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Synthesis, Characterization and Anthelmentic Activity of Indole Derivatives

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ABSTRACT

Indole is a planar molecule with a conjugated system of 10 π electrons. It exists in resonance form with resonance energy of 47-49 K cal/mole. It is a very weak base with Pka value 3.63. In structure a, b, and d the benzenoid 6-π system is preserved. The chemical structures of the synthesized compounds were established on the basis of physical, chemical, analytical data. The purification of the compounds was carried by purification methods like recrystallization. Physical constant like melting point, boiling point etc, of the new compounds were determined. All the intermediates and final synthesized products were inspected visually for physical appearance. It was physically characterized on the basis of organoleptic properties like color, odor and taste. This determination was obtained using a digital capillary melting point apparatus (Cambell Electronics, Bombay, India) by capillary fusion method. All the synthesized materials were further identified and confirmed by Thin Layer Chromatographic (TLC). UV/Visible spectra enables us o study the absorption pattern of the molecule and determination of λ_{max} which is useful for the quantitative estimation of the compound. The IR spectrum of all the synthesized materials was recorded, which showed stretching and bending vibration levels of molecules in potassium bromide pellet by FTIR Spectrophotometer. The synthesized compounds were screened for Anthelmintic activity by using Mathew et al method and Indian adult earthworms (Pheretima Posthuma). All substituted quinoxaline compounds have been screened for their anthelmentic activity. From the screening results it was observed that the presence of electron withdrawing group made the substituted quinoxaline compounds to exhibit moderate to significant anthelmentic activity in comparison to standard drug albendazole. Compound QX1 and QX5 exhibited promising anthelmentic activity. However other two compounds (QX2 & QX4) of the series also exhibited moderate to weak activity against the *Pheritma phosthuma.*

Keywords: *Pheritma phosthuma,* Indole, Anthelmentic activity.

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1. INTRODUCTION:

The word Indole [1-4] is coined from the word India, a blue dye imported from India known as Indigo. Bayer first prepared it in 1866 by zinc distillation of ox-indole. The I.U.P.A.C name of indole is 1 H-benzo [b] pyrrole. Indole is a planar molecule with a conjugated system of 10 π electrons. It exists in resonance form with resonance energy of 47-49 K cal/mole. It is a very weak base with Pka value 3.63. In structure a, b, and d the benzenoid 6-π system is preserved. The electrophilic attack results at 3rd position. Presence of high electron density at 3rd position has been also supported by the calculation of π electron density and by molecular orbital method. It undergoes all types of reactions for example:

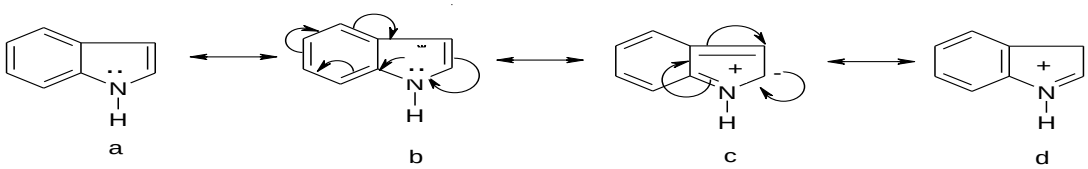
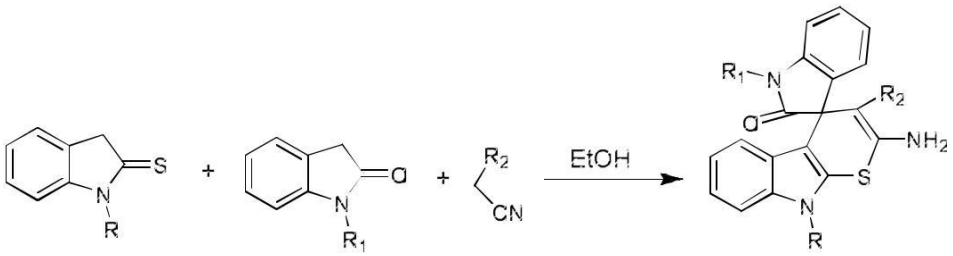


Figure 1: Indole: 1 H-benzo pyrrole

1.1 Methods for the synthesis of indole derivatives:

K.C. Majumdar and others [5] have developed a green and highly efficient one-pot three-component approach for the synthesis of spiro[indoline-3,40-thiopyrano[2,3-b]indole] derivatives by domino reaction of indoline-2-thione, isatin and ethyl cyanoacetate or malononitrile in ethanol.



2. MATERIALS & METHOD

2.1 Materials Used:

Table 1: List of materials used with manufacturer

Material Used	Manufacturer
Aniline	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
4-methyl aniline	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
4-chloro aniline	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
4-bromo aniline	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
Methanol	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
Toluene	S.D. Fine Chem. Ltd., Mumbai, India.
Ethanol	S.D. Fine Chem. Ltd., Mumbai, India.
Glacial acetic acid	S.D. Fine Chem. Ltd., Mumbai, India.
chloral hydrate	S.D. Fine Chem. Ltd., Mumbai, India.
Anhydrous sodium sulfate	S.D. Fine Chem. Ltd., Mumbai, India.
Concentrated Hydrochloric acid	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
Concentrated sulfuric acid	S.D. Fine Chem. Ltd., Mumbai, India.
Chloroform	S.D. Fine Chem. Ltd., Mumbai, India.
Tween 80	S.D. Fine Chem. Ltd., Mumbai, India.
DMSO	S.D. Fine Chem. Ltd., Mumbai, India.
Potassium dihydrogen phosphate	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
Potassium hydrogen phthalate	Ranbaxy Fine Chemicals Ltd, New Delhi, India.
Di sodium hydrogen phosphate	S.D. Fine Chem. Ltd., Mumbai, India.
Formalin	S.D. Fine Chem. Ltd., Mumbai, India.
Saubouraud dextrose	HI-media, Mumbai, India.

2.2 Methods:

Starting materials were identified by physical, chromatographic and spectral analysis. The chemical structures of the synthesized compounds were established on the basis of physical, chemical, analytical data. The purification of the compounds was carried by purification methods like recrystallization. Physical constant like melting point, boiling point etc, of the new compounds were determined. The purity and progress of the reactions were monitored by TLC, and column chromatography (if needed) by using suitable solvents and UV, FTIR, NMR, CHN analysis and MASS spectral data were used for the characterization of the synthesized compounds by sending the sample to various advanced research laboratory.

2.3 Synthesis of Substituted-Indole-2, 3 dione (Isatin) and Final Products:

A. Synthesis of substituted isonitroso acetanilide from substituted aniline

B. Synthesis of substituted-indole-2,3 dione (isatin) from substituted isonitroso acetanilide

C. Synthesis of substituted-1-(4-substituted benzyl) indole-2,3-dione.

D. Substituted-1-(4-substituted benzyl)-1H-indolo(2,3-b) quinoxaline N-benzyl indole-2,3-dione

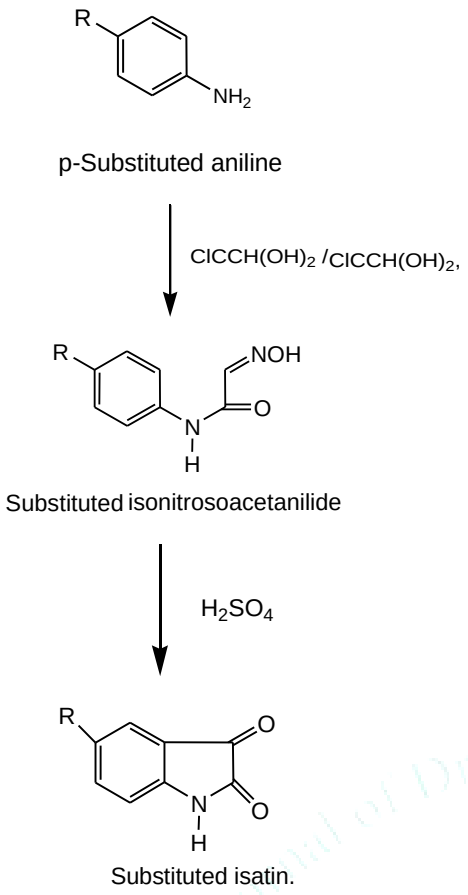


Figure 2: Scheme for synthesis of Substituted-1- (4-substituted benzyl)-1H- indolo (2,3-b) quinoxaline N-benzyl indole-2,3- dione from substituted aniline

2.4 Physical appearance:

All the intermediates and final synthesized products were inspected visually for physical appearance. It was physically characterized on the basis of organoleptic properties like color, odor and taste.

2.5 Determination of Melting points & Boiling point:

This determination was obtained using a digital capillary melting point apparatus (Cambell Electronics, Bombay, India) by capillary fusion method. A capillary was taken and bringing it near the burner flame then sealed its one end. The open end of the capillary tube was pushed in to a small heap of drug, so that a small plug of the powder was collected in the open end and the tube was tapped gently, so that collected material was settled down. This process was repeated several times. Then the capillary tube was placed in the melting point determination apparatus and observed the temperature at which sample changes its state from solid to liquid. The experiment was performed in triplicate. The temperature at which starts to melt was noted with the help of thermometer. Boiling points of all the intermediates and final synthesized products were determined by capillary method in liquid paraffin bath and all the data were matched with reported values (if available).

2.6 Thin layer chromatography:

All the synthesized materials were further identified and confirmed by Thin Layer Chromatographic (TLC) study on readymade TLC plate in a mixed solvent system and R_f values were matched with earlier reported values.

2.7 Ultraviolet spectrum:

UV/Visible spectra enables us o study the absorption pattern of the molecule and determination of λ_{max} which is useful for the quantitative estimation of the compound. 20 mg of all the synthesized materials were dissolved individually in a 100 ml of methanol. Then from this solution 10 ml was taken and volume was made up to 100 ml with methanol, to make the solution concentration of 20 µg/ml & the resulting solution was scanned between 200-600 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The UV Spectra of the drug was recorded.

2.8 IR Spectrum:

The IR spectrum of all the synthesized materials was recorded, which showed stretching and bending vibration levels of molecules in potassium bromide pellet by FTIR Spectrophotometer (Prestige-21, Shimadzu, and Tokyo, Japan) to monitor the identifications of drug between the ranges of 400 to 4000 cm^{-1}. The IR Spectra of the all the synthesized materials was recorded.

2.9 ^{1}HNMR spectra:

NMR spectroscopy enables us to record differences in magnetic properties of the various magnetic nuclei present and to deduce in the large measure about the position of these nuclei within the molecule. We can deduce how many different kinds of environments are there in the molecules and also which atoms are present in neighboring groups. The proton NMR spectra enable us to know different chemical and magnetic environments corresponding to protons in molecules.^{1}HNMR spectra of synthesized materials were recoeded in $CDCl_3$ and d6-DMSO on a Bruker ultraspec 500 HZ/AMX 400MHZ/300MHZ spectrometer at IISC Bangalore. The reported chemicals shifts were measured against TMS.

2.10 Mass spectra:

The advent of FABMS in which ion generation is achieved by bombardment of the sample by a beam of fast rare gas atoms resolved the limitation problem that the conventional electron impact mass spectrometry used to have in the characterization of high molecular weight cluster compounds.

2.11 Elemental CHN analysis:

Enables us to study the fragmentation pattern of the molecules and very important tool in determining the molecular mass of the unknown compound along with the C, H, N analysis. Perkin-Elmer CHN element analyzer was applied to ensure the accuracy of oxygenated percent in the liquid.

2.12 Anthelmentic activity on *Pheritma phosthuma:*

The synthesized compounds were screened for Anthelmintic activity [6-7] by using Mathew et al method and Indian adult earthworms (Pheretima Posthuma). The earthworms (collected from the water logged areas of soil in and around jaipur, Rajasthan) were washed with normal saline to remove all faecal materials. The earthworms in 4 - 5 cm in length and 0.1 - 0.2 cm in width were used for all experimental protocol. Earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity. Five earthworms of nearly equal size were placed in standard drug solution and test compounds solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 15 ml with normal saline

solution to get the concentration of 0.5 % w/v, 1.0 % w/v and 1.5% w/v. Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive.

3. RESULTS & DISCUSSION

3.1 Identification of Starting Materials:

Table 2: Physical appearance of starting material [8-13]:

Sl. No.	Ingredients	Physical appearance	Compliance with reported values
1.	Aniline	Colorless, characteristic amine odor and burning taste	Yes
2.	4-methyl aniline	colorless solid, wine-like odor, and burning taste	Yes
3.	4-chloro aniline	Colorless crystals, slightly sweetish characteristic amine odor	Yes
4.	4-bromo aniline	Brown solid with a sweet odor	Yes

Table 3: Melting points and/or Boiling point [8-13]:

Sl. No.	Ingredients	Melting points	Boiling point	Compliance with reported values
1.	Aniline	-	183 -185 ^{o}C	Yes
2.	4-methyl aniline	44-46°C	200-202°C	Yes
3.	4-chloro aniline	69-71°C	232-234°C	Yes
4.	4-bromo aniline	60-63 °C	219-221 °C	Yes

Table 4: Thin layer chromatography of starting material [14]:

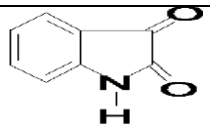
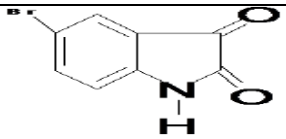
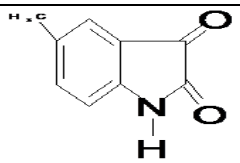
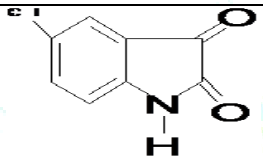
Sl. No.	Ingredients	Rf values (Toluene: Methanol -95:5)	Compliance with reported values
1.	Aniline	0.25±0.02	Yes
2.	4-methyl aniline	0.22±0.06	Yes
3.	4-chloro aniline	0.26±0.10	Yes
4.	4-bromo aniline	0.29±0.12	Yes

Table 5: Ultraviolet spectrum of starting material [8-12]:

Sl. No.	Ingredients	Maximum wave length (λ_{max})	Compliance with reported values
1.	Aniline	230 nm in ethanol	Yes
2.	4-methyl aniline	294 nm in cyclohexane	Yes
3.	4-chloro aniline	242 nm in ethanol	Yes
4.	4-bromo aniline	245 nm in ethanol	Yes

3.2 Identification and Characterization of the Intermediate Synthesized Compounds:

Table 6: Melting points and % yield of intermediate compounds [8-13]

Sl. No.	Substituted Isatin or 1H-indole-2,3-dione	Structure of compound	Melting points (°C) Observed / (Reported)*	% yield
1.	Un-substituted Isatin		196-198°C (194-198°C)*	75
2.	5-Bromo isatin		248-250°C (247-252°C)*	84
3.	5-Methyl isatin		186-188°C (184-188°C)*	79
4.	5-Chloro isatin		243-246°C (240-246°C)*	76

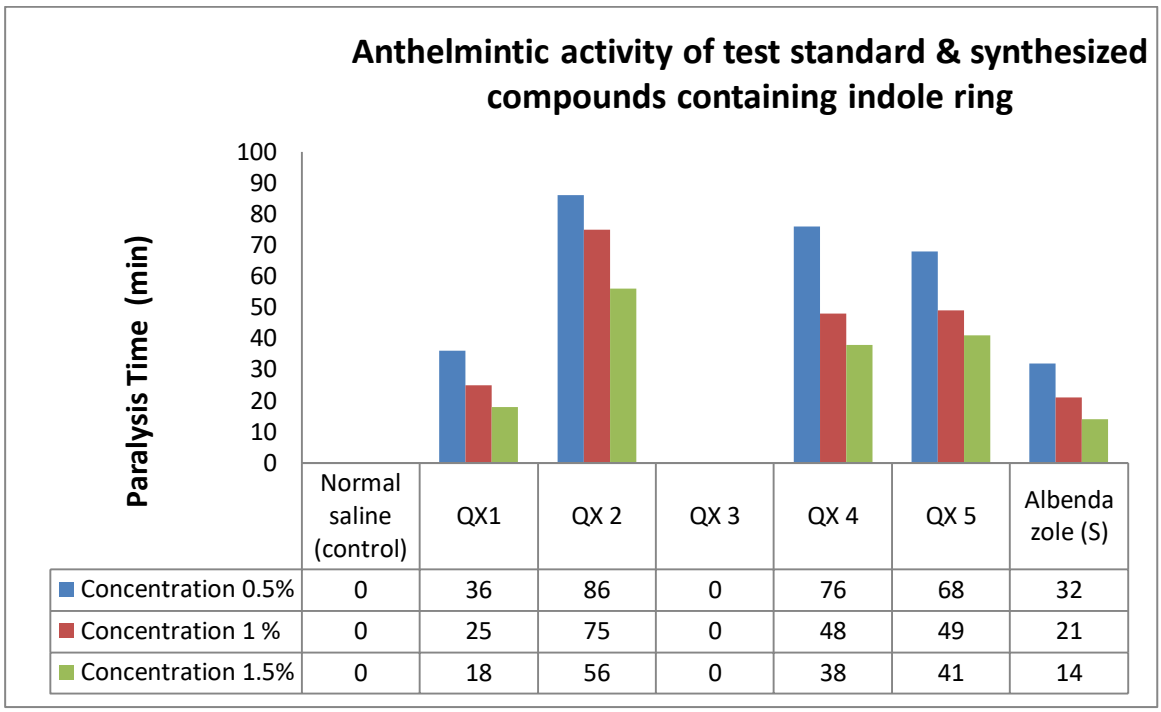
*Matched with reported values [8-13]

3.3 Anthelmentic activity on *Pheritma phosthuma:*

Table 7: Anthelmintic activity of test standard & synthesized compounds containing indole ring

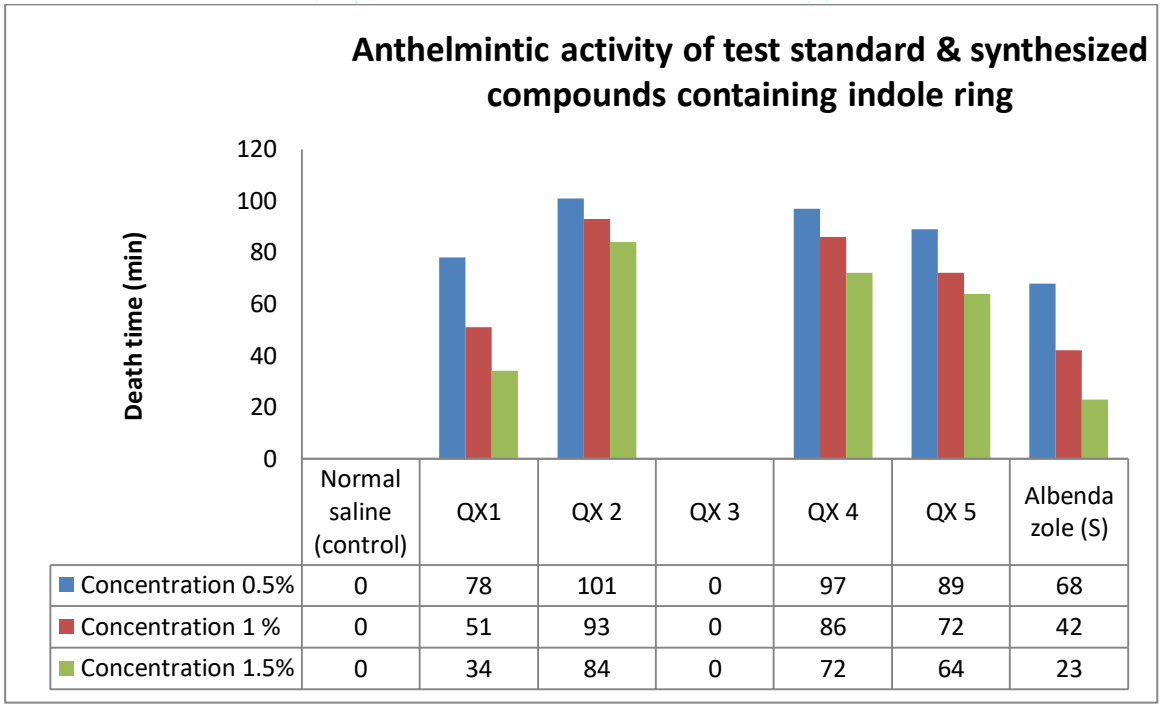
Test and standard compound	Concentration % w/v	Paralysis Time (min)	Death time (min)
Normal saline (control)	-	-	-
QX1	0.5	36±4	78±8
	1.0	25±3	51±4
	1.5	18±2	34±4
QX2	0.5	86±6	101±9
	1.0	75±5	93±6
	1.5	56±5	84±5
QX3	0.5	-	-
	1.0	-	-
	1.5	-	-
QX4	0.5	76±7	97±9
	1.0	48±4	86±8
	1.5	38±3	72±7
QX5	0.5	68±6	89±8
	1.0	49±4	75±7
	1.5	41±4	64±6
Albendazole (S)	0.5	32±3	68±5
	1.0	21±2	42±4
	1.5	14±2	26±3

Values are expressed as Mean ± SEM (n = 6)



	Normal saline (control)	QX1	QX 2	QX 3	QX 4	QX 5	Albenda zole (S)
Concentration 0.5%	0	36	86	0	76	68	32
Concentration 1 %	0	25	75	0	48	49	21
Concentration 1.5%	0	18	56	0	38	41	14

Figure 2: Histogram showing anthelmintic activity [Paralysis Time (min)] of standard & synthesized compounds containing indole ring



	Normal saline (control)	QX1	QX 2	QX 3	QX 4	QX 5	Albenda zole (S)
Concentration 0.5%	0	78	101	0	97	89	68
Concentration 1 %	0	51	93	0	86	72	42
Concentration 1.5%	0	34	84	0	72	64	23

Figure 3: Histogram showing anthelmintic activity [Death Time (min)] of standard & synthesized compounds containing indole ring

4. CONCLUSION:

All substituted quinoxaline compounds have been screened for their anthelmentic activity. From the screening results it was observed that the presence of electron withdrawing group made the substituted quinoxaline compounds to exhibit moderate to significant anthelmentic activity in comparison to standard drug albendazole. Compound QX1 and QX5 exhibited promising anthelmentic activity. However other two compounds (QX2 & QX4) of the series also exhibited moderate to weak activity against the *Pheritma phosthuma*. The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms [15].

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