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[Research article]

Synthesis and pharmacological evaluation of novel imidazole derivatives

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ABSTRACT

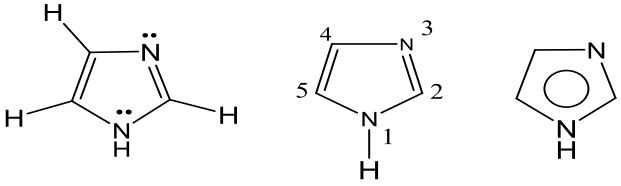
Literature survey reveals that in recent years several imidazole derivatives have been synthesized and reported to possess varied biological and pharmacological properties. They are found to be useful as antifungal, antibacterial, antiviral, anthelmintic, analgesic, antineoplastic, antidiabetic, antihistaminic and anti-inflammatory agents. In view of the biological importance of both imidazole moiety and isatin derivatives, we synthesized new novel derivatives containing imidazole acid hydrazide at the third position of the isatin ring.

Key words: Imidazole acid hydrazide, Isatin derivatives, anthelmintic, antifungal, antibacterial.

INTRODUCTION

A great number of drugs are heterocyclic compounds, mostly of synthetic origin and few of them are obtained from natural resources such as alkaloids, glycosides, xanthines, hormones and several antibiotics. Heterocycle is a cyclic organic compound containing one or more carbons and atleast one or two heteroatoms like O, N, S. Most of the research works in the discovery of newer and potent analogs of molecules are being carried out by bringing modifications in the parent compound having already established activities to enhance the activity and eliminate the adverse effects associated with the parent compound.

IMIDAZOLE: [**structural features of the ring**]



Isatin (20)[16](1H-indole-2,3-dione) and its analogues are versatile substrates which can be used for the synthesis of various heterocyclic substances. Isatins are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry.

In recent years, Schiff and mannich bases of isatin are reported to exhibit broad spectrum chemotherapeutic properties such as antiviral, anti

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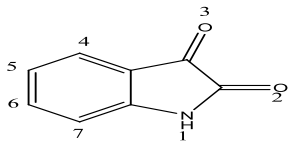
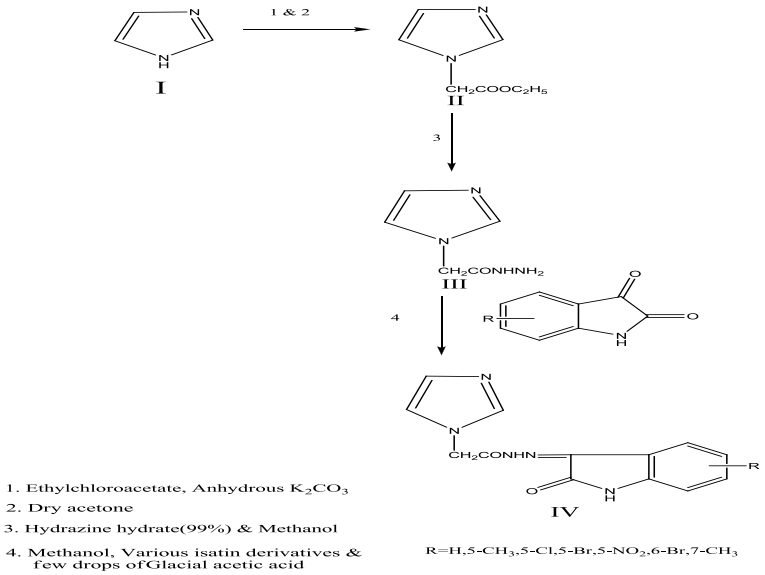
T.B, antifungal, antibacterial and various CNS activities as they are capable of crossing the blood brain barrier. Isatins have also been found in mammalian tissues and functions as modulator of biochemical processes.



EXPERIMENTAL PROCEDURE SCHEME



Synthesis of ethyl 2-(1H-imidazol-1-yl) acetate (II)

An appropriate amount of imidazole (6.8g, 0.1mole) was taken and dissolved in dry acetone. To this solution of imidazole in dry acetone, ethyl chloroacetate (16.7g, 0.1mole) was slowly added under constant stirring in presence of 5g of anhydrous K_2CO_3. The resulting mixture was then stirred and refluxed for 8-10 hrs using a electromagnetic heating mantle. Then the reaction mixture was cooled and the desired ester was separated and the resultant light brownish yellow liquid was collected. Yield; 65%

The purity of the compound was checked by thin layer chromatography using chloroform and methanol (1.5:0.5) solvent system using Iodine chamber.

Synthesis of 2-(1H-imidazol-1-yl) aceto hydrazide (III)

The ester obtained, ethyl-2-(1H-imidazol-1-yl)acetate (8g, 0.05mole) was dissolved in 15ml of anhydrous methanol, to this solution, hydrazine hydrate (99%, 0.1mole) was added slowly under constant stirring, it was refluxed for about 9-10h. Then the excess solvent was removed and the required yellow liquid was collected.

Yield; 70%

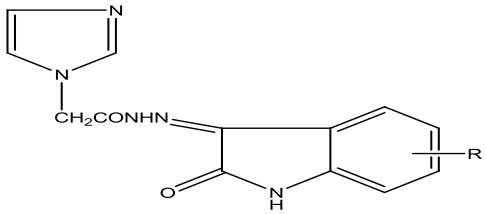
Synthesis of 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (IV)

An equimolar quantities of 2-(1H-imidazol-1-yl) acetohydrazide (0.01mole) and isatin (R=H, 0.01mole) are dissolved in methanol and few drops of catalytic amount of glacial acetic acid was added and then it was refluxed for 2-3hrs resulting in the formation of a Schiff base. The product obtained was then recrystallized using methanol and yellow crystals are collected and dried. The purity of the compound is determined by TLC and spectral data.

M.P: 218-221°c, Yield: 75%

As many as seven different compounds have been synthesized using different isatins adopting the above procedure and physical data is presented in Table-I

Table-I: Physical Data Of 2-(1h-Imidazol-1-Yl)-N'-(2-Oxoindolin-3-Ylidene) Acetohydrazide (Iv_{A-G})

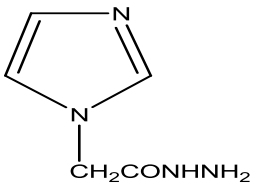


IV

S. No.	Compound	R	Mol. Formula	M. Wt	M.P(°c)	Yield(%)
1	IV_a	H	$C_{13}H_{11}N_5O_2$	269	218-221	75
2	IV_b	5-Cl	$C_{13}H_{11}N_5O_2Cl$	304.5	158-163	60
3	IV_c	5-NO_2	$C_{13}H_{11}N_6O_4$	315	192-195	64
4	IV_d	6-Br	$C_{13}H_{11}N_5O_2Br$	348.9	234-237	75
5	IV_e	5-Br	$C_{13}H_{11}N_5O_2Br$	348.9	212-216	68
6	IV_f	7-CH_3	$C_{14}H_{14}N_5O_2$	284	194-196	73
7	IV_g	5-CH_3	$C_{14}H_{14}N_5O_2$	284	201-203	82

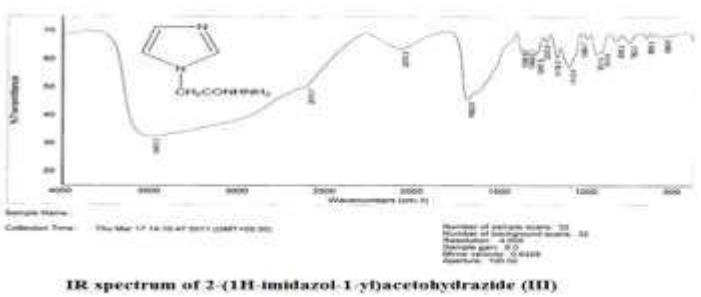
SPECTRAL DATA

Spectral data of 2-(1H-imidazol-1-yl) acetohydrazide (III)



(III)

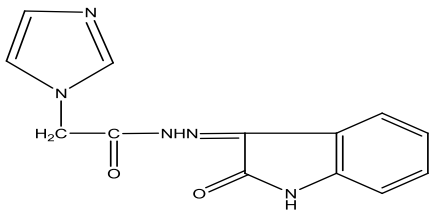
Molecular formula: $C_5H_8N_4O$
Molecular weight: 140
Solubility: Methanol, Ethanol
The IR spectra of the compound (III) exhibited its absorption bands (in cm^{-1}) at 3493 (NH), 1692.8 (C=O) and 1388 (C-N).



IR spectrum of 2-(1H-imidazol-1-yl)acetohydrazide (III)

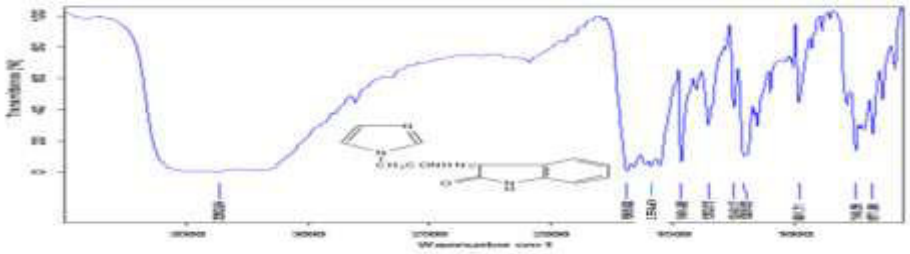
SPECTRAL DATA

Spectral data of 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (IV_a)

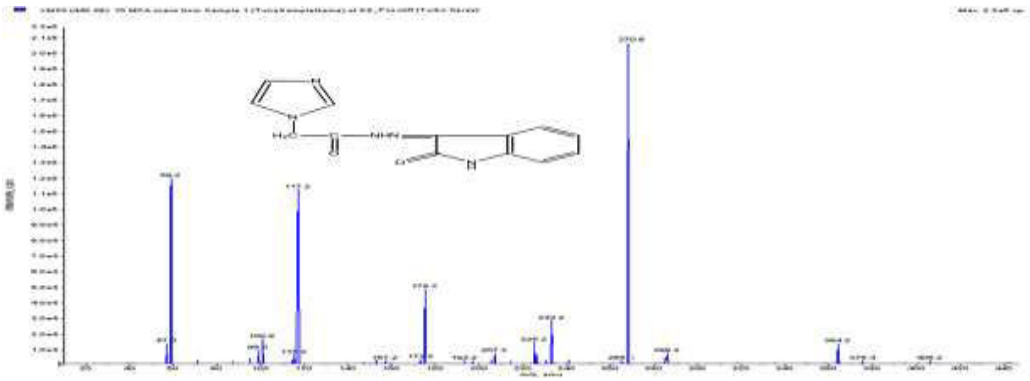


2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene) acetohydrazide

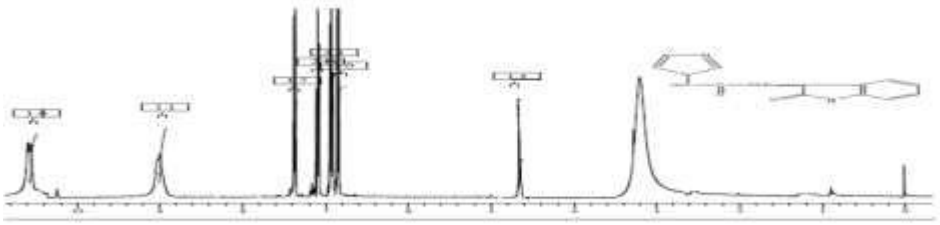
Molecular formula: $C_{13}H_{11}N_5O_2$
Molecular weight: 269
Melting point: 218-221°c
Solubility: Methanol, Dimethyl sulfoxide
The IR spectra of the compound **IV** $_a$ exhibited its absorption bands at (in cm^{-1}) at 3362 (NH), 1686 (C=O), 1554 (C=N), 1362 (C-N), 745- 677 (Aromatic CH bend).
The Mass Spectrum of the Compound showed its Molecular ion peak (M+1) at m/Z 270.6
PNMR spectrum of the Compound exhibited the Characteristic Signals (δ ppm) at 10.6 (S, 1H, Indole NH), 9 (S, 1H, hydrazide NH), 6.8-7.0 (m, 4H, Indole Aromatic-H), 7.15 (d, 2H, Imidazole Ar-H at 4th and 5th positions) and 7.6 (S, 1H, Imidazole Aromatic-H at 2nd position), 4.6 (s, 2H, methylene protons).



IR spectrum of 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (IV_a)



Mass spectrum of 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (IV_a)



^{1}HNMR spectrum of 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (IV_a)

PHARMACOLOGICAL EVALUATION ANTHELMINTIC ACTIVITY [13]

Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research.

Imidazole nucleus has proved to be a versatile moiety for a number of medicinal agents. Metronidazole an imidazole nucleus was first reported in 1961 and used as an effective class of anthelmintic in human and animal pathogenic helminthes. Structural modification of metronidazole led to the discovery of tinidazole, nimorazole and panidazole and their clinical trials established the value of tinidazole in the treatment of intestinal and hepatic amoebiasis in humans.

The aim of the present revision is to investigate the anthelmintic activity of newly synthesized 1-substituted imidazoles. Therefore a search of these novel 1-substituted imidazoles and its derivatives leads to the evaluation of prototype compounds with anthelmintic activity.

Drugs and Chemicals

Albendazole (pure drug)

Saline water

Vehicle (2% v/v Tween 80 in distilled water)

Test compound: 2-(1H-imidazol-1-yl)-N'-(2-oxoindolin-3-ylidene) acetohydrazide

All the prototypes were dissolved in minimum quantity of 2%v/v Tween80 and then the volume was adjusted to 10ml with normal saline for making the concentrations of 5, 10 and 20mg/ml.

Standard drug: Albendazole is taken as a reference standard and the concentration of the standard drug is prepared in 2% v/v Tween80 in normal saline to give 20 mg/ml.

Experimental model

Adult earthworms of genus and species Pheretima posthuma were used for the in vitro anthelmintic bioassay of all newly synthesized prototypes and they are washed with normal saline to remove all the fecal matter and waste surrounding their body.

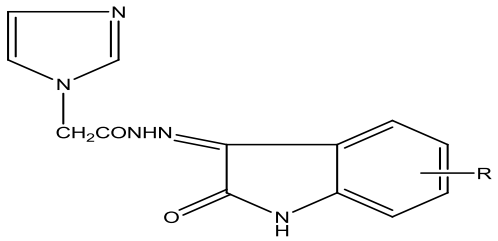
The earthworms of nearly equal size (7±1 cm) were used for all the experiment protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity.

The worms were divided into the respective groups containing six earthworms in each group.

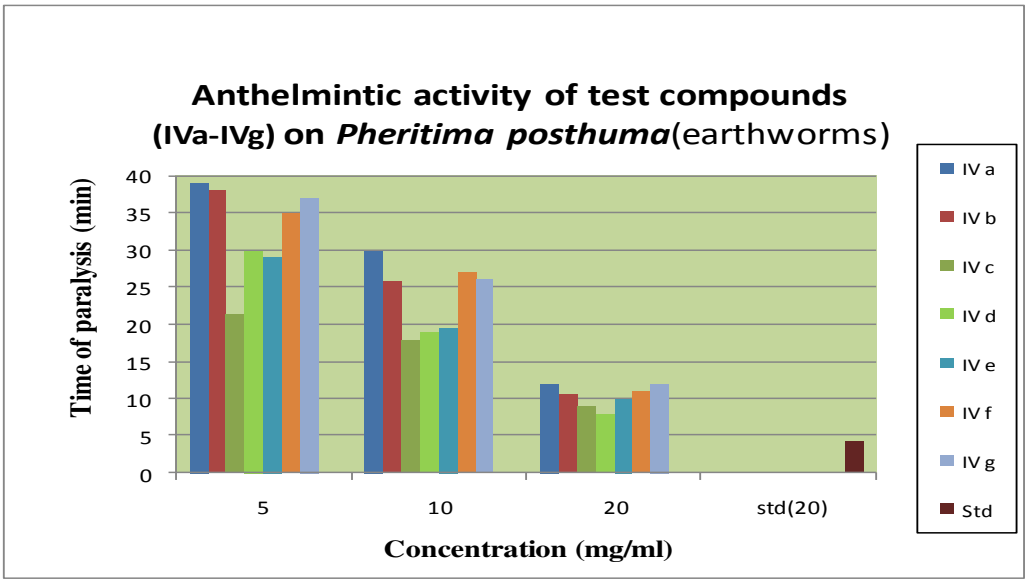
All the prototypes were dissolved in minimum quantity of 2%v/v Tween80 and the volume was adjusted to 10ml with normal saline for making the concentration of 5, 10 and 20mg/ml. All the concentrations of drug were freshly prepared before the commencement of the experiment and the earthworms are released into 10ml of the respective formulation as follows, vehicle, albendazole (20mg/ml) and prototypes (5,10 and 20mg/ml) and the activity was determined in five observations. Six worms of about same size per petridish were used and observed for their spontaneous motility and evoked responses. Time taken to paralysis and death of individual worms were noted down. The mean paralysis time and lethal time for each sample was calculated (each reading was taken in a triplicate)

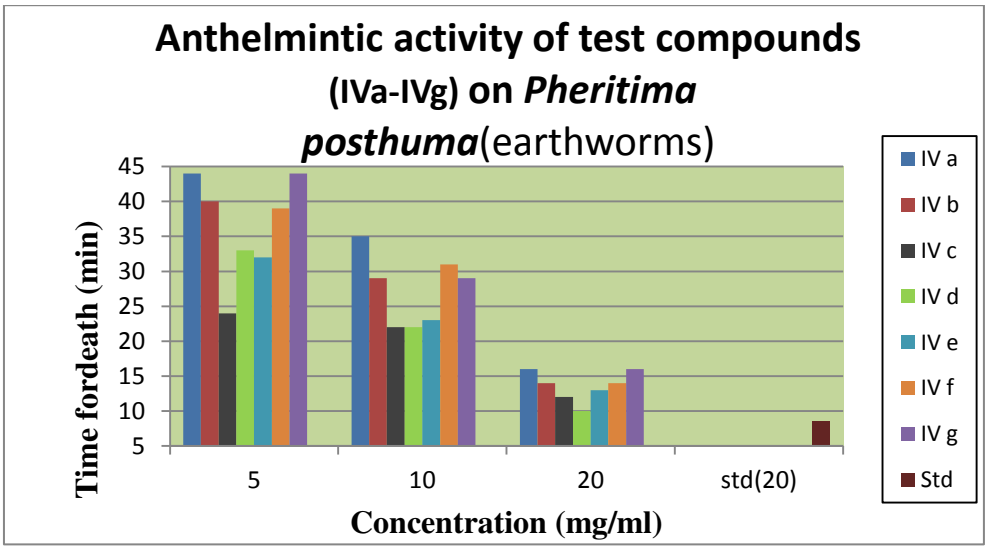
Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour No paralysis or death was occurred with the control group.

Table-ii:Anthelmintic activity of 2-(1h-imidazol-1-yl)-n'-(2-oxoindolin-3-ylidene) acetohydrazide (iv $_{a-g}$)



S. No.	Compound	Substituent R	Conc. (mg/ml)	Time (min, Mean ± SEM)	
				Paralysis	Death
1	**IV_a**	H	5	39±1.05	44±0.36
			10	30±0.25	35±0.48
			20	12±0.33	16±0.56
2	**IV_b**	5-Cl	5	38±0.91	40.56±087
			10	25.93±0.25	29±0.37
			20	10.58±0.27	14.6±0.18
3	**IV_c**	5-NO_2	5	21.4±1.02	24±0.24
			10	18±0.87	22±0.25
			20	9±0.75	12±0.29
4	**IV_d**	6-Br	5	30±1.15	33±0.54
			10	19±0.88	22±0.29
			20	8±0.43	10±0.44
5	**IV_e**	5-Br	5	29±0.21	32±035
			10	19.5±0.28	23±0.55
			20	10±0.21	13±0.43
6	**IV_f**	5-CH_3	5	35±0.53	39±0.64
			10	27±0.91	31±0.78
			20	11±0.44	14±0.03
7	**IV_g**	7-CH_3	5	37±0.32	44±0.26
			10	26±0.43	29±0.89
			20	12±0.88	16±0.22
8	**Albendazole**		20	4.32±0.28	8.56±0.05





ANTIBACTERIAL ACTIVITY[14]

The antibacterial activity of the title compounds was assayed against two different strains of bacteria i.e., a Gram- positive bacteria Bacillus subtilis and a Gram-negative bacteria Escherichia coli using **"**Agar diffusion method".

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 one is Serial dilution method and the other is diffusion method. The serial dilution method is very useful for the determination of antimicrobial activity. It is not much useful for the quantitative detection tests and 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 employed in this investigation was cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of zone of inhibition was measured.

PROCEDURE OF ANTIBACTERIAL STUDIES

Materials and method used

Method employed: Agar diffusion method

Requirements: Petridishes, glass syringes, cork borers (all sterilized by dry heat)

WORKING PROCEDURE

PREPARATION OF TEST AND STANDARD SOLUTIONS

The test solutions were prepared in distilled DMSO by dissolving 10mg of the respective samples each in dimethylsulfoxide (DMSO 10ml) and prepare 25, 50,100µg/ml concentrations. Ampicillin was used as standard and was dissolved in distilled DMSO to get a final concentration 10µg/ml. DMSO (0.1ml) was used as solvent control.

MICROORGANISMS USED

Two bacterial test organisms such as Bacillus subtilis (MTCC441) (Gram positive bacteria) and Escherichia coli (MTCC 722) (Gram negative bacteria) were selected and obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in Petri dishes prior to testing. The media used was nutrient agar, nutrient procured from HiMedia Laboratories, Mumbai.

PREPARATION OF INOCULUM

A 24 hour old culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9% w/v)

CULTURE MEDIUM

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

Composition of nutrient broth

Peptone	0.5gm
Sodium chloride	0.5gm
Beef extract	1.5gm
Yeast extract	1.5gm
Distilled water	1000ml

Composition of nutrient agar

Peptone	5.0gm
Sodium chloride	5.0gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water	1000ml
p^H	7.0±0.2

The test organism was subcultured 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 cultures were maintained and the bacterial inoculums were prepared by transferring a loopful of stock culture to nutrient broth. The flasks were incubated at 37 ± 1°c for 48 hours before the experimentation.

STERILIZATION OF MEDIUM

The nutrient agar medium was sterilized by autoclaving at 121°c for 15 minutes and the petriplates, tubes, flasks plugged in cotton were sterilized in hot air oven at 160°c for an hour.

PROCEDURE

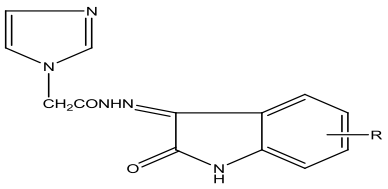
Into each sterilized Petri-plate (10 cm diameter), about 27 ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of inoculum into each plate) was transferred

aseptically. The plates were left at room temperature to allow solidification. A sterile borer was used to prepare cups of 10 mm diameter in the agar media. Accurately measured (0.1 ml) solution of each test and standard samples were added to the cups with a micropipette aseptically and labeled accordingly.

The plates were kept undisturbed for 1 hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. Then the plates are incubated at 37 ± 1ºC for 24 hours.

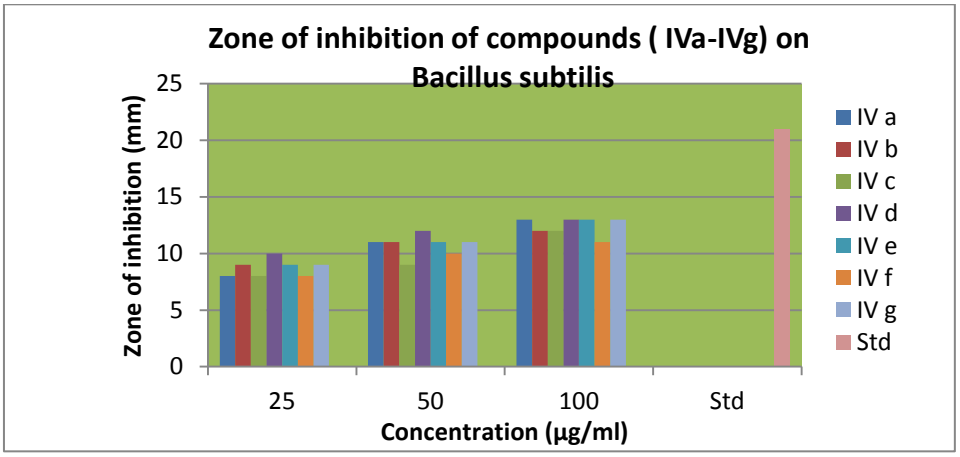
The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for test solutions. The diameter of zone inhibition surrounding each of discs was measured with the help of an antibiotic zone reader and the results were represented in Table-III.

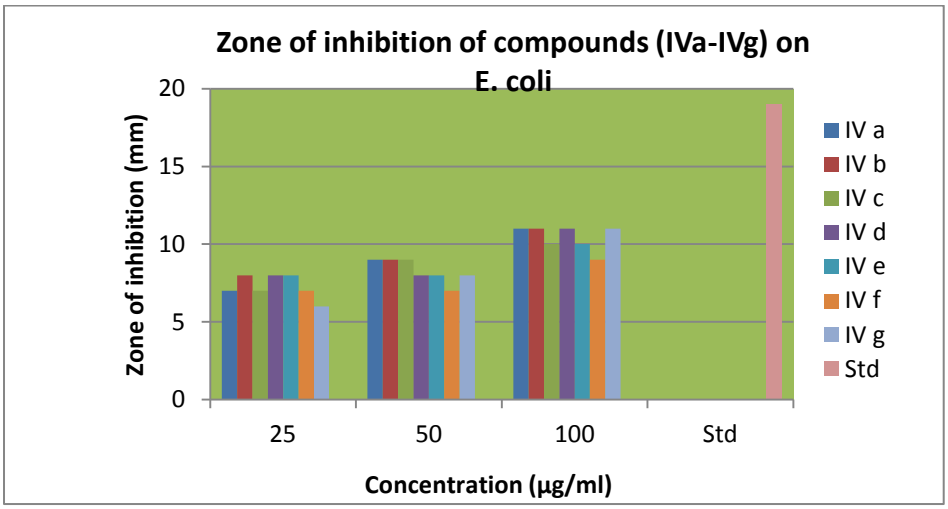
Table-iii:antibacterial activity of 2-(1h-imidazol-1-yl)-n'-(2-oxoindolin-3-ylidene) acetohydrazide (iv $_{a-g}$)



IV

S. No.	Compound	Substituents R	Conc. in µg/ml	Zone of inhibition B. subtilis	(in E. coli
1	**IV_a**	H	25	8	7
			50	11	9
			100	13	11
`2	**IV_b**	5-Cl	25	9	8
			50	11	9
			100	12	11
3	**IV_c**	5-NO_2	25	8	7
			50	9	9
			100	12	10
4	**IV_d**	5-Br	25	10	8
			50	12	8
			100	13	11
5	**IV_e**	6-Br	25	9	8
			50	11	8
			100	13	10
6	**IV_f**	5-CH_3	25	8	7
			50	10	7
			100	11	9
7	**IV_g**	7-CH_3	25	9	6
			50	11	8
			100	13	11
8	**Ampicillin**		10	21	19





ANTIFUNGAL ACTIVITY[15]

The antifungal screening of the test compounds were assayed against Candida albicans and Saccharomyces cerevisiae (Yeast) using agar diffusion method (cup plate method).

Preparation of test and standard solutions

The test solutions were prepared in distilled DMSO by dissolving 10mg of the respective samples each in dimethyl sulfoxide (DMSO, 10ml) and prepare 25, 50,100µg/ml concentrations. Clotrimazole was used as standard and was dissolved in distilled DMSO to get a final concentration 10µg/ml. DMSO (0.1ml) was used as solvent control.

Composition of the medium used

Sabouraud Dextrose Agar medium (SDA):

Meat peptone	5gm
Casein peptone	5gm
Dextrose	40g
Agar	15gm
Distilled water	1000ml
p^H	5.6±0.2

PREPARATION OF INOCULUM

The test organisms were subcultured using SDA medium. The tubes containing sterilized medium were inoculated with test fungi and after inoculation at 25°c for 48hrs, were stored at 4°c in refrigerator.

The inoculum was prepared by taking a loopful of stock culture to about 5ml of sabouraud dextrose

broth in a test tube. The tubes were incubated at 25°c for 48hours before use.

STERLIZATION OF MEDIUM

The SDA medium was sterilized by autoclaving at 121°c for 15minutes. The petri plates were sterilized in hot air oven at 160°c for an hour.

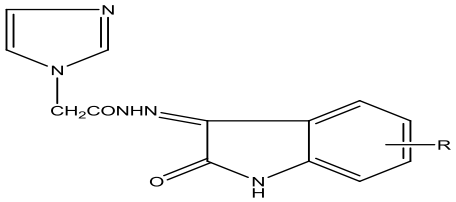
PROCEDURE

Into each sterilized petri plate, about 27ml of molten SDA medium was added and incubated at 30°c for two days. After two days of incubation, the medium free of contaminations was spreaded with 50µl of 48 hrs culture. After solidification, cups of 6mm diameter were made in each plate with sterile borer.

Accurately 50µl of 25, 50 and 100µg/ml of test solutions were transferred to the respective petri plates aseptically and labeled accordingly. The reference standard 50µl was also added to the discs in each plate.

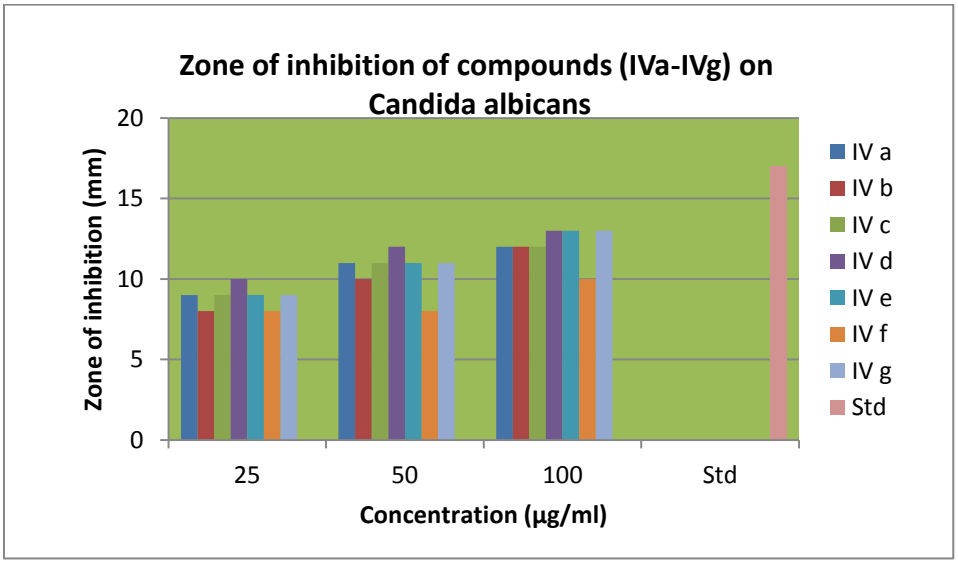
The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then, the plates were incubated at 25°c for 48hours in inverted position. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiment was performed in triplicate and the results were represented in table-IV.

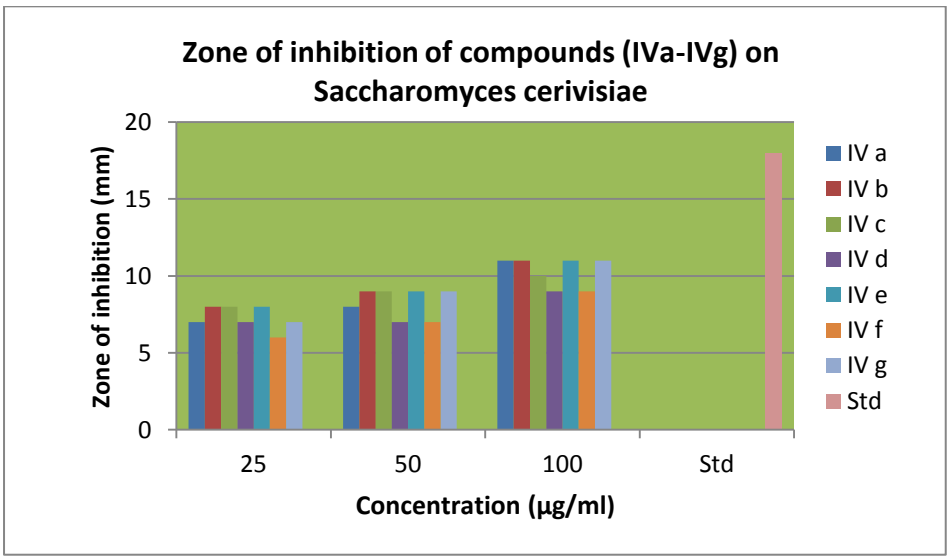
Table-iv:antifungal activity of 2-(1h-imidazol-1-yl)-n'-(2-oxoindolin-3-ylidene) acetohydrazide (iv $_{a-g}$)



IV

S. No.	Compound	Substituents R	Conc. in µg/ml	Zone of inhibition mm)	(in
				Candida albicans	Yeast
1	IV_a	H	25	9	7
			50	11	8
			100	12	11
`2	IV_b	5-Cl	25	8	8
			50	10	9
			100	12	11
3	IV_c	5-NO_2	25	9	8
			50	11	9
			100	12	10
4	IV_d	5-Br	25	10	7
			50	12	7
			100	13	9
5	IV_e	6-Br	25	9	8
			50	11	9
			100	13	11
6	IV_f	5-CH_3	25	8	6
			50	8	7
			100	10	9
7	IV_g	7-CH_3	25	9	7
			50	11	9
			100	13	11
8	Clotrimazole		10	17	18





RESULTS AND DISCUSSION

The objective of this work was to study the anthelmintic activity of 1-substituted imidazoles on Pheretima posthuma (earthworm) to explore the beneficial use of these derivatives in gastro intestinal disorders.

As evident from the available literature, compounds possessing imidazole nuclei viz., metronidazole, tinidazole, nimorazole and panidazole showed significant anthelmintic activity. Anthelmintic activities of all prototypes were tested in this bioassay at various concentrations of 5, 10 and 20mg/ml [TABLE II].

All the investigational compounds IV_{a-g} acquired the anthelmintic activity at minimal dose of 5mg/ml. Compounds IV_c, IV_d had shown good anthelmintic activity nearly as that of standard which was confirmed from the paralysis and death time. These compounds showed paralysis 9 & 8 min and death at 12 & 10 min respectively at the concentration of 20mg/ml which is nearly equal to that of albendazole at the same concentration.

The other prototypes IV_a, IV_b, IV_e, IV_f and IV_g showed moderate to good anthelmintic activity with paralysis times being in the range of 10 to 13min and death occurred within 16 min at the range of 20mg/ml. All the prototypes showed minimal activity at low concentrations like 5 and 10mg/ml whereas they possessed greater activity at higher concentration (20mg/ml).

Hence the synthesized compounds were found to possess mild to moderate anthelmintic activity from the above discussion.

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