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In-Vitro Cytotoxicity and Anthelmintic Evaluation of Various Synthesized Novel Imidazoles

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Abstract

Synthesized novel imidazole derivatives as potential therapeutic agents for *in-vitro* cytotoxicity and anthelmintics. Firstly, Pyridine-2-amine was condensed with substituted benzaldehydes to give corresponding Schiff's base. These Schiff's bases further on treatment with Ammonium acetate and isatin yielded corresponding novel imidazoles. The synthesized compounds were analyzed by physical & analytical methods. The synthesized compounds were evaluated for *in-vitro* cytotoxicity activity and anthelmintic activity. All the synthesized novel substituted imidazoles showed moderate to good anthelmintic activity. It also possessed significant *in-vitro* cytotoxicity against HEp2 cell lines (Human larynx cancer cell line) against standard using *5*-fluorouracil. The compounds 1b, 2b, 4b, 6b and 8b possessed higher anthelmintic activity in comparison to standard Mebendazole. The synthesized compounds 1b, 2b, and 8b possessed significant *in-vitro* cytotoxicity against HEp2 cell lines.

Keywords
Imidazoles, Anthelmintic activity, Cytotoxicity

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INTRODUCTION

Due to the advances in modern medicine, overall life expectancy is being greatly extended; however, the unintended consequence is a large increase in reduced immune system and immunocompromised cancer and organ transplant patients. This extension of life expectancy resulted in a lot of opportunistic fungal and bacterial infections often leading to patient mortality. Imidazole and indole based anticancer agents have been the mainstay of the treatments for cancer. With the advent of resistance to many of the clinically used drugs, novel candidate compounds that can overcome the resistance are urgently required. In this regard, we envisioned to develop novel imidazole and Indole based small molecules that could be easily synthesized and densely functionalized for structure activity relationship studies [1].

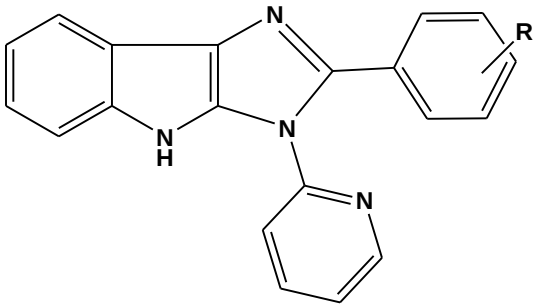
Synthesis of heterocyclic compounds from readily available reagents by simple and efficient method is the major requirements of heterocyclic chemistry. Imidazole-Indole combined to form newer tetra aryl imidazoles with higher efficacy and low side effects. The simplest member of the azole family is imidazole

with molecular formula $C_3H_4N_2$. The systemic name for the compound is 1, 3 diazole, one of the annular N having a H atom and can be regarded as a pyrole type Nitrogen & it is soluble in polar solvents. 'It exists in two equivalent tautomeric forms. The Indole having a benzene ring with pyrole nucleus taken from Isatin which provide link with imidazole to form newer tetra aryl imidazoles. Individually both imidazole and indole nucleus possess significant anticancer and anthelmintic agents, so we have made to synthesized newer tetra aryl imidazole which is a combination of imidazole and indole [2].

A survey of the pertinent literature discloses that, imidazole derivatives possess diverse biological activities independently from their synthetic interests. They are reported to display pharmacological activities such as antimicrobial [3,4], anthelmintic [5,6], cognitive enhancers [7,8], and anticancer [9-12]. Some of the best-selling therapies today contain this adaptable heterocycle in their core structures. So, it would be difficult to underestimate the importance of imidazoles in the pharmaceutical industry.

In 1858, Debus reported the reaction between glyoxal and ammonia, ever since this reaction became a novel route to the syntheses of imidazole derivatives. Later, a number of articles have described the syntheses of various imidazole derivatives [13-23]. In view of these observations, this study is, hence, focused to investigate the *in-vitro* cytotoxicity and anthelmintic activities of synthesized Imidazoles derivatives.

The synthesized compounds were evaluated for their anthelmintic and *in-vitro* anticancer activity.



(1b-8b)

MATERIAL AND METHOD

In-vitro cytotoxicity studies

Cell cultures were taken from National centre for cell sciences, Puna. HEp-2 cells were grown in Earl's Minimal essential medium supplemented with 2mM L-glutamine, 10% Fetal Bovine Serum, Penicillin (100µg/ml), Streptomycin (100µg/ml) and Amphoterecin B (5µg/ml) and the cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 and subculture two times a week. The compound shows significant cytotoxicity activity against these cell lines. Determinations of CTC50 value by SRB assay are given in results.

Anthelmintic studies

Anthelmintic activity studies were performed against earthworms at 2 mg/ml concentration using Garg and Atal method [24]. Suspensions of samples were produced by triturating synthesized compounds (100 mg) with Tween80 (0.5%) and distilled water and the resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of the test samples. Suspension of standard drug, mebendazole, was produced with the same concentration in a similar way. Three sets of five earthworms of almost similar sizes (2 inch in length) were placed in Petri plates of 4-inch diameter containing 50 ml of suspension of test sample and reference drug at room temperature. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and Tween 80 (0.5%). The paralyzing and death times were noted, and their mean was calculated for triplicate sets. The death time was checked by placing the earthworms in warm water (50°C) which stimulated the movement, if the worm was alive. Determination of paralyzing and death time is given in results.

General procedure for the preparation of newer tetra aryl imidazoles was completed in two steps as follows:

General procedure for the preparation of Schiff's bases (1a- 8a) Step-I

Same amounts (0.01 M) of pyridine-2-amine and substituted benzaldehydes were taken in to a 250 ml round bottom flask containing 15 ml of glacial acetic

acid & refluxed it for 6 h. The reaction mixture was allowed to cool to give the product. The reactions were monitored through TLC. The completed reactions were taken directly for the step-II.

General procedure for the preparation of Newer imidazoles (1b- 8b) Step-II

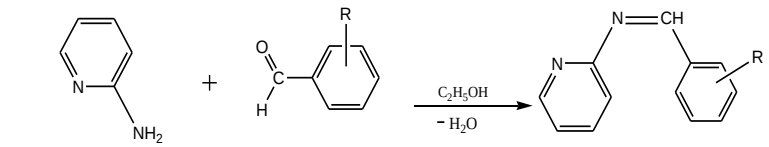
Isatin (0.01M) was taken along with ammonium acetate (0.1 M) into a flask containing the Schiff's base (~0.01M) obtained by step-I. The reaction mixture was refluxed with stirring on heating plate with magnetic stirrer for about 11-13 h. The reaction was monitored through TLC.

The reaction mixture was cascaded into 250 ml of water to remove excess of ammonium acetate and acetic acid then it was filtered and dried in hot air oven. The product was washed with 2 x 20 ml of benzene to remove traces of any unreacted isatin and products were recrystallized by ethyl acetate give the corresponding novel imidazoles 1b—8b **(Table 1)**.

On the basis of above facts (as illustrate in the introduction) that the novel series of synthesized derivative of aryl imidazoles containing Indole moiety may yield compounds with high therapeutic potential. The newer compounds were analyzed and illustrated by physical and analytical data.

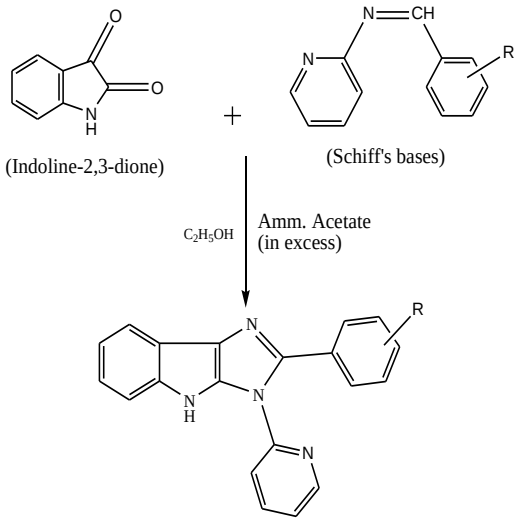
<SCHEME>

Step-I:



(Pyridin-2-amine) / (Primary aromatic amine) (Sustituted Benzaldehyde) (Schiff's bases)

Step-II:



(Indoline-2,3-dione) (Schiff's bases)

(Tetraaryl imidazoles)

2.1 3, 4-Dihydro-2-(3-Nitrophenyl)-3-(pyridine-2-yl) imidazo[4,5-*b*] indole **(1b)**

Yellow solid, Mp (ºC): 123-124; R_f value: 0.63; IR (KBr): 3322.12 (N–H), 3082.04 (Ar C-H), 1595.09 (C=N), 1151.42 (C-N), 858.77 (C-N stretching for NO_2), cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 355.96 (M+1 peak calculated, 355.35); Anal. Calcd. for: C, 67.52; H, 3.69; N, 19.70; O, 9.00. Found: C, 67.48; H, 3.57; N, 19.68; O, 8.97.

2.2 2-(3,4-dihydro-3-(pyridin-2-yl)imidazo[4,5-b]indol-2-yl)phenol **(2b)**

Yellow solid, Mp (ºC): 127-128; R_f value: 0.71; IR (KBr): 3570.42 (O-H), 3330.71 (N–H), 3080.17 (Ar C-H), 1560.10 (C=N), 1195.78 (C-N), cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable), 4.9 (s, 1H, OH, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 326.01 (M+1 peak calculated, 326.35); Anal. Calcd. for: C, 73.62; H, 4.32; N, 17.17; O, 4.08. Found: C, 73.59; H, 4.29; N, 17.12; O, 4.05.

2.3 3-(3,4-dihydro-3-(pyridin-2-yl)imidazo[4,5-b]indol-2-yl)phenol **(3b)**

Yellow solid, Mp (ºC): 118-119; R_f value: 0.68; IR (KBr): 3570.42 (O-H), 3330.71 (N–H), 3080.17 (Ar C-H), 1560.10 (C=N), 1195.78 (C-N), cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable), 4.9 (s, 1H, OH, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 326.01 (M+1 peak calculated, 326.35); Anal. Calcd. for: C, 73.63; H, 4.32; N, 17.17; O, 4.09. Found: C, 73.59; H, 4.29; N, 17.12; O, 4.05.

2.4 2-(2-chlorophenyl)-3,4-dihydro-3-(pyridin-2-yl)imidazo[4,5-b]indole **(4b)**

Pale solid, Mp (ºC): 137-138; R_f value: 0.62; FTIR (KBr): 3328.83 (N–H), 3063.62 (Ar C-H), 1569.66 (C=N), 1151.42 (C-N), 756.04 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 344.02 (M+1 peak calculated, 344.8); Anal. Calcd. for: C, 69.65; H, 3.80; Cl, 10.26; N, 16.25. Found: C, 69.63; H, 3.76; Cl, 10.22; N, 16.20.

2.5 2-(4-chlorophenyl)-3,4-dihydro-3-(pyridin-2-yl)imidazo[4,5-b]indole **(5b)**

Yellow solid, Mp (ºC): 134-135; R_f value: 0.64; FTIR (KBr): 3328.83 (N–H), 3063.62 (Ar C-H), 1569.66 (C=N), 1151.42 (C-N), 756.04 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 344.02 (M+1 peak calculated, 344.8); Anal. Calcd. for: C, 69.66; H, 3.80; Cl, 10.28; N, 16.25. Found: C, 69.63; H, 3.76; Cl, 10.22; N, 16.20.

2.6 2-(3-chlorophenyl)-3,4-dihydro-3-(pyridin-2-yl)imidazo[4,5-b]indole **(6b)**

Pale solid, Mp (ºC): 141-142; R_f value: 0.67; FTIR (KBr): 3328.83 (N–H), 3063.62 (Ar C-H), 1569.66 (C=N), 1151.42 (C-N), 756.04 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 344.02 (M+1 peak calculated, 344.8); Anal. Calcd. for: C, 69.67; H, 3.80; Cl, 10.27; N, 16.25. Found: C, 69.63; H, 3.76; Cl, 10.22; N, 16.20.

2.7 3, 4-Dihydro-2-(4-Nitrophenyl)-3-(pyridine-2-yl) imidazo[4,5-*b*] indole **(7b)**

Yellow solid, Mp (ºC): 121-122; R_f value: 0.59; IR (KBr): 3322.12 (N–H), 3082.04 (Ar C-H), 1595.09 (C=N), 1402.15 (NO_2), 1151.42 (C-N), 858.77 (C-N stretching for NO_2), cm^{-1}; ^1H NMR (DMSO-d_6): δ = 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 355.96 (M+1 peak calculated, 355.35); Anal. Calcd. for: C, 67.54; H, 3.69; N, 19.71; O, 9.00. Found: C, 67.48; H, 3.57; N, 19.68; O, 8.97.

2.8 3,4-dihydro-2-(3-methoxyphenyl)-3-(pyridin-2-yl)imidazo[4,5-b]indole **(8b)**

Yellow solid, Mp (ºC): 144-145; R_f value: 0.74; FTIR (KBr): 3350.76 (N–H), 3072.39 (Ar C-H), 2925.81 (Ali. C-H), 1593.09 (C=N), 1236.29 (C-N), 1149.50 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 3.7 (s, 3H, OCH_3), 7.12-8.48 (m, 12H, Ar-H), 10.1 (s, 1H, N-H, D_2O exchangeable) ppm; MS (ESI) m/z: M+1 peak found, 340.02 (M+1 peak calculated, 340.38); Anal. Calcd. for: C, 74.10; H, 4.74; N, 16.46; O, 4.70. Found: C, 74.08; H, 4.71; N, 16.42; O, 4.67.

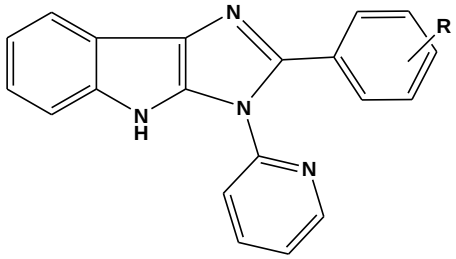
RESULTS AND DISCUSSION

The results and discussion for the synthesis substituted novel imidazoles are as follows:

Firstly, the pyridine-2-amine was condensed with substituted benzaldehydes afforded the corresponding Schiff's base. To produce aryl imidazoles, the Schiff's base was further directly reacted with ammonium acetate and isatin (cyclization steps involving diketone) in the presence of glacial acetic acid as a solvent, gave a corresponding novel imidazoles 1b—8b. On the basis of literature, the novel series of synthesized derivative of aryl imidazoles containing Indole moiety may yield compounds with high therapeutic potential.

Structures of all the newly synthesized imidazoles were confirmed by FTIR, ^1H NMR and Mass spectral analysis. The IR spectra of the newly synthesized compounds showed the presence of characteristic absorption in the region 3310-3350 cm^{-1} for N-H in NH_2, 3012-3096 cm^{-1} for aromatic C-H stretching, 1500-1600 cm^{-1} for C=N stretching respectively. ^1H NMR spectra of synthesized compounds showed the characteristic peaks in the region 6.67-7.80 ppm for aromatic protons and 10.1-11.0 ppm for N-H.

Table 1: Data of all synthesized novel imidazoles (1b-8b)



(1b-8b)

Compound name	R	Molecular Formula	Molecular Weight	Reaction Time (hr)	% Yield
1b	3-nitro	$C_{20}H_{13}N_5O_2$	355.35	11.5	64.82
2b	2-hydroxy	$C_{20}H_{14}N_4O$	326.35	12	58.24
3b	3-hydroxy	$C_{20}H_{14}N_4O$	326.35	11.5	55.32
4b	2-chloro	$C_{20}H_{13}ClN_4$	344.8	12.5	66.36
5b	4-chloro	$C_{20}H_{13}ClN_4$	344.8	12	65.25
6b	3-chloro	$C_{20}H_{13}ClN_4$	344.8	13	64.38
7b	4-nitro	$C_{20}H_{13}N_5O_2$	355.35	11	65.48
8b	3-methoxy	$C_{21}H_{16}N_4O$	340.38	12.5	62.32

The synthesized compounds were screened for *in vitro* cytotoxicity studies against HEp2 cell lines by SRB assay. The compounds 1b, 2b, and 8b shows significant cytotoxicity against HEp2 cell lines in comparison to standard 5-Fluorourecil. Presence of phenolic group in compound 2b and 3b significantly affect activity due to the binding capability to the cytoplasmic hormone receptors [25]. Compound 7b containing sulfur in its structure decreases the melting temperature of DNA in EAC cells and thereby showing significant activity. Presence of methoxy group also increases the prospects of compound 8b. Compounds 4b, 5b and 6b having the chlorine in their structure showing the compounds more toxic as compare with other. Anticancer activities of all the synthesized compounds at the concentrations of 500, 250, 125, 62.5, 31.25m g/ml were performed. The percentage growth inhibition was calculated by using the following formula: - % growth inhibition= [(total cells-live cells)*100]/total cells. The results of determination of CTC50 value by SRB method are given in **table 2**.

Table 2: Determination of CTC_{50} value by SRB assay

S. No	Sample No.	CTC_{50}c value (µg/ml)
1.	1B	36.25
2.	2B	32.41
3.	3B	36.32
4.	4B	93.45
5.	5B	>150
6.	6B	115.23
7.	7B	48.41
8.	8B	34.46
9.	5-Fluorouracil	31.76

Anthelmintic activity of the synthesized novel imidazoles were accomplishes against *Eudrilus* species at 4mg/ml concentration. All the novel imidazoles showed good activity at 100mg in tween 80 (0.5%) and distilled water. Comparison of anthelmintic data revealed that derivative 1b, 2b, 4b, 6b, and 8b possessed higher activity in comparison to standard Mebendazole.

Presence of nitro and methoxy group in compounds 1b and 8b make them more potent with low toxicity and several times more potent than Mebendazole. Substituent's at R' position was introduced to prevent metabolic inactivation. Presence of hydroxyl group in compound 2b and 3b make them lacking side effects. Hydroxyl and carboxyl group are accountable for inhibition of respiration and blocking

glucose absorption by the intestinal adult worms. The overall results are given in **table 3**.

Table 3: Anthelmintic Activity od synthesized compound

Compound No.	Mean paralyzing time (min) *Eudrilus species*	Mean death time (min) *Eudrilus species*
1b	19.75 ± 0.50	27.35 ± 0.50
2b	22.38 ± 0.76	28.80 ± 0.76
3b	20.36 ± 0.57	29.58 ± 0.76
4b	17.40 ± 0.50	27.61 ± 0.57
5b	26.31 ± 0.57	38.83 ± 0.76
6b	20.00 ± 1.00	28.35 ± 0.57
7b	26.63 ± 0.76	40.33 ± 0.57
8b	20.33 ± 1.52	29.00 ± 1.00
Control	-	-
Mebendazole	24.83 ± 0.76	29.33 ± 1.52

In the fig 1 the paralysis time of helminthes (*Eudrilus* sp.) was plotted against taking standard mebendazole. In the fig 2 the death time of helminthes was plotted against mebendazole as standard.

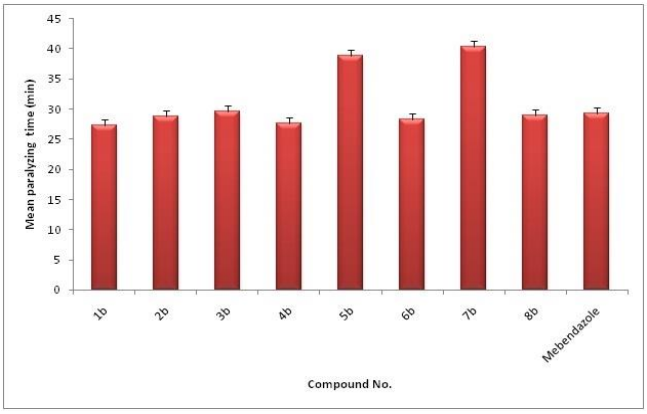


Fig 1: Paralysis time of helminthes

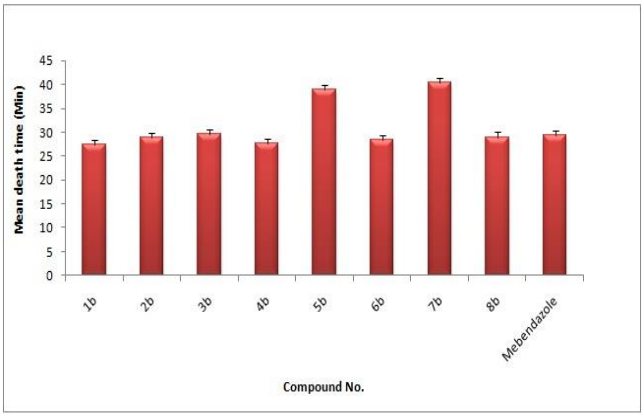


Fig 2: Death time of helminthes

CONCLUSION

All the novel imidazoles were analyzed by physical and analytical data and evaluated for *in-vitro* cytotoxicity and anthelmintic screening. All the synthesized novel imidazoles have shown moderate to good anthelmintic activity. The compounds 1b, 2b, 4b, 6b, and 8b acquired higher activity in comparison to standard Mebendazole. The synthesized novel

imidazoles obtained significant *in-vitro* cytotoxicity against HEp2 cell lines.

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