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Synthesis, spectrum characterization and estimation of biological activity of new heterocycylic compound

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ABSTRACT

This present work deals with the synthesis of new azo derivative based on reaction between Amino pyridine and Para hydroxy benzoic acid. The synthesized compound was determined by spectrum characterization and screened for Antimicrobial and anthelmintic activity.

Keywords: Amino pyridine; Para hydroxy benzoic acid; Azo derivative; Antimicrobial; Anthelmintic

INTRODUCTION

In the branch of organic chemistry, the medicinal chemistry occupies the chief position because it involves design, development and synthesis of many new drugs. It is the major field of pharmaceutical science which the applies the principle of the chemistry and biology to the creation of knowledge leading to introduction of new drugs. The main objective of this field is to discover a new lead compounds or drug derivatives for use as a drugs. In ancient period many medicinal drugs were synthesized from natural sources but now a days these natural sources still important. Thus the majority of drug compounds are synthesized in the laboratory.

Heterocyclic chemistry is the branch of the medicinal chemistry it deals with the synthesis, properties and application of heterocyclic compounds. heterocyclic compounds are the organic compounds which contains heteroatoms (O,S,N,P,Si). The source of this compounds in drug provides a useful tool for modification of its properties. These compounds are widely distributed in nature for example-chlorophyll, vitamin-E, etc,. Pyridine is one of the basic organic compound of molecular formula C_5H_5N and its structure is similar to benzene in which one carbon is replaced by the nitrogen atom. pyridine nucleus present in niacin, vitamin B6,.^(1,2,3)



MATERIALS

All the materials such as 2 amino pyridine and para hydroxy benzoic acid were purchased from

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DOLPHIN PHARMACY INSTRUMENT Pvt limited, Mumbai. The IR spectra of the compounds were recorded on FT-IR spectrometer 4100 type A with potassium bromide pellets (BRUCKER ALPHA II). The absorption of compounds to be visualized in UV- chamber (Systronics AU 2701 DOUBLE BEAM). The anthelmintic activity performed on Indian earthworm and antimicrobial activity performed against some bacteria.

METHODS

Preparation of the new azo derivatives 4-[Pyridine-2yl diazenyl] phenol(0.1)(9.6gm) of 2-amino pyridine dissolved in 16 ml of Hydrochloric acid then this mixture is cooled to $0-5^{0}$ C and then add sodium nitrite (6gm) NaNO₂ (0.1mole) Dissolved in 20ml of distilled water.to this add the solution of (0.1mole) of para hydroxyl benzoic acid (13.8gm) and 4gm of NaOH dissolved in 140ml of distilled water. This process was carried out at PH 5 and kept this solution aside for 24 hrs after which the precipitate was filtered and the precipitate was collected and washed with the distilled water, after drying it should be recrystallized with the ethanol.⁽⁴⁾



Instrumentation

The melting point of newly synthesized product determined by the open capillary tube method. The completion of the reaction is confirmed by the using TLC method. The UV spectrum of newly synthesized compound is determined by using Systronics AU 2701 DOUBLE BEAM spectroscopy. the structure of compound is confirmed by FT-IR spectrometer 4100 type A with KBr (BRUCKER ALPHA II)

DETERMINATION OF BIOLOGICAL PROPERTIES

Antimicrobial activity: A different concentration azo compounds were tested against the microorganisms to determine their MIC, for their anti-microbial activity three different types of bacteria used namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* using agar well diffusion method . For this test, culture media is prepared aseptically using standard procedure. A bacteria to be inoculated on the three petri dish using strike method. After 24 hrs of incubation inhibition zones are measured. Results are compared with amoxicillin antibiotics.⁽⁵⁾

Anthelmintic activity: The anthelminthic activity was carried out on adult Indian earthworm *Lumbricina terrestris*. The worms were collected from a local area of Dharmapuri district. They were washed with distilled water to remove dirt, soil, and faecal matter from the skin of the worms. They were divided into groups in three Petri dishes with each containing one worms. Dimethyl sulphoxide [DMSO]was used as the control, while Albendazole used as a standard. Different concentration of azo compounds is prepared and

test against the worms. the time take for paralyzing and death of worms to be noted.⁽⁶⁾



The preparation of azo compound is shown in scheme 1. The compound are chemically named using Chemdraw ultra software . The complete of the reaction is confirmed by the TLC method using iodine chamber. The structure of the compound is determined by the spectrum characterization. Azo groups are the organic compounds of molecular fornula of A-N=N-B where A and B are the aromatic rings that conjugates with the azo group.



4 [pyridin 2yl diazenyl]phenol

Chemical name: 4 [pyridine 2yl diazenyl] phenol						
Boiling point : 788.16 k						
Appearance : appears like a crystal form						
Colur	: light brownish colour					
Uv/ visible Spectrum	: Maximum absorbance in 290nm in NaOH solvent Maximum absorbance in 300 nm in DMF solvent					
Ir spectrum	: Peaks are obtained and indicates the confirmation	of structutre				

Spectrum characterization

UV/VISIBLE Systronics AU 2701 DOUBLE BEAM spectroscopy were used to determine the absorption spectrum of azo compounds at room temperature using different solvents such as NaOH and DMF shown in fig 1 and fig 2 within the range of 200- 400nm.

In fig 1 describes the absorption spectrum of the azo compounds using sodium hydroxide as a solvent gives a maximum wavelength at 290 nm and in fig 2 describes the absorption spectrum of the azo compounds using dimethyl foramide as a solvent which gives a maximum wavelength at a 300 nm. The overlay of these two peaks were described in figure 3 respectively.



FIGURE 3 ovaerlay of peak of azo compounds using NaOH and DMF as solvent



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FTIR spectrum data for a new compound is described above, which show band at 3613 cm $^{-1}$ for OH group ,1594 cm $^{-1}$ for C=N, 1030cm $^{-1}$ for C-N, 843cm $^{-1}$,760cm $^{-1}$ for C-H, the presence of aromatic ring is indicated by shown band at 1594

IR SPECTRUM

 cm^{-1} ,1483 cm^{-1} , the presence of azo group N=N shown band in 1594 cm^{-1} , 760 cm^{-1} indicates the presence of azo group at the ortho substitution of benzene group.

RADICAL	WAVELENTH LITERATURE	FTIR READING	INFERENCE				
C=C	1700-1500cm ⁻¹	1594.54cm ⁻¹ , 1661.05cm ⁻¹	C=C bond in a molecule				
С-Н	860-680 cm ⁻¹	760.79cm ⁻¹ , 843.90cm ⁻¹	C-H bond in a molecule				
О-Н	3640-3530 cm ⁻¹	3613cm ⁻¹	OH bond in a molecule				
N=N	$1630 - 1575 \text{ cm}^{-1}$	1594cm ⁻¹	N=N bond in a molecule				
C-N	1200-1025cm ⁻¹	1030.05cm ⁻¹ , 1152.70cm ⁻¹	C-N bond in a molecule				

FT IR ANALYSIS OF AZO COMPOUND

Based on the interpretation of FTIR readings the structure of the newly synthesized compounds are confirmed.

ANTIMICROBIAL ACTIVITY

Different concentration of azo compounds are tested against three different types of bacteria to

determine their minimum inhibition concentration [MIC] as shown as below and amoxicillin used as standard.

Bacteria	Concentration of azo compound	
	50mg	100 mg
Amoxicillin(standard)	15mm	19mm
Gram negative bacteria Escherichia coli	7mm	11mm
Klebsiella pneumonia	8mm	10mm
Gram positive bacteria Staphylococcus aureus	11mm	14mm



In the minimum inhibition concentration, the azo compounds are screened against different types of bacteria namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* using agar well diffusion method. It was found to be a newly synthesized product shows highly activity against *Escherichia coli , Staphylococcus aureus,* where its shows moderate activity against *Klebsiella pneumonia* when compared to reference/ standard drug



ANTHELMINTIC ACTIVITY

The anthelmintic activity carried on Indian earthworm. in these two different concentrations of compounds are used [5mg/ml and 15 mg/ml], DMSO used as a control and albendazole used as a standard drug. Time taken for death and paralyse of worms to be noted down.

COMPOUNDS	5mg/ml		15 mg/ml	
	Paralysis time	Death time	Paralysis time	Death time
	[min]	[min]	[min]	[min]
Control	-	-	-	-
Albendazole	9	10	5	8
Azo compound	15	19	10	9



For the anthelmintic activity it was notice that the as the concentration of the azo compound increases as time taken for paralysis and death of worms decreases and its proved that the azo compounds have a greatest anthelmintic activity against the Indian earthworms.

CONCLUSION

We conclude that, a successful synthesis of new azo derivatives of aminopyridine was achieved. The biological activity of a compound reveals that the new azo moiety has good bioactive. These compounds exhibit better anthelmintic activity against Indian earthworms at concentrations of 5 and 15 mg/ml when compared to the standard reference drug albendazole. Then in vitro antimicrobial assay performed against gram positive and gram-negative bacteria has shown a better inhibition effect when compared the reference drug thus a newly synthesized azo compound can be used as dyes with to pharmacogical properties in various sectors

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