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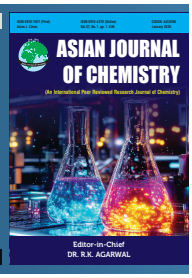

Synthesis, Characterization, *in vitro* Antimicrobial and Antiproliferative Potentials of Novel Carvacrol Based Schiff Base Derivatives

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A series of novel carvacrol based Schiff base derivatives (5-Cl, Nap, 3,5-Dibr, DEAS and 5-Br) were synthesized and characterized through FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods. The FT-IR confirmed the characteristic peak of imine (–CH=N–) at 1619-1514 cm^{-1}, while the NMR spectra confirmed the arrangement of aromatic and aliphatic protons and carbons with halogen substitutions. The synthesized Schiff Base derivatives had significant biological activity, particularly Nap, 5-Br, and 3,5-Dibr, which exhibited high cytotoxicity against the A-549 lung cancer cell line. Antimicrobial assay indicated a strong lethality in brine shrimp assays and acceptable antibacterial activity, especially for 5-Cl and 3,5-Dibr, with minimum inhibitory concentrations (MIC). These findings confirm the structural integrity and pharmacological significance of Schiff bases, highlighting their usefulness in the development of anticancer and antibacterial drugs.

Keywords: Carvacrol, Schiff base, Azomethine, Anticancer activity, Brine shrimp lethality assay.

INTRODUCTION

Carvacrol is a natural compound found in essential oils, specifically belonging to the monoterpene family a class that has a phenolic structure [1]. It is a food flavouring compound for human consumption that is widely considered to be safe (GRAS) [2]. Owing to its significant biological efficacy in the treatment of various diseases like hypertension and immune disorders system modulators, diabetes, cancer, *etc*. [3-6], this compound is in high demand. Carvacrol can also be synthesized in the laboratory using numerous ways, including chemical and microbiological procress involving genetically modified bacteria [7]. There were reports about derivatives of carvacrol, where various esters replaced the acidic proton (H^+) in carvacrol ethers and acetic acid functional groups showed antioxidant and cytotoxic effects opposed to HeLa cells [8].

Now days, drug resistance to antibiotics is becoming another emerging global issues and difficult to treat and contribute to a general increase in the morbidity and mortality. Thus, novel synthetic moieties with improved and tolerable therapeutic indices are urgently needed to address these issues [9]. The significance of Schiff base compounds has been widely acknowledged and discussed in various sectors [10-13]. The imine group (–CH=N–) in Schiff bases plays a crucial role in the synthesis of several new compounds, which exhibit extensive biological activity [14-16]. Schiff bases are frequently encountered in the organization of products found in nature. Thus, efforts are made to synthesize using new compounds comprising Schiff base derived from natural compounds for effective biological activites. In this work, a series of novel carvacrol-based Schiff bases was synthesized with substituted aldehydes and characterized with FT-IR, ^{1}H NMR and ^{13}C NMR. The antibacterial, antifungal and brine shrimp assays were also conducted on the synthesized compounds.

EXPERIMENTAL

Laboratory grade chemicals and solvents *viz.* carvacrol, sodium nitrite, substitued aldehydes, ammonia solution, ethanol, methanol and dimethyl sulfoxide were procured from Merck Ltd. India and used as such. ^{1}H & ^{13}C NMR were recorded on a Bruker AVANCE 400 spectrometer (Bruker Corporation, MA, USA) operating at 400 MHz and 25 ºC, with samples dissolved

in deuterated DMSO. Elemental analysis of carbon, nitrogen and hydrogen were carried out on a CHN elemental analyzer (Thermo Scientific). Infrared (IR) spectra were recorded at room temperature from 4000 to 500 cm^{-1} with KBr pellets, a using Thermo-Nicolet 8700 spectrometer (Thermo-Scientific).

Synthesis of carvacrol based Schiff base (1-5): To an ethanolic solution of carvacrol, sodium nitrite was added in the presence of excess of HCl at below 5 ºC to obtain the nitroso derivative. The nitroso derivative was converted into the equivalent amine by passing H_2S gas through an ammonical reaction mixture. The produced amine was reacted with substituted alde-hydes to obtain carvacrol based Schiff base ligand [17] (**Scheme-I**).

4-[(5-Chloro-2-hydroxy-benzylidene)amino]-5-isopropyl-2-methyl-phenol (1): Yellow; yield: 84%; m.p.: 122-123 ºC; Elemental analysis of $C_{17}H_{18}NO_2Cl$; calcd. (found) %: C, 67.21 (67.20); H, 5.97 (5.91); Cl, 11.67 (11.65); N, 4.61 (4.65); O, 10.53 (10.60).

1-[(4-Hydroxy-2-isopropyl-5-methyl-phenylimino)-methyl]naphthalen-2-ol (2): Orange; yield: 89%; m.p.: 216-217 ºC; Elemental analysis of $C_{21}H_{21}NO_2$; calcd. (found) %: C, 78.97 (78.91); H, 6.63 (6.59); N, 4.39 (4.38); O, 10.02 (10.10).

4-[(5-Bromo-2-hydroxy-benzylidene)amino]-5-isopropyl-2-methyl-phenol (3): Orange; yield: 75%; m.p.: 218-219 ºC; Elemental analysis of $C_{17}H_{17}NO_2Br_2$; calcd. (found) %: C, 47.80 (47.78); H, 4.01 (4.00); Br, 37.42 (37.40); N, 3.28 (3.26); O, 7.49 (7.55).

4-[(4-Diethylamino-2-hydroxy-benzylidene)amino]-5-isopropyl-2-methyl-phenol (4): Greenish yellow; yield: 83%; m.p.: 213-214 ºC; Elemental analysis of $C_{21}H_{28}N_2O_2$; calcd. (found) %: C, 74.08 (74.12); H, 2.89 (2.88); N, 8.23 (8.25); O, 9.40 (9.45).

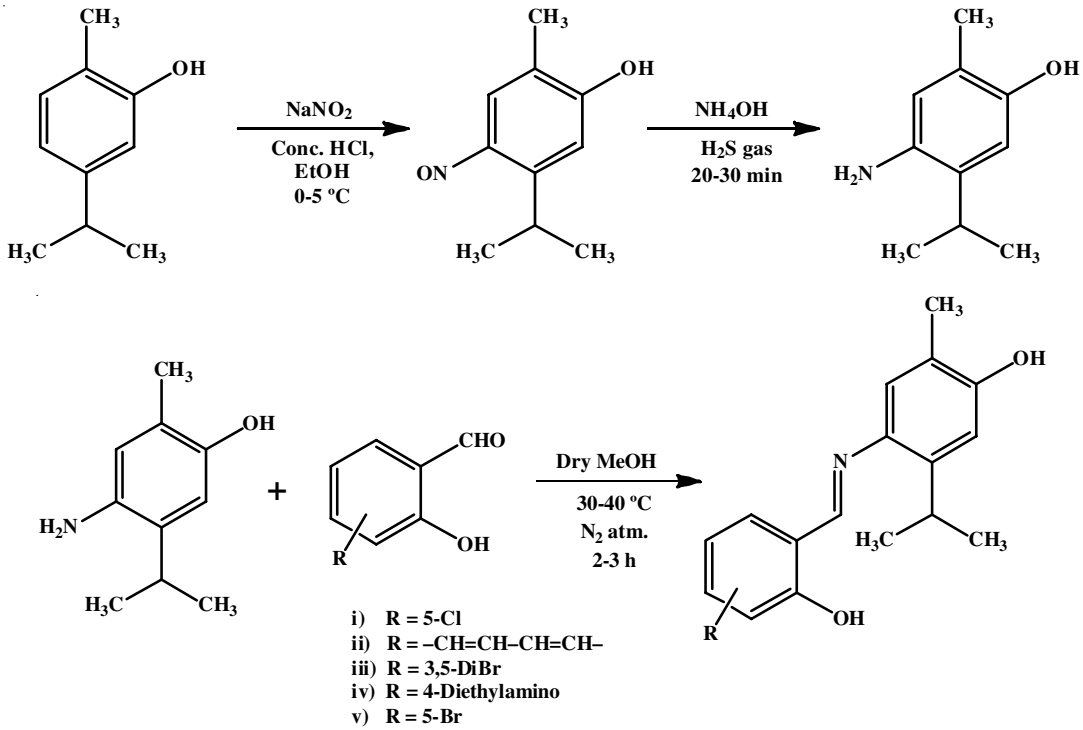
4-[(5-Bromo-2-hydroxy-benzylidene)-amino]-5-isopropyl-2-methyl-phenol (5): Yellow; yield: 87%; m.p.: 198-199 ºC; Elemental analysis of $C_{17}H_{18}NO_2Br$; calcd. (found) %: C, 58.63 (58.65); H, 5.21 (5.25); N, 4.02 (4.01); Br, 22.95 (22.96); O, 9.19 (9.24).

Brine shrimp lethality assay

Sample preparation: In order to conduct the brine shrimp lethality assay, the samples were prepared by dissolving 10 mg of sample in 20 µL of DMSO. The volume was then made up to 10 mL with distilled water, resulting in a 1000 µg/mL stock solution to achieve final drug concentrations of 10 µg/mL, 100 µg/mL and 1000 µg/mL, respectively. For each dose level, three replicates were prepared. Control vials were also prepared by adding equal volumes of distilled water.

Preparation of sea water: Dried Brewer's yeast (6 mg/L) was added to the solution of 25 g of crude sea salt per litre of distilled water for use as a brine shrimp feeding. Prior to use, it was filtered with filter paper.

Hatching of brine shrimp eggs: After being rinsed with water, shrimp eggs (each weighed ~ 40 mg) were evenly distributed into the darkened chamber after 2.0 L of sea water was poured into it. The phototropic nuclei were employed for the bioassay after being capillarily extracted from the lighter side after 48 h. The bioassay experiment was carried out following the reported procedure [18]. The Nauplli were inserted into a glass capillary together with water. After transferring ten shrimp to each vial, a brine solution containing 4.5 mL of appropriate quantities of brine and yeast suspension was added. The stems of capillaries were counted against a background that was illuminated. Combined 0.5 mL of sample with 4.5 mL of salt water for each trial. After 1 day, 4.5 mL of artificial sea water and



Scheme-I: Synthetic route of novel carvacrol based Schiff base derivatives

0.5 mL of artificial sea water treated with 0.2% DMSO were introduced to the control vial. Three pairs of magnifying glasses were used to count the survivors. Using eqn. 1, the percentage of fatalities were calculated against a light-illuminated background.

$$\text{Mortality (\%)} = \frac{\text{Total naupii} - \text{Alive naupii}}{\text{Total naupii}} \times 100 \quad (1)$$

Assessment of minimal inhibition concentrations: For both primary and secondary screening, serial dilutions were prepared. The antibiotic-free control tube was promptly subcultured by evenly spreading a loopful across a fourth of a medium plate suitable for the test organism's growth, followed by incubation at 37 ºC overnight. The tubes were incubated overnight. The minimum inhibitory concentration (MIC) of the control organism was assessed to confirm the accuracy of the drug concentrations [19]. A comparison was conducted between the growth from the control tube, which represents the initial inoculum before incubation.

Primary and secondary screening methods: A stock solution of 2000 µg/mL was prepared by diluting each compound. **Primary screen:** The synthesized drug concentrations in 1000 µL, 500 µL and 250 µL volumes were tested. In a second round of dilution tests, the active synthetic drugs identified in this primary screening were examined further against all the studied bacteria. **Secondary screen:** The drugs identified as effective in the first screening were diluted in a similar way to yield concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.50 µg/mL and 6.25 µg/mL.

RESULTS AND DISCUSSION

The novel carvacrol based Schiff bases with different substituted aldehydes were synthesized by following the reported procedure [17]. All the five compounds were characterized using spectroscopic methods with yields ranging from 83% to 89%.

FT-IR spectral studies: FT-IR spectra of the synthesized Schiff base derivatives showed prominent absorption bands associated with the characteristic functional groups, confirming the formation of the target compounds. The characteristic azomethine (C=N) stretching vibrations occurred prominently between 1619-1514 cm^{-1}, across all samples (Table-1), therefore confirming the successful condensation reaction between the amine and carbonyl components. The variation of the C=N stretching frequency results from the electronic characteristics of the substituents present on the aromatic ring structure. Phenolic C–O stretching vibrations were exhibited between 1366 and 1345 cm^{-1} successfully confirming the presence of hydroxyl groups on the target compounds, while aromatic C–N (Ph–N) stretches were observed in the 1175-1135 cm^{-1} region confirming a substituted aromatic system. Furthermore, the broad O–H stretching bands were exhibited within the 3367-3027 cm^{-1} region highlighting the presence of hydrogen-bonded hydroxyl groups. Schiff base compounds **1** and **4** displayed stronger O–H interaction with O-H bands around 3367 cm^{-1} confirming stronger hydrogen interactions. Overall, the FT-IR spectroscopic characteristics demonstrated the functional moieties linked to the biological activity.

TABLE-1
FT-IR SPECTRAL DATA OF SYNTHESIZED SCHIFF BASES

Schiff base	Azomethine peak (cm^{-1})	C–OH	Ph–N	OH
1	1611.62	1351.21	1175.12	3367.01
2	1543.66	1347.23	1134.24	3057.03
3	1618.70	1366.64	1139.41	3052.70
4	1514.21	1345.15	1139.28	3027.43
5	1606.79	1349.59	1174.49	3367.32

^{1}H NMR spectral studies: The ^{1}H NMR analysis of the synthesized compounds invariably lays out some main structural characteristics. The imine proton (-CH=N-) showed up as a distinguishable singlet between 8.25-9.60 ppm and was strongly deshielded due to the conjugation and electrophilic nitrogen. Multiplets arise between 6.60 and 8.50 ppm, with shifts due to substituents like hydroxy (-OH), bromo (-Br), blocking and methyl (-CH_3). Hydroxy protons (-OH) appeared, again as singlets, between 9.50-5.20 ppm, indicating hydrogen bonding influences. The aliphatic methyl (-CH_3) protons give rise to the shielded peaks between 2.50-1.20 ppm, indicating that this methyl group is situated onto the phenolic ring (Table-2).

^{13}C NMR spectral studies: The ^{13}C NMR spectra revealed that the synthesized Schiff base ligands are organized with respect to their carbon atoms. The imine carbon (-CH=N-) is highly deshielded and therefore appears at about 196 ppm due to the electronegativity and conjugation of nitrogen. The aromatic carbons are found in the region of 154-122 ppm, with those carbons adjacent to substituents, like -OH or -Br, appearing even further downfield (more deshielded). The methyl (-CH_3) carbons remain well shielded in the range between 40-16 ppm, thus reflecting their aliphatic environment. Table-3 supports the distinct structural characteristics of the compounds, including aromatic systems, hydroxyl activators and aliphatic methyl substitutions.

Anticancer activity on human lung cancer cell line A-549: As illustrated in the growth curve (Fig. 1), the effectiveness among the synthesized carvacrol based Schiff base complexes varied significantly. Schiff base compounds containing bromo (**5**) and naphthyl (**2**) groups showed strong anticancer potential as early as 10 µg/mL, with growth inhibition levels

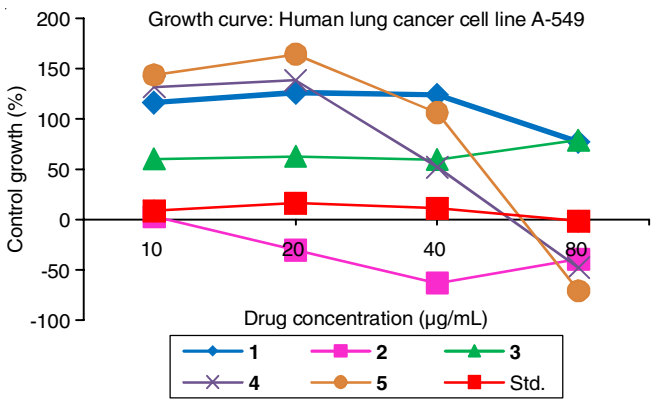


Fig. 1. Growth curve and cytotoxicity

TABLE-2
^{1}H NMR SPECTRA OF SCHIFF BASES

Schiff base	Chemical Shift (ppm)	Integration	Multiplicity	Assignment
1	9.60	1	Singlet	Imine proton (–CH=N–)
	8.80	1	Singlet	Aromatic proton
	7.30–7.60	4	Multiplet	Aromatic protons
	2.20–2.40	2–3	Multiplet	Methylene (–CH_2) protons
	1.80–1.20	6	Multiplet	Methyl (–CH_2) protons
2	9.55, 9.51	2	Singlet	Hydroxyl (–OH) protons
	8.51	1	Singlet	Imine proton (–CH=N–)
	7.99–7.54	4–5	Multiplet	Aromatic protons
	6.98, 6.90, 6.69	3	Multiplet	Aromatic protons
	2.55–2.18	6	Singlet	Methyl (–CH_2) protons
3	8.39	1	Singlet	Aromatic (N–Ar)
	7.56, 7.46	2	Doublets	Aromatic protons
	~7.00	4	Doublets	Aromatic protons
	5.00	1	Broad singlet	Hydroxyl (–OH) proton
	3.12	4	Quartet	Methylene (–CH_2) protons
	1.29	6	Triplet	Methyl (–CH_2) protons
4	8.25	1	Singlet	Imine proton (–CH=N–)
	7.90–6.60	4–5	Multiplet	Aromatic protons
	5.20	1	Singlet	Hydroxyl (–OH) proton
	3.66–3.30	2–3	Multiplet	Methylene (–CH_2) protons
	2.18–0.75	6–9	Multiplet	Methyl (–CH_2) protons
5	9.60	1	Singlet	Imine proton (–CH=N–)
	8.82	1	Singlet	Aromatic proton
	7.28–7.67	4–5	Multiplet	Aromatic protons
	6.94, 6.87	2	Doublet	Aromatic protons
	2.29–2.41	2–4	Multiplet	Methylene (–CH_2) protons
	1.78–1.16	3–6	Multiplet	Methyl (–CH_2) protons

TABLE-3
^{13}C NMR OF SCHIFF BASES

Schiff base	Chemical shift (ppm)	Carbon type	Assignment
1	155.0–159.0	Deshielded aromatic carbons	Carbons adjacent to -Cl and -OH on the aromatic ring
	141.5	Imine carbon (-CH=N-)	Imine group connecting aromatic rings
	112.1–120.0	Aromatic carbons	Other aromatic carbons
	28.0	Methyl carbon (-CH_3)	Aliphatic chain
2	196.0	Highly deshielded carbon	Imine carbon (-CH=N-)
	154.2	Aromatic carbon with -OH	Carbon bonded to -OH on phenolic ring
	129.4–122.1	Aromatic carbons	Naphthalene system
	49.5–39.9	Aliphatic carbons (-CH_3)	Methyl carbons on phenolic ring
3	157.95	Deshielded aromatic carbon	C-OH (phenolic ring)
	137.91, 137.59	Aromatic carbons	C-Br substituted aromatic ring
	133.15	Aromatic carbon	Phenyl ring carbon (C-N connection)
	122.19, 120.29	Aromatic carbons	Unsubstituted phenyl carbons
	112.56	Aromatic carbon	Phenyl ring carbon
	28.03	Methyl carbon	Aliphatic methyl carbon
	23.20	Methyl carbon	Aliphatic methyl carbon
	15.46	Methyl carbon	Methyl carbon adjacent to aromatic ring
4	159.4–158.4	Deshielded aromatic carbons	Carbons adjacent to -OH on aromatic rings
	141.5	Imine carbon (-CH=N-)	Imine group connecting aromatic rings
	136.5–120.7	Aromatic carbons	Carbons on aromatic rings
	40.4	Aliphatic carbon (-CH_2)	Methylene group attached to aromatic rings
	28.0, 16.4	Methyl carbons (-CH_3)	Terminal methyl groups
5	158.4–155.0	Deshielded aromatic carbons	Carbons near -Br and -OH substituents
	141.5	Imine carbon (-CH=N-)	Imine group connecting aromatic rings
	112.1–120.0	Aromatic carbons	Other aromatic carbons
	40.2–28.0	Aliphatic carbons (-CH_2/-CH_3)	Methylene and methyl carbons

reaching -70.9% at 80 µg/mL. The latter showed similar results to adriamycin, a standard chemotherapy, which also displayed significant cytotoxicity. Compound having bromo group (**5**), in particular, displayed a really good dose-dependent relationship, thus becoming one of the most promising options for further lung cancer research.

On the contrary, Schiff base complex containing chloro group (**1**) proved moderately effective with growth inhibition reaching a maximum of 77.4% at 80 µg/mL. This indicated that growth inhibition had lasting and less aggressive effects on the cancer cell growth. Similarly, compound **4** exhibited moderate concentration, consistently demonstrating efficacy, particularly at higher doses, suggesting its potential as an adjuvant in combination therapy (Table-4).

While, the Schiff base (**2**) has strong effects on certain cell lines at low concentrations (< 10 µg/mL), it has strong growth inhibition over all tested concentrations, indicating its relatively powerful cytotoxicity against lung cancer cells. The significant property of Schiff base (**2**) makes it one of the most active molecules ever synthesized, opening up promising field for future drug development. Most of the LC_{50} results indicated that Schiff base (**2**) and standard adriamycin are quite active anticancer agents with relatively low concentrations. While standard adriamycin was compared to the rest with respect to LC_{50}, synthesized Schiff bases (**3** and **4**) displayed similarly promising results indicating strong anticancer activity at 80 µg/mL.

The tumour growth inhibition (TGI) values of Schiff bases (**3** and **5**) were high, measuring 63 µg/mL and 62 µg/mL, respectively. These results indicated strong inhibition of tumor growth by the synthesized Schiff base compounds and positioned them as effective inhibitors with possible application in the clinical studies. Further validation for GI_{50} values was demonstrated through the external release of synthesized compounds. Schiff base (**2**) and standard adriamycin registered low GI_{50} values, denoting those compound systems were capable of effectively inhibiting growth when administered at lower doses. Likewise, Schiff bases (**4** and **5**) exhibited good potential in growth inhibition with GI_{50} values of 44 µg/mL and 48 µg/mL, respectively, concluding them as potential subjects of further development in lung cancer therapies.

Among the synthesized Schiff bases, Schiff bases **2**, **3** and **5** showed effective anticancer action much stronger or less than standard adriamycin in some cases. As a reference, standard adriamycin showed excellent anticancer action at lower concentration, whereas Schiff bases **2** and **5** have shown considerable cytotoxicity at low doses and Schiff base **3** showed moderate yet significant tumor inhibition, thus confirming their therapeutic potential. These results reinforce the rationale for using the synthesized compounds as potential lead compounds alone or along with standard adriamycin in the treatment of lung cancer. Their action in tumor and cell growth suppression at much lower concentrations further enhances their potency as targeted anticancer therapies. In addition to their decreased toxicity compared to efficacy, these compounds may offer additional benefits in reducing the adverse effects typically associated with chemotherapy.

Brine shrimp lethality assay: The results bioassay show that the synthesized carvacrol based Schiff base compounds lead to shrimp mortality at three concentrations (10, 100 and 1000 µg/mL). Shrimp mortality was zero in the control group, with all 30 shrimps surviving (0% mortality). However, all the test compounds exhibited 100% mortality across all concentrations, with absolutely no shrimps surviving in any treatment groups (Table-5). It appears that the above compounds are highly toxic as basis of total mortality has been observed even for the lowest concentration (10 µg/mL). From these results, it can be understood that these compounds possess considerable bioactivity, which can be investigated in the future for further applications in cytotoxicity or antimicrobial studies.

Antibacterial activity: The comparative analysis of synthesized Schiff base derivatives against standard antibiotics (gentamycin, ampicillin, chloramphenicol, ciprofloxacin and norfloxacin) exhibited moderate antimicrobial property. In the broth dilution method, the Schiff bases displayed an MIC range of 62.5-250 µg/mL, with Schiff bases **3** and **1** exhibiting high activity against *E. coli* (62.5 µg/mL) and *S. aureus* (100 µg/mL). Their activity was moderate compared to that of gentamycin, which showed significant MIC values (0.05-1 µg/mL), the Schiff bases demonstrated some activity which is either comparable or slightly lower than that of nopfloxacin (10 µg/mL) having moderate compatibility with ciprofloxacin and chloramphenicol (50 µg/mL) (Table-6). These results under-

TABLE-4
ANTICANCER ANALYSIS OF COMPOUNDS
Human lung cancer cell line A-549; % Control Growth; Drug Concentrations (µg/mL)

Schiff base	Experiment 1				Experiment 2				Experiment 3			
	10	20	40	80	10	20	40	80	10	20	40	80
1	105.0	114.3	114.9	74.1	112.4	125.3	119.9	78.5	131.2	138.3	135.9	79.6
2	3.0	-35.5	-67.5	-43.2	-2.2	-31.6	-65.0	-40.7	7.9	-24.1	-56.9	-34.6
3	48.6	46.8	51.9	73.0	58.0	65.8	63.7	85.2	73.5	75.1	62.3	78.6
4	118.1	101.7	25.3	-52.6	125.5	146.6	59.7	-46.5	151.3	166.8	71.9	-44.1
5	129.5	132.6	79.9	-76.2	140.7	176.6	117.0	-69.1	160.9	183.4	122.4	-67.2
Std.	3.2	5.4	0.7	-15.3	8.1	20.9	15.5	-0.6	15.1	22.6	17.8	11.3

Schiff base	Average values				Drug concentrations (µg/mL)		
	10	20	40	80	LC_{50}	TGI	GI_{50}*
1	116.2	126.0	123.5	77.4	NE	NE	>80
2	2.9	-30.4	-63.1	-39.5	74	<10	<10
3	60.1	62.6	59.3	78.9	NE	NE	>80
4	131.6	138.4	52.3	-47.7	80	62	44
5	143.7	164.2	106.4	-70.9	78	63	48
Std.	8.8	16.3	11.3	-1.5	NE	NE	<10

TABLE-5
BRINE SHRIMP ASSAY OF SYNTHESIZED COMPOUNDS

Schiff base	Conc. of extract	Total no of shrimps used/tube	Shrimp survived			Total no of shrimp survived	Percentage mortality
			T1	T2	T3		
Control	–	30	10	10	10	30	00
1	10 µg	30	00	00	00	30	100
	100 µg	30	00	00	00	00	100
	1000 µg	30	00	00	00	00	100
2	10 µg	30	00	00	00	00	100
	100 µg	30	00	00	00	00	100
	1000 µg	30	00	00	00	00	100
3	10 µg	30	00	00	00	00	100
	100 µg	30	00	00	00	00	100
	1000 µg	30	00	00	00	00	100
4	10 µg	30	00	00	00	00	100
	100 µg	30	00	00	00	00	100
	1000 µg	30	00	00	00	00	100
5	10 µg	30	00	00	00	00	100
	100 µg	30	00	00	00	00	100
	1000 µg	30	00	00	00	00	100

TABLE-6
COMPARATIVE ANTIBACTERIAL ACTIVITY OF SCHIFF BASES

Schiff base	Minimal inhibition concentration (µg/mL)			
	E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyrogenus* MTCC 442
1	125	62.5	100	125
2	250	250	125	125
3	62.5	100	100	100
4	100	125	125	250
5	62.5	100	250	250
Gentamycin	0.05	1	0.25	0.5
Ampicillin	32	-	40	25
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Nopfloxacin	10	10	10	10

score the promise of carvacrol based Schiff base derivatives as possible antibacterial agents, conspicuously in cases represented by the lack of effectiveness exerted by standard antibiotics.

The antibacterial mechanism of Schiff base derivatives probably involves various processes, mainly modified by their structural characteristics. The imine group (-CH=N-) and aromatic rings contribute to bacterial membrane disruption through addition to lipophilicity. The hydroxyl (-OH) and halogen substituents (-Cl, -Br) enhance bioactivity by producing oxidative stress, mainly through the formation of reactive oxygen species (ROS) and the chelation of essential metal ions, thereby disrupting bacterial enzyme systems. Furthermore, the planar aromatic systems in the Schiff bases may intercalate into bacterial DNA or inhibit crucial enzymes blocking bacterial replication and leading to cell death. The presence of halogen atoms in Schiff base compounds **3** and **5** substantially increases their activity through increased penetration and interaction with the membrane. These synthesized Schiff base derivatives are significant as they could serve as scaffolds for developing new antibiotics due to increasing antimicrobial resistance. Structural versatility, ease of synthesis and moderate antibacterial activity render them prime candidates for further optimization. Although they may not yet outperform potent antibiotics such as gentamycin, the interesting mechanisms of action and modifiable structures serve as strong foundations for further exploration towards newer classes of antibacterial agents effective against resistant strains of bacteria (Table-6).

Antifungal activity: The synthesized carvacrol based Schiff bases were screened for antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) using the broth dilution technique, the MIC values ranged between 250-1000 µg/mL. The most active were found with Schiff bases **1**, **3** and **5**, exhibiting inhibition at 250 µg/mL against *C. albicans* and 500 µg/mL against *A. clavatus*; hence indicating moderate antifungal activity. Schiff bases **2** and **4** are comparatively less active with an MIC in the range of 500-1000 µg/mL; the activities are much pronounced against *A. niger* and *A. clavatus* (Table-7). The results define the special significance of halogen-substituted Schiff bases compared with other derivatives, which may be due to their higher interactions with the cell structures of the fungus.

Potential mechanisms for the antifungal action of Schiff bases involve the interaction of imine (-C=N-) groups or halogen substituents (-Cl, -Br) to enhance lipophilicity and subsequently better penetration through the fungal cell membranes,

TABLE-7
COMPARATIVE ANTIFUNGAL ANALYSIS OF SCHIFF BASES

Schiff base	Minimal inhibition concentration (µg/mL)		
	C. albicans MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
1	250	250	500
2	500	1000	1000
3	250	500	500
4	500	1000	1000
5	250	500	500
Nystatin	100	100	100
Greseofulvin	500	100	100

leading to membrane disruption and interference with metabolic pathways through metal ion chelation. Contribution to antifungal activity is probably due to induction of oxidative stress through generation of reactive oxygen species (ROS). Inclusion of halogens in Schiff bases (**3** and **5**) may enhance their antifungal action by better interaction with fungal membranes as compared to Nap and DEAS.

While nystatin was the most potent among five drugs with 100 µg/mL MICs against different strains of fungi, griseofulvin showed 100 µg/mL MIC against *A. niger* and *A. clavatus*, but a higher 500 µg/mL MIC against *C. albicans*. The Schiff bases compounds **1**, **3** and **5** were as follows: *C. albicans* (MIC 250 µg/mL); whereas for the other strains, they had weaker effects with MICs of 500 µg/mL or higher. Although they are considered to be less active than nystatin, Schiff base derivatives have still provided possibilities for further development in exploring other features owing to greater structural variations.

Conclusion

The synthesized carvacrol based Schiff bases (**1-5**) were found to provide a compelling collation of structural stability by virtue of their various biological activities. Structural confirmation methods through ^{1}H NMR and ^{13}C NMR revealed the presence of key bioactive moieties such as imine (-CH=N-) groups, aromatic rings and halogen (-Cl, -Br) substituents, which impart appraised the biological properties. The synthesized compounds, particularly Schiff bases **1**, **3** and **5**, showed significant promise as anticancer agents against the A-549 lung cancer cell line. Their effective cytotoxicity, coupled with the growth inhibition data, indicates their potential as effective treatments, with the added advantage of being able to function at low concentrations. Compared to standard adriamycin, the synthesized Schiff bases offer the possibility of a more targeted approach, reducing the need for high-dose chemotherapy. The biological evaluations showed these compounds to be relatively effective on various strains. The Schiff bases tested in the brine shrimp lethality assay displayed 100% mortality at concentrations as low as 10 µg/mL, portending to a highly cytotoxic characteristic. In antibacterial investigations, Schiff bases **1** and **3** against *E. coli* and *S. aureus*, exhibiting MIC values as low as 62.5 µg/mL, potentially comparable to those of the standard ciprofloxacin. Antifungal studies demonstrated an impressive efficacy, as Schiff bases **1**, **3** and **5** exhibited significant activity against *C. albicans* and *A. clavatus*, with MIC values ranging from 250 to 500 µg/mL, comparable to griseofulvin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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