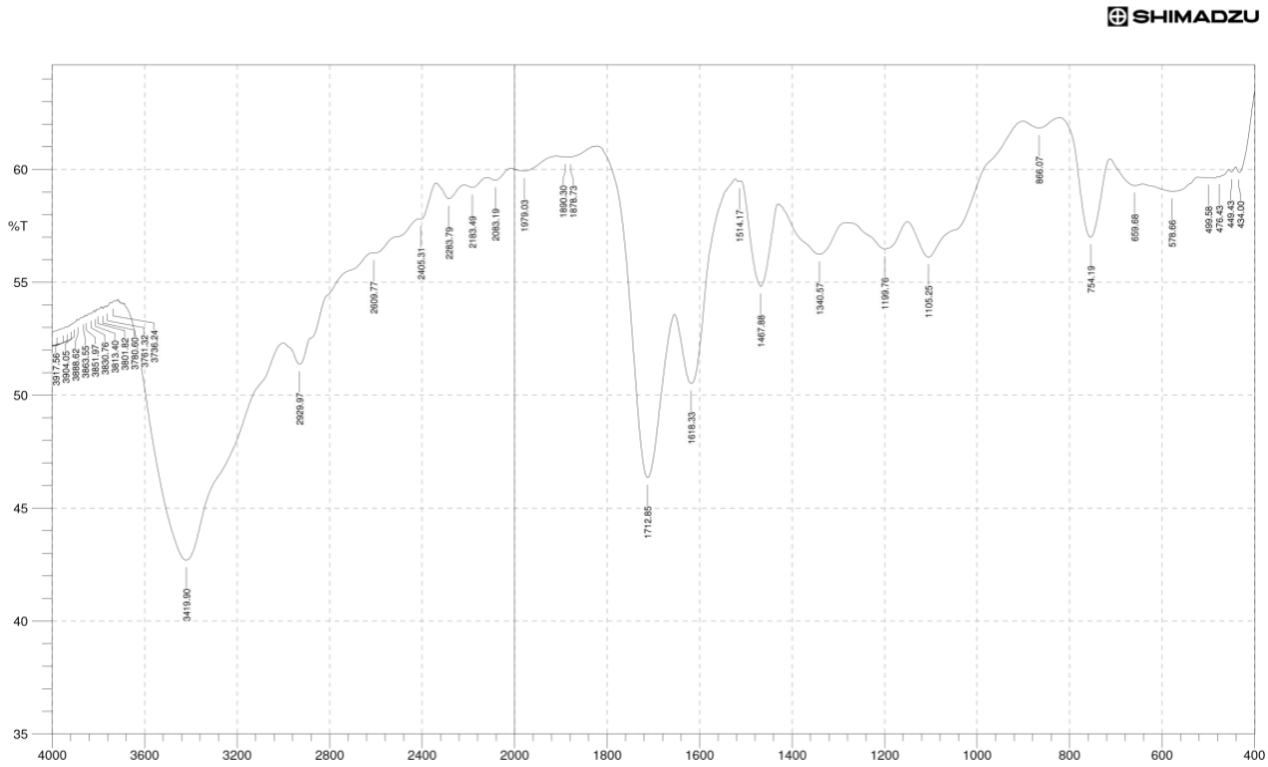
Melting Point:

S.No	Compound Code	Melting Point
1	IST-1	208-212°C
2	IST-2	209-210°C
3	IST-3	214-220°C
4	IST-4	214-218°C
5	IST-5	214-216°C
6	IST-6	214-216°C
7	IST-7	223-227°C

Solubility

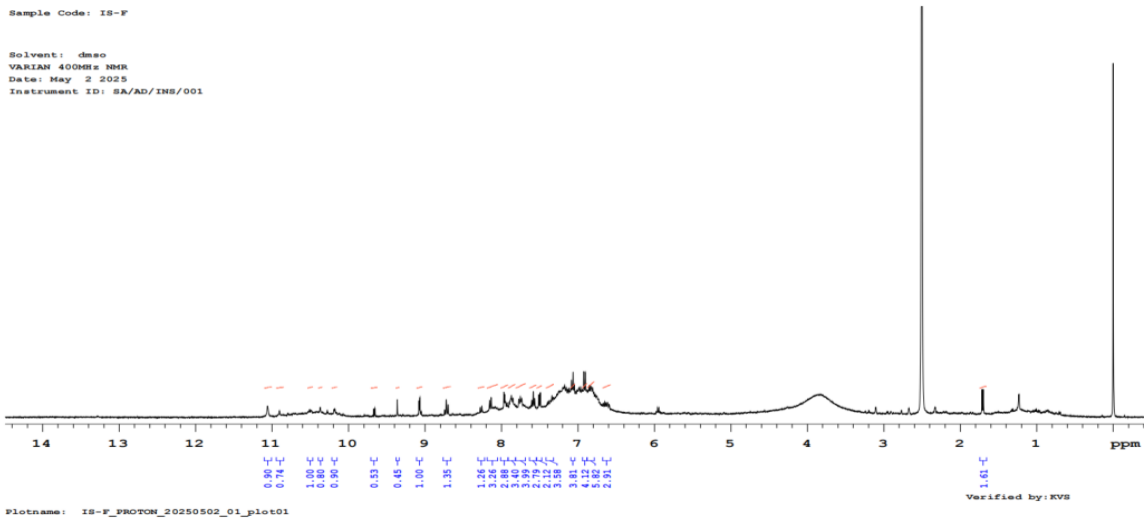
Solvent	Solubility
Ethanol	Soluble
Water	Insoluble
Sulphuric acid	Soluble
Hydrochloric acid	Soluble
Acetone	Insoluble
Chloroform	Soluble
Ether	Partially soluble

IR INTERPRETATION OF COMPOUNDS



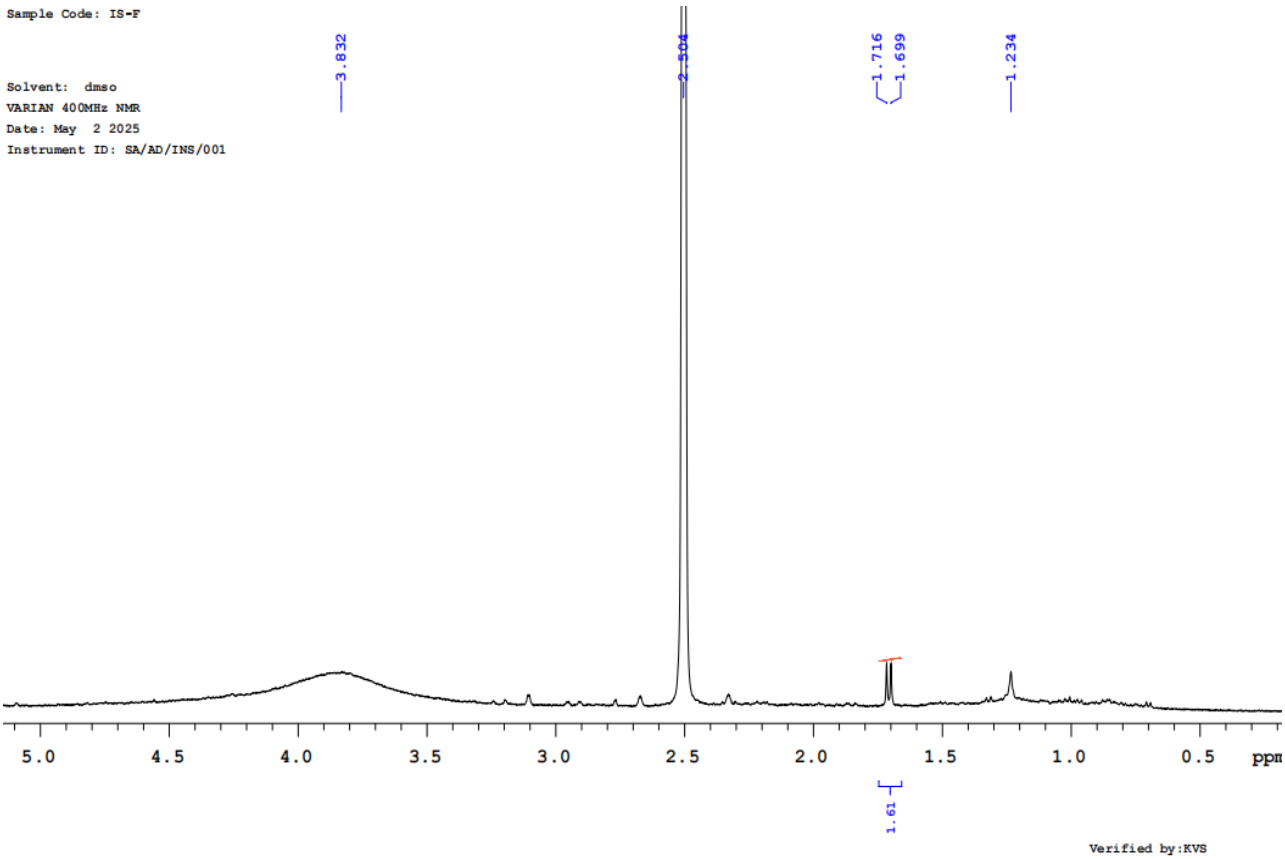
The IR spectrum (KBr, vmax, cm^{-1}) showed absorption bands at 3419 (N–H stretching), 2929 (sp^3 C–H stretching), 1712 (C=O stretching of ketone), 1618 (aromatic C=C stretching), 1340 (symmetric stretching of NO_2 group), 1105 (C–O stretching), and 754 cm^{-1} (aromatic C–H out-of-plane bending of mono substituted benzene ring, such as Benzene derivatives).

NMR SPECTRA



400 MHz ^1H NMR (DMSO-d_6),δ11.0 (NH or NH /OH),δ8.2–6.8 ppm(Ar–H-Substituted aromatic ring),**δ**6.4–6.6 ppm(heterocyclic thiazolidine CH),δ2.3ppm(–CH_3, attached to aromatic ring),δ8-9ppm(Imine moiety–N=CH).

MASS INTERPRETATION



400

MHz ^{1}H NMR (DMSO-d_6),δ3.8ppm (OCH_3, NCH_3, CH attached to O/N),δ1.7ppm(Aliphatic CH_2/CH_3),δ1.23ppm(terminal CH_3).

Thin Layer Chromatography (TLC):

Thin Layer Chromatography (TLC) is a useful analytical technique used to monitor the progress of an organic chemical reaction by observing the spots that represent reactants and products. By comparing these spots, it is possible to determine whether the reaction has been completed. The disappearance of the starting material spot and the appearance of a new product spot indicate the progress of the reaction.

Procedure

Preparation of the Initial TLC Plate:

A small amount of the reaction mixture is spotted onto the TLC plate using a capillary tube. Along with the reaction mixture, a separate spot of the pure starting material is also applied on the same plate for comparison.

Developing the TLC Plate: The spotted TLC plate is placed in a developing chamber containing a suitable solvent system (mobile phase). The solvent rises up the plate by capillary action and

separates the compounds based on their affinity for the stationary phase (silica gel) and the mobile phase (solvent mixture)**.**

Observing and Interpreting the Results

Reaction Complete: If the reaction is complete, the following observations are made:

The starting material spot disappears**,** indicating that it has been consumed during the reaction.

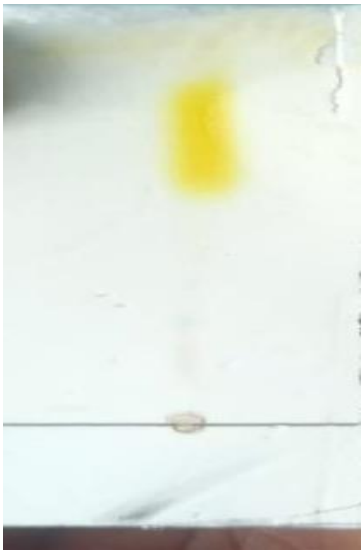
New spot(s) appear**,** corresponding to the product(s) formed.

Reaction Not Complete

If the reaction is not complete, the following observations are seen: **The** starting material spot is still visible**,** indicating that it is still present in the reaction mixture. Additional spot(s) corresponding to product(s) may appear, but the starting material spot remains prominent.

Preparation of Mobile Phase:

To prepare the mobile phase for TLC, accurately measure and mix the required solvents in the desired ratio. A 1:1:1 solvent system consisting of n-hexane, ethyl acetate, and acetone is prepared by mixing equal volumes of each solvent in the TLC developing chamber. The solvents are mixed thoroughly before placing the TLC plate inside the chamber.

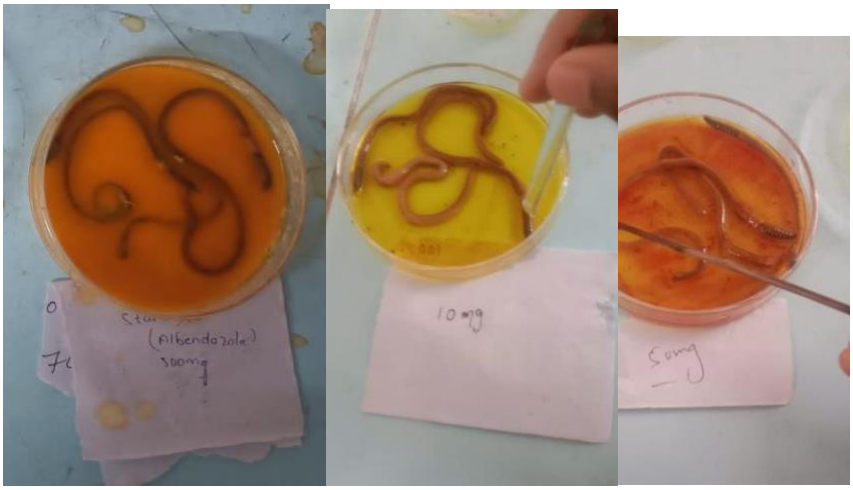


Reaction complete of Schiff base Derivatives by TLC.

Reaction complete of thiazolidine-4-one

Evaluation of Anthelmintic Activity Using Earthworms

The anthelmintic activity of the test drug was evaluated using earthworms as an experimental model. Earthworms of approximately 6 cm in length were selected for the study. The worms were placed in Petri dishes containing 2 mL of different concentrations of the test drug (10, 20, 30, and 50 mg/mL).A solution of Albendazole (500 mg/mL) was used as the reference standard, while normal saline (NaCl solution) served as the control. All Petri dishes were incubated at 37 °C. The worms were observed periodically for motility. After incubation, the contents of the Petri dishes were gently poured into a wash basin, and the worms were stimulated by tapping their posterior end with a glass rod and applying slight pressure. Worms showing movement were considered alive, whereas those showing no movement were considered dead. The motile worms were returned to their respective Petri dishes containing the drug solutions, and incubation was continued. In the control group, worms remained viable for at least 12 days, consistent with previously reported findings. The time taken for paralysis and death was recorded. Paralysis was confirmed when the worms did not show any movement unless shaken vigorously. Death was confirmed when the worms failed to move even after vigorous shaking or when dipped in warm water (50 °C).



Albendazole (standard) 10mg/ml conc 20mg/ml conc 50mg/ml conc.

Figure: Anthelmintic Activity of Synthesized Compounds on Earthworms

Anthelmintic activity of synthesized compounds at different concentrations compared with the standard drug Albendazole

(a) Standard drug (Albendazole, 500 mg/mL)

(b) Test compound – 10 mg/mL

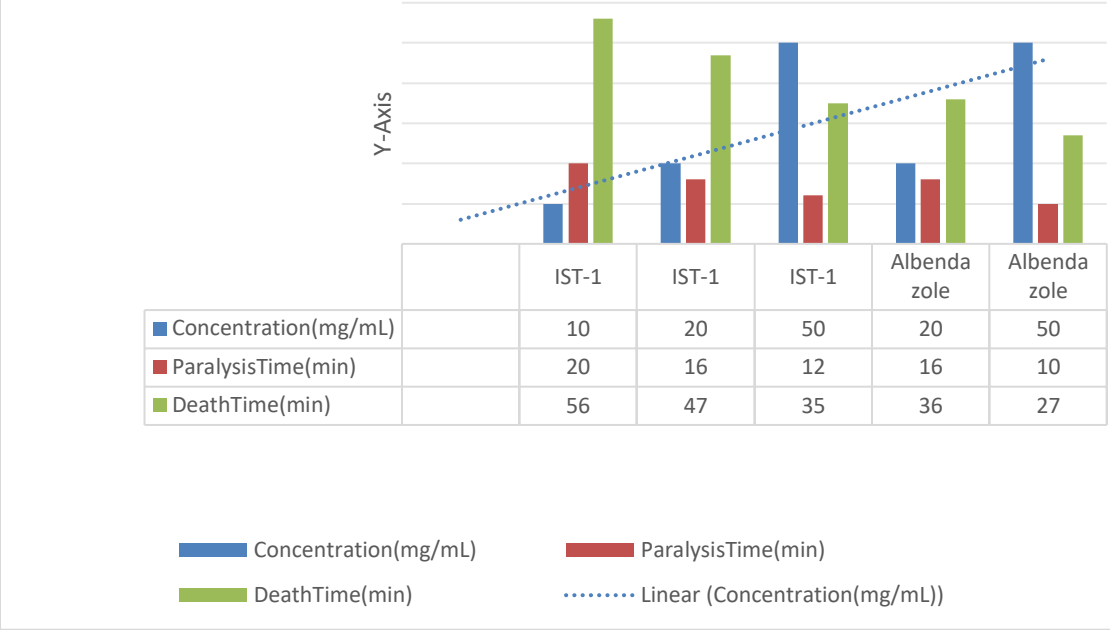
(c) Test compound – 20 mg/mL

(d) Test compound – 50 mg/mL

Earthworms (average length 6 cm) were exposed to different concentrations of the test compound and standard drug. The time required for paralysis and death was recorded. Increased concentration showed enhanced anthelmintic activity compared to lower concentrations. Comparative anthelmintic activity of synthesized compounds at varying concentrations against earthworms.

The synthesized compounds exhibited dose-dependent anthelmintic activity. At 50 mg/mL concentration, the test compound showed faster paralysis and death time compared to 10 mg/mL and 20 mg/mL concentrations. The activity was comparable to the standard drug Albendazole.

Compound	Concentration (mg/mL)	Paralysis Time (min)	Death Time(min)
IST-1	10	20	56
IST-1	20	16	47
IST-1	50	12	35
Albendazole	20	16	36
Albendazole	50	10	27



		IST-1	IST-1	IST-1	Albenda zole	Albenda zole
Concentration(mg/mL)		10	20	50	20	50
ParalysisTime(min)		20	16	12	16	10
DeathTime(min)		56	47	35	36	27

RESULTS AND DISCUSSION:

The synthesis of isatin-based Schiff base derivatives and thiazolidine derivatives was successfully carried out using simple and efficient synthetic procedures. The reaction of isatin with various amines and thioglycolic acid resulted in the formation of the desired compounds in good yields. The synthesized compounds were purified by recrystallization to obtain products of high purity.

The structural characterization of the synthesized compounds was performed using Nuclear Magnetic Resonance (NMR) spectroscopy, Mass spectrometry, and Infrared (IR) spectroscopy. The NMR spectra of the synthesized compounds showed characteristic signals corresponding to the formation of the isatin Schiff base framework, confirming the successful condensation reaction. The mass spectrometry data further confirmed the molecular weights of the synthesized compounds, supporting the proposed molecular structures. Additionally, the IR spectra exhibited characteristic absorption bands corresponding to important functional groups such as C=N (imine), C=O (carbonyl), and other substituent-related vibrations, confirming the formation of the targeted derivatives.

The biological evaluation of the synthesized compounds was carried out using earthworms as the experimental model to determine their anti-helminthic activity. The results indicated that several compounds exhibited significant anti-helminthic activity at a concentration of 10 mg/mL. Among the synthesized derivatives, the compounds containing specific substitution patterns on the isatin ring showed comparatively higher biological activity.

These findings suggest that structural modification of the isatin nucleus plays an important role in enhancing anti-helminthic activity, indicating the potential of these derivatives as promising candidates for further pharmacological investigation.

CONCLUSION:

In conclusion, isatin remains an important scaffold in synthetic and medicinal chemistry. In this study, isatin-based Schiff base and thiazolidine derivatives were successfully synthesized and characterized using NMR and mass spectroscopy, confirming their structures. The synthesized compounds showed promising anthelmintic activity in the earthworm model. Isatin derivatives are also known to possess anticancer, antiviral, anticonvulsant, antibacterial, and anti-tubercular activities. These findings indicate that isatin is an important nucleus for the development of new biologically active compounds and provides opportunities for further research.

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