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Synthesis And Evaluation Of The Anti-Microbial Activity Of Some Schiff Bases Of *1H*-Indazole

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Abstracts

The objective of this work was to perform the preparation of indazole nucleus and condense it with various aldehydes to obtain Schiff's bases and evaluate the *in vitro* antimicrobial action of these compounds against bacteria. The preparation of the target compounds ($\mathbf{SB}_{\mathbf{a\text{-}j}}$) was done by condensation of 2,4-dichloro benzonitrile with hydrazines to yield *1H*-imidazolyl followed by the imine linkage formation by interaction with the active carbonyl group of the aromatic aldehyde. The compounds were checked for solubility, melting point, and yield while the structural confirmation was done by IR and ^{1}H-NMR. The antibacterial action of the prepared imidazolyl Schiff bases was tested against *E. coli*, *P. aeruginosa, B. subtilis,* and *S. aureus.* The zone of inhibition obtained from the disc diffusion method was used as the indication of antibacterial action.

Keywords: Indazole, Schiffs base, antimicrobial, disc diffusion, zone of inhibition, hydrazine

Introduction

Mounting drug resistance between bacterial strains is the propulsion for new molecules that are effective against them[1]. Continual research for antimicrobial and antitubercular agents fascinates medicinal chemists who strive towards the development of molecules that might possess potential actions, broad spectrum, and harmless therapeutic profiles in comparison to the presently available agents.

Indazoles are isomeric heterocyclic compounds having chemical formula $C_7H_6N_2$, and have pyrazole condensed with benzene ring[2]. Indazole is a significant heterocyclic nucleus in medicinal research owing to its association with a vivid range of biological actions including anti-inflammatory, antiprotozoal, antihypertensive, anticancer, antitumor, antifungal, antibacterial, anti-HIV, and antiplatelet among others[3-6].

Quite a lot of reports have spelled out the significance of Schiffs bases as antimicrobial and antitubercular agents[7]. Moreover, the antimicrobial and antitubercular activity of indazole nuclei with a broad range of substituted functional groups has also been reported[8,9]. Taking these considerations into account, synthesis of the Schiffs base with indazole nucleus was envisaged to possess good antibacterial action.

Material: The materials utilized in the research work included a variety of chemicals and reagents sourced from reputable suppliers. These included 2,4-dichlorobenzonitrile and phenyl hydrazine from Avra, diglyme and potassium butoxide from CDH, hydrazine from Sigma, and benzaldehyde and 2-chloro benzaldehyde from SD Fine. Additionally, various benzaldehyde derivatives such as 2-nitro, 3-nitro, and 4-hydroxy benzaldehyde were obtained from CDH, along with ethanol from Merck, and solvents like ethyl acetate, petroleum ether, and chloroform from Rankem. Nutrient agar media and nutrient broth media, used for microbiological studies, were supplied by Microgen. All glassware employed during the experiments was of borosilicate grade, ensuring durability and resistance to chemical reactions. Prior to use, the glassware was thoroughly cleaned using a chromic acid cleaning mixture, followed by rinsing with distilled water and drying in a hot air oven. Precoated TLC plates were used throughout the study to monitor the progress of reactions effectively.

Methodology: In the present work, the synthesis of a few indazole derivatives was achieved using a modified synthetic strategy reported by Esmaeili-Marandi et al[11]. The derivatization of indazole to yield Schiff's was achieved using the method reported by Malviya et al[35]. The synthetic scheme is presented in Figure 1.

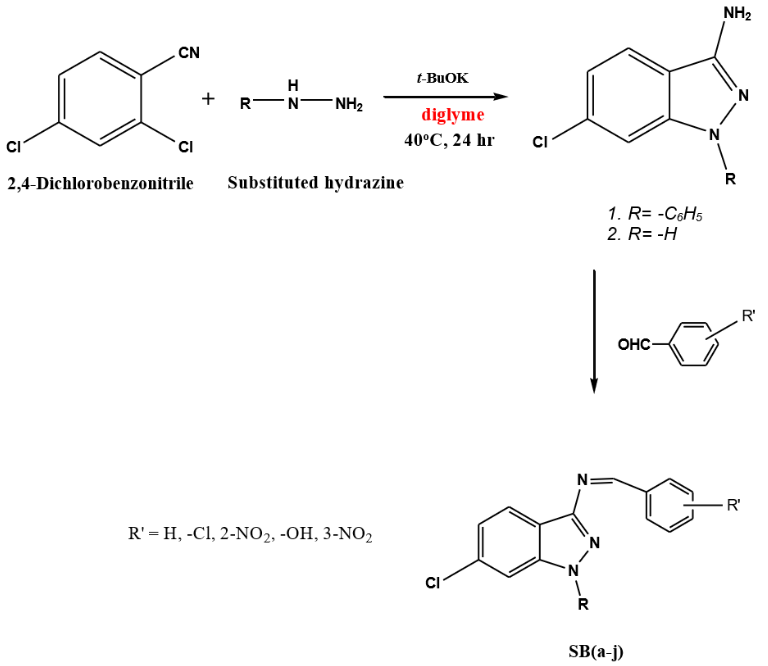

Figure1: Synthetic scheme for indazole derivatives

The synthesis was achieved in two steps:

Step 1: Synthesis of indazole
Step 2: Preparation of Schiff's base

A general method for synthesis of *1H*-Indazole: 2,4-dichloro benzonitrile (0.01 mol) was dissolved in 20 mL of diglyme and potassium- tert- butoxide (0.01 mol) was added to it. To the mixture was added substituted hydrazine (0.01 mol) and the reaction mixture was refluxed at 100°C for 2 to 3 h. The excess of hydrazine was removed from the reaction mixture by evaporating at 50°C. The crude product was filtered and recrystallized from hot ethanol to obtain the indazole derivative. The completion of the reaction was monitored by TLC using petroleum ether-ethyl acetate (7:3) as the developing solvent. The spots were visualized under UV light using a TLC cabinet.

Synthesis of 6-chloro-1H-indazol-3-amine: 2,4-dichloro benzonitrile (0.01 mol) was dissolved in 20 mL of diglyme and potassium- tert- butoxide (0.01 mol) was added to it. To the mixture was added a slight excess of hydrazine hydrate (0.012 mol) and the reaction mixture was refluxed at 100°C for 2 to 3h. The excess of hydrazine was removed from the reaction mixture by evaporating at 50°C. The crude product was filtered and recrystallized from hot ethanol to obtain the indazole derivative. The completion of the reaction was monitored by TLC using petroleum ether-ethylacetate (7:3) as the developing solvent. The spots were visualized under UV light using a TLC cabinet.

Synthesis of 6-chloro-1-phenyl-1H-indazol-3-amine: 2,4-dichloro benzonitrile (0.01 mol) was dissolved in 20 mL of diglyme and potassium- tert- butoxide (0.01 mol) was added to it. To the mixture was added a slight excess of phenylhydrazine (0.012 mol) and the reaction mixture was refluxed at 100°C for 2 to 3h. The crude product was filtered, and washed with cold water to remove excess of phenylhydrazine. The crude product obtained was recrystallized from hot ethanol to obtain the indazole derivative. The completion of the reaction was monitored by TLC using petroleum ether-ethyl acetate (7:3) as the developing solvent. The spots were visualized under UV light using a TLC cabinet.

General method for synthesis of 1H-Indazolyl Schiff's base (SBa-j): To a solution of 1H-indazole/1-phenyl-1H-indazole (0.01 mol) in ethanol (60 mL), substituted aromatic aldehyde (0.01 mol) and with a few drops of glacial acetic acid were added. The resulting mixture was then refluxed for 7- 8 hours. The excess of the ethanol was distilled off and the remaining mixture was cooled, poured onto crushed ice, and filtered. The completion of the reaction was monitored by TLC using petroleum ether-ethyl acetate (7:3) as the developing solvent. The spots were visualized under UV light using a TLC cabinet. The crude product obtained was recrystallized by using hot ethanol.

Synthesis of (Z)-N-benzylidene-6-chloro-1H-indazol-3-amine (SBa): To a solution of 1H-indazole (0.01mol) in ethanol (60mL), benzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(2-chlorobenzylidene)-6-chloro-1H-indazol-3-amine (SBb): To a solution of 1H-indazole (0.01mol) in ethanol (60mL), 2-chlorobenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(2-nitrobenzylidene)-6-chloro-1H-indazol-3-amine (SBc): To a solution of 1H-indazole (0.01mol) in ethanol (60mL), 2-nitrobenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(3-nitrobenzylidene)-6-chloro-1H-indazol-3-amine (SBd): To a solution of 1H-indazole (0.01mol) in ethanol (60mL), 3-nitrobenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(4-hydroxy benzylidene)-6-chloro-1H-indazol-3-amine (SBe): To a solution of 1H-indazole (0.01mol) in ethanol (60mL), 4-hydroxybenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-benzylidene-6-chloro-1-phenyl-1H-indazol-3-amine (SBf): To a solution of 1-phenyl-1H-indazole (0.01mol) in ethanol (60mL), benzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(2-chlorobenzylidene)-6-chloro-1-phenyl-1H-indazol-3- amine (SBg): To a solution of 1-phenyl-1H-indazole (0.01mol) in ethanol (60mL), 2- chlorobenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(2-nitrobenzylidene)-6-chloro-1-phenyl-1H-indazol-3- amine (SBh): To a solution of 1-phenyl-1H-indazole (0.01mol) in ethanol (60mL), 2-nitrobenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(3-nitro benzylidene)-6-chloro-1-phenyl-1H-indazol-3- amine (SBi): To a solution of 1-phenyl-1H-indazole (0.01 mol) in ethanol (60 mL), 3- nitrobenzaldehyde (0.01 mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Synthesis of (Z)-N-(4-hydroxy benzylidene)-6-chloro1-phenyl-1H-indazol-3- amine (SBj): To a solution of 1-phenyl-1H-indazole (0.01mol) in ethanol (60mL), 4- hydroxybenzaldehyde (0.01mol) and with a few drops of glacial acetic acid were added and the product was obtained using the above-mentioned procedure.

Chemical Characterization of the Synthesized Compounds: The indazole derivatives were characterized for solubility, yield, physical appearance, melting point, and spectral characteristics. The melting points were determined on the melting point apparatus and are uncorrected. IR spectra are recorded on Bruker spectrophotometer, and mass and proton NMR spectra were recorded on the Jeol system.

Antimicrobial action: Bacterial strains used in this study are *E. coli* MTCC 3261, *P. aeruginosa* MTCC 647 as Gram-negative bacteria and *B. subtilis* MTCC 1134, *S. aureus* MTCC 3382 as the Gram-positive strains. The MTCC strains of microorganisms used in this study were obtained from the Institute of Microbial Technology, Chandigarh, India.

Preparation of Nutrient broth:

- Nutrient broth powder – 37.2 g
- Distilled water - 1000 ml

The nutrient broth was prepared by dissolving all the ingredients and adjusting the pH to 7.2 and autoclaved at 15 lbs. pressure for 20 min in an autoclave. One day before the testing, the microorganisms were sub-cultured into sterile nutrient broth and incubated at 37°C for 24 h. The culture growth thus obtained was used as inoculum for the antibacterial testing.

Preparation of nutrient agar media: The nutrient agar media was prepared by using the following ingredients.

- Nutrient agar – 28.6 g
- Distilled water - 1000 ml

The specified amount of nutrient agar powder was dissolved by heating a water bath and the volume of the final solution was made up to 1000 ml with distilled water. The above

Prepared nutrient agar media was sterilized by autoclave at 121°C for 20 minutes at 15 lbs pressure.

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Preparation of test solutions: The synthesized Schiff's bases were dissolved in dimethyl sulfoxide (DMSO) and further dilutions of the test compounds were prepared in nutrient broth to obtain concentrations of 100, 50, and 25 µg/mL.

Antimicrobial Assay: All the synthesized compounds were evaluated for antimicrobial action using the disc diffusion method. The lyophilized cultures for all the bacterial strains were revived in nutrient broth by incubation for 24 h at 37 ± 1°C. Ciprofloxacin (10µg/mL) was used as the standard drug. Discs impregnated with the test solution and standard drug were prepared by soaking a 1cm diameter circular disc made of Whatman filter paper with the 1 mL solution of the respective compounds. The sterilized media (nutrient agar) was cooled to 45°C with gentle shaking for uniform cooling and then inoculated with 18-24 h old bacterial subculture under aseptic conditions in a laminar airflow bench and mixed well by gentle shaking. This was poured into sterile Petri dishes and allowed to set. After solidification, the impregnated discs were placed on top of the nutrient agar, and the plates were kept in the laminar airflow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The zone of inhibition after 24 h was measured in millimeters (mm).

RESULTS AND DISCUSSION

Chemical Characterization: The synthesized compounds were subjected to determination of yield, melting point, solubility, and structure elucidation. The physicochemical properties are shown in Tables 1 and 2.

Table 1: Yield and color of synthesized compounds

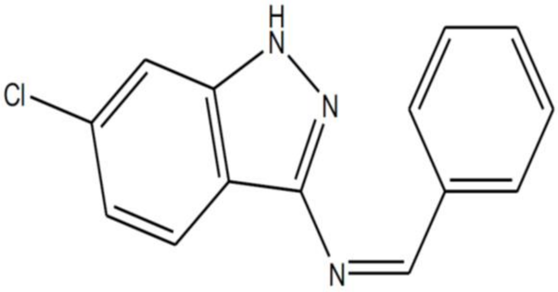
Compound	Melting point (°C)	Yield (%)	Color
SBa	114-117	71	Yellow
SBb	96-99	73	Brownish Yellow
SBc	115-116	69	Yellow
SBd	79-81	75	Yellow
SBe	96-98	71	Brown
SBf	106-108	71	Yellow
SBg	87-89	72	Yellow
SBh	117-120	73	Pale yellow
SBi	114-116	71	Yellow
SBj	95-97	70	Yellow

Table 2: Solubility characteristics of the indazole Schiff's bases

Compound	Water	Ethanol	Chloroform
SBa	Insoluble	Soluble	Soluble
SBb	Insoluble	Soluble	Soluble
SBc	Insoluble	Soluble	Soluble
SBd	Insoluble	Soluble	Soluble
SBe	Insoluble	Soluble	Soluble
SBf	Insoluble	Soluble	Soluble
SBg	Insoluble	Soluble	Soluble
SBh	Insoluble	Soluble	Soluble
SBi	Insoluble	Soluble	Soluble
SBj	Insoluble	Soluble	Soluble

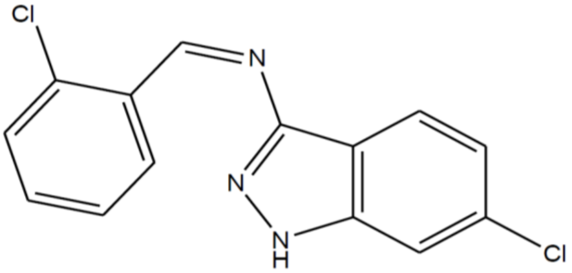
Structure Elucidation: The structure elucidation of the synthesized compounds was confirmed by interpretation of the IR, ^{1}HNMR, and Mass spectra of the compounds. The IR spectra were observed for the characteristic peaks obtained due to the presence of the functional groups. All the compounds exhibited the peaks of aromatic C-H bending, C-N, and C=N stretching. The vibrational stretch or bends for C-O, O-H, and N-O could also be located in the spectra. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen and imine hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH.

SBa



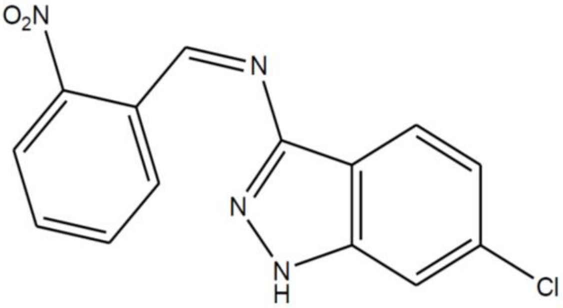
S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 5.3-6.5 H of C=C	1693.49-1770.55	Ar C-H bend
2		1647.07	C=N Str
3		1456.90	N-N Str
4		1340.77	C-N Str (Ar amine)
5		815.57	C-Cl str

SBb



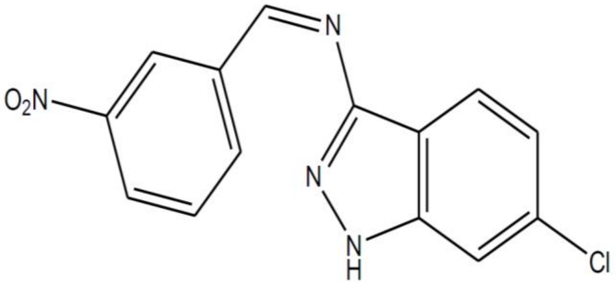
S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H	1693.44-1990.57	Ar C-H bend
2		1647.13	C=N Str
3		1464.71	N-N Str
4		1340.88	C-N Str (Ar amine)
5		844.74	C-Cl str

SBc



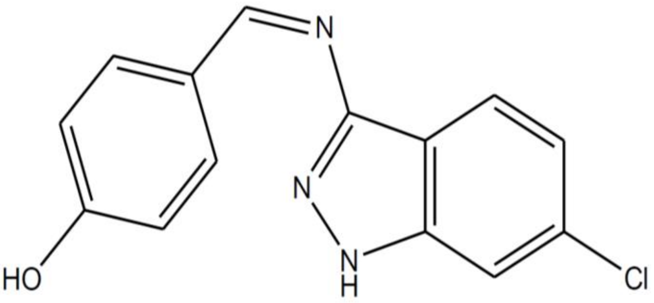
S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 8.2 H adj to NO_2	1693.25-1916.86	Ar C-H bend
2		1647.26	C=N Str
3		1464.53	N-N Str
4		1316.03	C-N Str (Ar amine)
5		749.73	C-Cl str
6		1515.78	N-O str

SBd



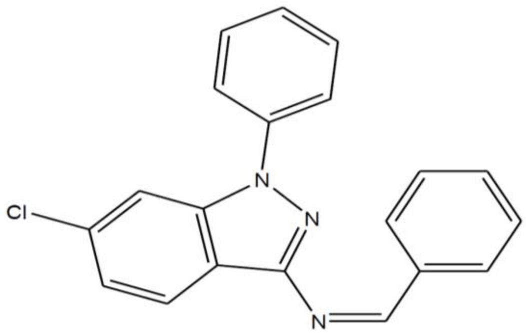
S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 8.2 H adj to NO_2	1708.73-1917.10	Ar C-H bend
2		1647.21	C=N Str
3		1463.48	N-N Str
4		1340.25	C-N Str (Ar amine)
5		812.87	C-Cl str
6		1516.01	N-O str

SBe



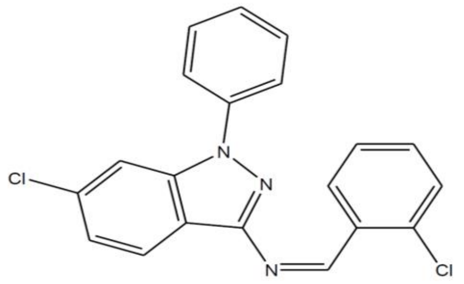
S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 6.8 H adj to OH, 5.0 OH	1693.40-1990.87	Ar C-H bend
2		1647.00	C=N Str
3		1464.69	N-N Str
4		1340.69	C-N Str (Ar amine)
5		749.91	C-Cl str
		3743.60	O-H str

SBf



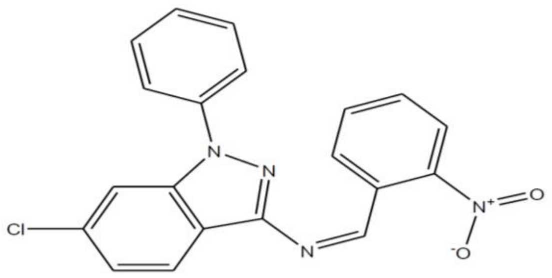
S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.2-7.69 Ar H, 8.1 imine H	1693.48-1917.02	Ar C-H bend
2		1647.33	C=N Str
3		1463.99	N-N Str
4		1340.18	C-N Str (Ar amine)
5		804.81	C-Cl str

SBg



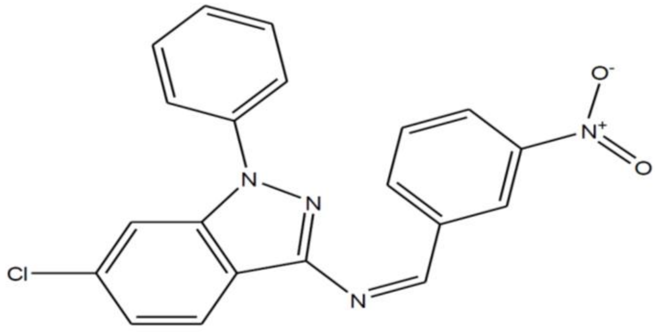
S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.69 Ar H, 8.1 imine H	1693.00-1917.05	Ar C-H bend
2		1646.68	C=N Str
3		1464.44	N-N Str
4		1366.62	C-N Str (Ar amine)
5		667.06	C-Cl str.

SBh



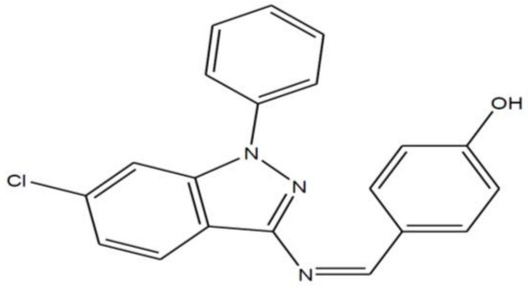
S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 8.2 H adj to NO_2	1706.61-1990.95	Ar C-H bend
2		1646.58	C=N Str
3		1464.52	N-N Str
4		1340.25	C-N Str (Ar amine)
5		752.99	C-Cl str
6		1515.62	N-O str

SBi



S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 8.2 H adj to NO_2	1693.46-1916.96	Ar C-H bend
2		1646.95	C=N Str
3		1464.39	N-N Str
4		1340.42	C-N Str (Ar amine)
5		751.20	C-Cl str
6		1515.82	N-O str

SBj



S. No.	NMR signals (ppm relative to TMS)	Wave number (Cm^{-1})	Due to…
1	7.2-7.9 Ar H, 8.1 imine H, 6.8 H adj to OH, 5.0 OH	1706.64-1916.91	Ar C-H bend
2		1625.37	C=N Str
3		1464.25	N-N Str
4		1339.24	C-N Str (Ar amine)
5		762.13	C-Cl str
6		3678.44	O-H str

Antibacterial action

The antibacterial activity of the synthesized compounds against four tested microorganisms was determined. The zone of inhibition was obtained as the primary indicator of the antibacterial action. Tables 3 and 4 present the zone of inhibition obtained for the indazole Schiff's bases Figures 1 and 2 represent the comparative zone of inhibition obtained from the test compounds and standard at the three different concentrations in different bacteria.

Table 3: Zone of inhibition by compounds SB(a-e)

Compound	***Conc (µg/mL)***	**Zone of inhibition (mm)**			
		E. coli	***B.subtilis***	***P.aeuroginosa***	***S.aureus***
SBa	25	9	9	10	10
	50	10	10	10	10
	100	11	11	13	13
SBb	25	11	10	12	11
	50	13	11	13	13
	100	14	13	14	14
SBc	25	10	11	11	11
	50	11	12	12	11
	100	12	12	13	13
SBd	25	10	10	10	10
	50	10	11	10	10
	100	11	12	12	12
SBe	25	13	13	14	14
	50	14	15	15	16
	100	14	15	16	16
Ciprofloxacin	10	17	19	19	18

Table 4: Zone of inhibition by compounds SB(f-j)

Compound	***Conc (µg/mL)***	**Zone of inhibition (mm)**			
		E. coli	***B.subtilis***	***P.aeuroginosa***	***S.aureus***
SB f	25	10	11	11	11
	50	11	12	12	11
	100	12	12	13	13
SB g	25	14	13	13	14
	50	14	16	15	15
	100	14	15	16	16
SB h	25	12	12	11	12
	50	13	13	13	12
	100	13	14	15	13
SB i	25	10	10	10	10
	50	10	11	11	10
	100	12	12	11	11
SB j	25	14	13	14	15
	50	15	15	14	16
	100	15	15	16	16
Ciprofloxacin	10	17	19	19	18

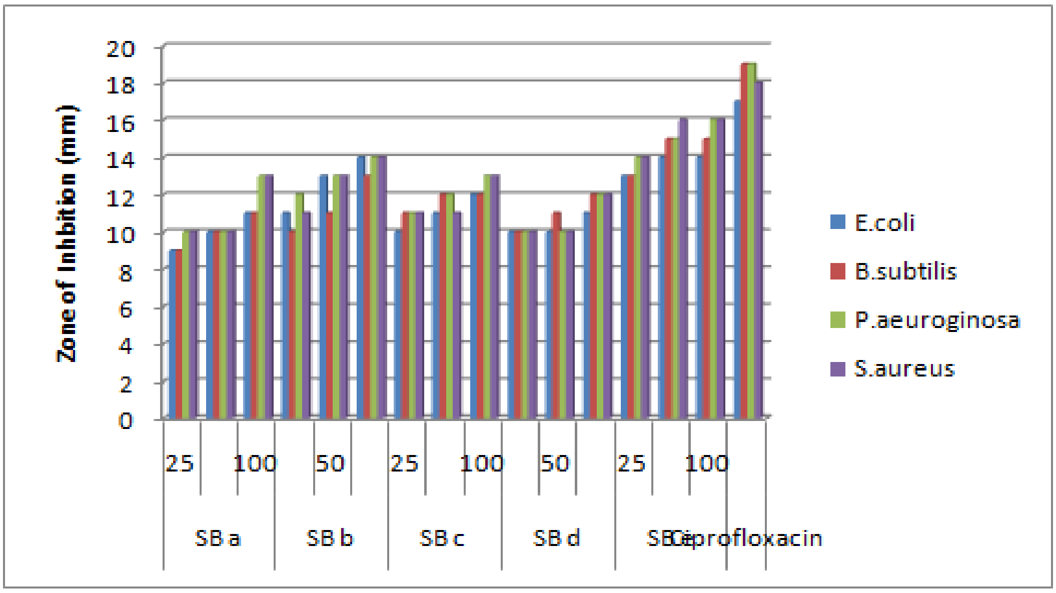


Figure 2: Comparison of the zone of inhibition exhibited by SB(a-e)

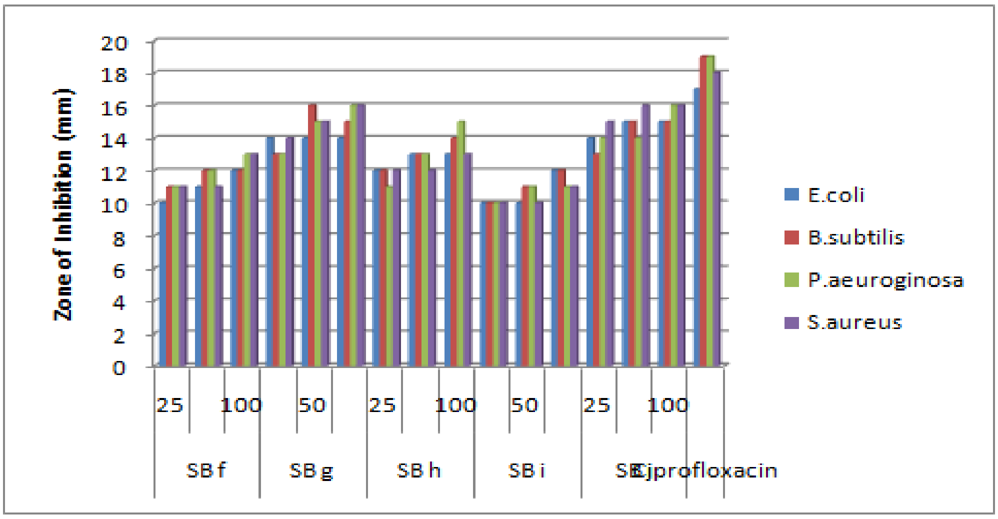


Figure 3: Comparison of the zone of inhibition exhibited by SB(f-j)

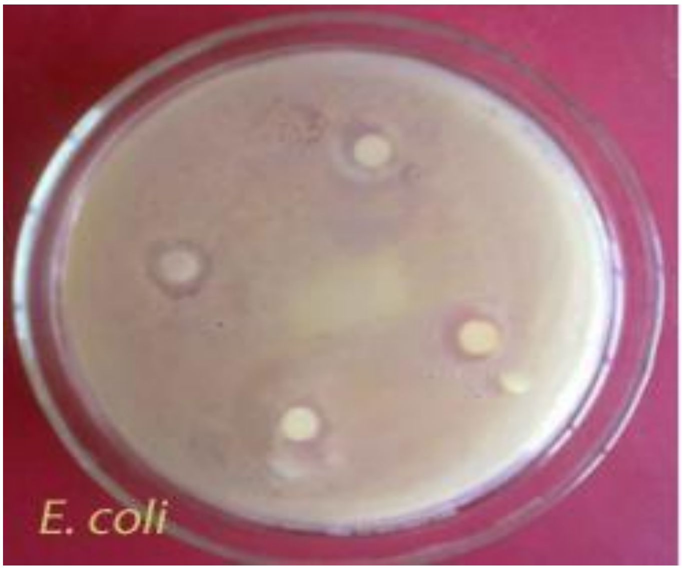


Figure 4: Plate showing growth inhibition of *Escherichia coli* by SBa

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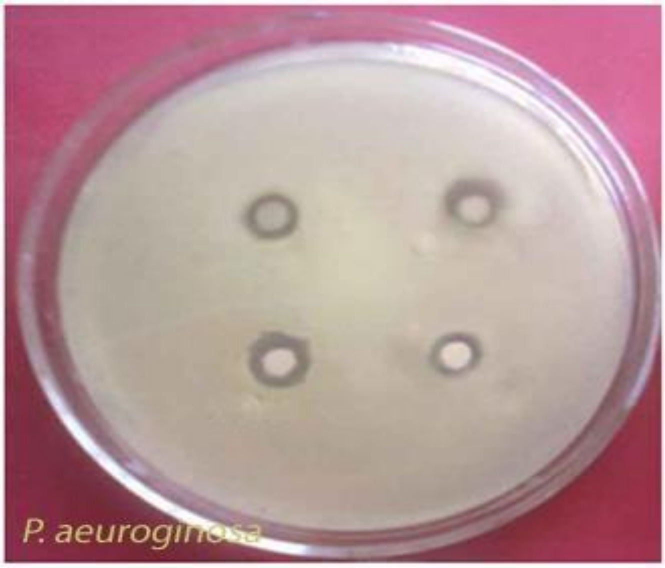

Figure 5: Plate showing growth inhibition of *Pseudomonas aeuroginosa* by SBa

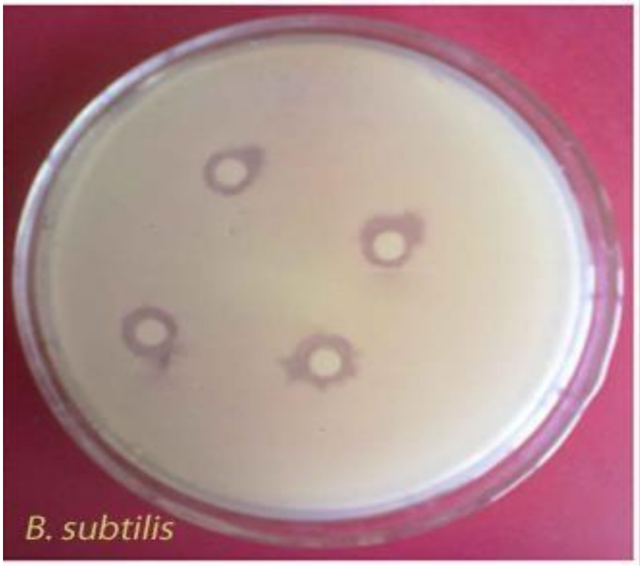

Figure 6: Plate showing growth inhibition of *Bacillus subtilis* by SBa

Figure 7: Plate showing growth inhibition of *Staphylococcus aureus* by SBa

The results of antibacterial testing reveal the importance of substitution on activity. It was found that the compounds having electron-withdrawing substitutions (**SBb, SBe, SBg & SBi**) on the phenyl ring exhibited higher activity than those either unsubstituted or having electron-donating substitution. It was also found that 1-phenyl- indazolyl derivatives had a higher zone of inhibition than the 1-indazolyl Schiff's bases. The presence of the phenyl ring on the 1-position of indazole might have caused an increase in permeation of the molecules across the bacterial cell membrane thereby causing higher activity.

SUMMARY AND CONCLUSION

Summary

The objective of the present investigation was to synthesize the indazole nucleus and condense it with various aldehydes to obtain Schiff's bases and evaluate the *in vitro* antimicrobial action of these compounds against bacteria.

The synthesis of the target compounds (**SBa-j)** was achieved by condensation of 2,4—dichloro benzonitrile with hydrazine to yield imidazolyl, followed by the formation of the imine linkage by interaction with the active carbonyl group of the aromatic aldehyde. The compounds were characterized for solubility, melting point, and yield, and the structural confirmation was done by IR and ^{1}H-NMR.

The IR spectra exhibited the peaks of aromatic C=C stretching, C-H stretching, C-N, and C=N stretching. The vibrational stretch or bends for C-O, O-H, and N-O could also be located in the spectra. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen and imine hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH.

Antibacterial activity of the synthesized compounds against four microorganisms (*E. coli* MTCC 3261, *P. aeruginosa* MTCC 647 as Gram-negative bacteria, and *B. subtilis* MTCC 1134, *S. aureus* MTCC 3382) was determined. The zone of inhibition obtained from the disc diffusion method was obtained as the primary indicator of the antibacterial action.

The results of antibacterial testing reveal the importance of substitution on activity. It was found that the compounds having electron-withdrawing substitutions (**SBb, SBe, SBg & SBi**) on the phenyl ring were able to exhibit higher activity in comparison to those either unsubstituted or having electron-donating substitution. It was also found that 1-phenyl-indazole derivatives had a higher inhibition zone than the 1-indazolyl schiffs bases.

Conclusion

The objective of the present investigation was to develop newer antibacterial molecules containing the *1H*-imidazolyl nucleus. The objective was accomplished by preparing Schiff's base of *1H*-imidazolyl nucleus. A few of the synthesized Schiff's bases were able to exhibit anti-bacterial activity comparable to that of the standard drug ciprofloxacin used during the study against microbial growth (in vitro). It could be concluded from the study that the *1H*-indazolyl nucleus can be modified and various designing approaches may be used to prepare more potent congeners of *1H*-indazolyl that might be able to present activity even against the resistant strains.

Conflict of interest: The authors declare no conflict of interest.

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