

Synthesis, characterization and *in vitro* antibacterial activity of cinnamyl amine derivatives

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A series of cinnamyl amine derivatives have been synthesized and characterized by their MS, ^1H and ^{13}C NMR spectral data. Antibacterial activity has been evaluated against three Gram-positive (*Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96 and *Staphylococcus epidermidis* MTCC 435) and two Gram-negative (*Escherichia coli* MTCC 723 and *Pseudomonas aeruginosa* MTCC 741) bacteria. The Schiff base derivatives benzyl-(3-phenylallylidene)amine **3**, phenyl-(3-phenylallylidene)amine **4** and amine derivatives 2-[benzyl-(3-phenylallyl)-amino]-1-(4-methoxyphenyl)ethanone **8c** and 1-(4-bromophenyl)-2-[phenyl-(3-phenylallyl)-amino]ethanone **9c** showed remarkable antibacterial activity against *B. subtilis*, *S. aureus* and *S. epidermidis* bacterial strains even at low concentration and are close to the standard antibiotic streptomycin (MIC 3.3-7.0 $\mu\text{g mL}^{-1}$). Furthermore, methoxy substitution at phenacyl nucleus increased the antibacterial activity as compared to the methyl and bromo substituents under identical conditions.

Keywords: Cinnamaldehyde, Schiff base, cinnamyl amine derivatives, antibacterial activity

The discovery and development of antibiotics to control the infectious diseases are among the notable achievement of modern synthetic methods. But, the increasing microbial resistance to antibiotics necessitated the search for new effective molecules.

Cinnamaldehyde (3-phenyl-2-propanal) is a common constituent of several essential oils, existing in *cis* and *trans* isomeric forms. *trans*-Cinnamaldehyde, a common constituent of the stem/ bark of *Cinnamomum cassia* and leaves of *C. tamala* is part of traditional medicines and pharmaceutical preparations for cold, anti-aggregation of platelets, anti-mutagenic and bactericidal effects^{1,2}. It is considered to be a safe fragrant constituent with no acute and chronic toxicity. No case of mutagenicity or genotoxicity and carcinogenicity due to cinnamaldehyde has been reported in mammalian studied³. 2'-Hydroxy cinnamaldehyde derivative isolated from *C. cassia* reported to have an inhibitory effect on farnesyl protein transferase activity and inhibited the

proliferation of several human cancer cell lines including breast, leukemia, ovarian, lung and colon tumor^{4,5}. Cinnamaldehyde has also been proven to have strong antifungal activities against a wide variety of wood decay fungi^{6,7}.

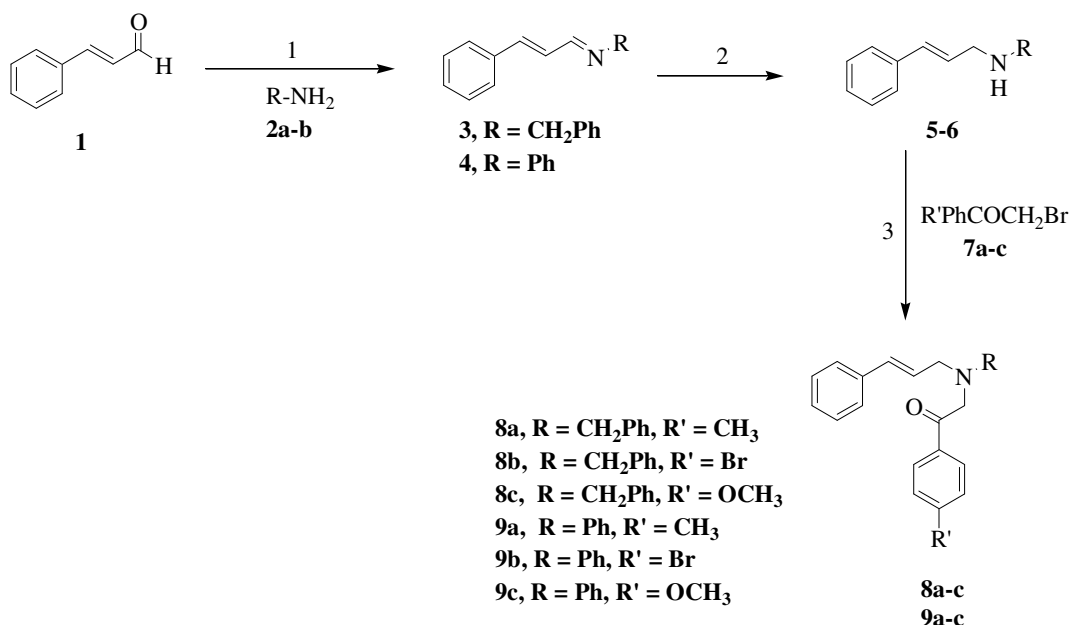
Schiff bases and their amine analogues derived from various aromatic aldehydes and ketones were reported to possess cytotoxic, anticonvulsant and antimicrobial activity⁸⁻¹⁰. Due to diverse structural aspects of Schiff bases and their analogues, a wide range of these compounds have been synthesized. Salicylaldehyde derivatives, with one or more halo atoms in the aromatic ring showed significant antibacterial and antifungal activities¹¹. Various Schiff bases derived from 5-chloro-salicylaldehyde have shown broad spectrum antibacterial and antifungal activities¹².

Keeping the diverse therapeutic activities of Schiff base analogues derived from aldehydes in view, it was contemplated to synthesize a series of cinnamyl derivatives and screen them for their *in vitro* antibacterial activities. We report herein the synthesis and antibacterial evaluation of substituted cinnamyl compounds.

Results and Discussion

Chemistry

A series of substituted cinnamyl derivatives have been synthesized by the reductive amination of cinnamaldehyde followed by the nucleophilic substitution reaction of resulting amine with different phenacyl bromides **7a-c** (Scheme I). Primary amines **2a-b** condense with a carbonyl group of cinnamaldehyde under specific condition giving Schiff bases (imine derivatives of cinnamaldehyde) **3** and **4** (Ref. 13). The resulting imine derivatives **3** and **4** were then reduced with sodium borohydride to afford **5** and **6**, respectively. Nucleophilic substitution reaction of 1.0 equiv of **5** or **6** with 1.1 equiv of 2-bromo-1-(4-bromo-phenyl)ethanone **7a**, 2-bromo-1-*p*-tolylethanone **7b**, 2-bromo-1-(4-methoxyphenyl)-ethanone **7c** in presence of K_2CO_3 and in dry acetonitrile afforded corresponding cinnamyl amines **8a-c** and **9a-c** in 55-75% yield. Among different solvents used, acetonitrile was most favourable in nucleophilic substitution reaction¹⁴⁻¹⁷. All the synthesized compounds were purified over silica gel column and



Scheme I — Reaction condition and reagents: (1) RNH₂, Mol Sieves, MeOH, 5-6 hr, RT. (2) NaBH₄, MeOH, 0°C-RT, 10 hr (3) K₂CO₃, acetonitrile, 8-10 hr, RT

characterized by their MS and NMR (¹H and ¹³C) spectral data. The spectral data confirm the formation of desired products.

Antibacterial activity

Antibacterial activity of all the synthesized cinnamyl-imine and cinnamyl-amine analogues was evaluated against three Gram positive *viz.* *B. subtilis* (MTCC-121), *S. aureus* (MTCC-96) and *S. epidermidis* (MTCC-435) and two Gram negative *viz.* *E. coli* (MTCC-723) and *P. aeruginosa* (MTCC 741) pathogenic bacterial strains using disc diffusion and broth dilution methods^{18,19}. Streptomycin was used as standard. The zone of inhibition (IZ) and MIC of the compounds against above bacterial strain are summarized in **Table I**. The results obtained showed that most of the compounds possess high to moderate activity as compared to the reference drug streptomycin. Schiff base analogues **3** and **4** showed significant to moderate activity against both gram positive and gram negative strains. Compound **3** showed high activity against *B. subtilis* (IZ = 21 mm and MIC = 6.6 µg/mL) while moderate activity against other Gram positive strains with IZ ranging 17-20 mm and MIC value ranging 7.8-13.2 µg/mL. Removal of CH₂ from amine derivative caused enhancement in activity of Schiff base **4** against gram positive bacterial strains while diminished against gram negative bacterial strains. Compound **4** showed potent activity against *S. epidermidis* (IZ = 21 mm,

MIC = 3.9 µg/mL) and *S. aureus* (IZ = 21 mm, MIC = 3.3 µg/mL) while moderate activity against others. Reduction of Schiff base **3** and **4** into amine derivatives **5** and **6** resulted in poor activity against tested bacterial strains as compared to Schiff base. Nucleophilic substitution of **5** and **6** with various phenacyl bromide derivatives **7a-c** displayed variable activity profile. Bromo and methoxy substitution at phenacyl nucleus exhibited significant to moderate activity while the methyl substituted **8a** and **9a** showed poor activity. Bromo substituted analogues **8b** and **9b** showed moderate activity against *E. coli* (IZ = 19 mm, MIC = 10.1 and 13.2 µg/mL respectively) and poor activity against others. Compounds **8c** and **9c** having methoxy substitution at phenacyl nucleus showed high activity. Compound **8c** showed significant activity against *S. aureus* (IZ = 18 mm and MIC = 6.6 µg/mL) and *B. subtilis* (IZ = 18 mm and MIC = 6.6 µg/mL) while **9c** showed significant activity against *B. subtilis* (IZ = 18 mm and MIC = 6.6 µg/mL) and *S. epidermidis* (IZ = 21 mm, MIC = 3.9 µg/mL) and moderate activity against *E. coli* and *P. aeruginosa*. Compounds **3**, **4**, **8c** and **9c** revealed better activity in comparison with other compounds used in this study. Schiff bases showed better activity as compared to their amine analogues except **8c** and **9c**. Enhanced activity of compounds **8c** and **9c** could be attributed to the presence of methoxy group at *para* position of phenacyl nucleus.

Table I— Antimicrobial activity of cinnamyl compounds **3-6**, **8a-c** and **9a-c**

Compd*	Zone of Inhibition (mm) and Minimum Inhibitory Concentrations ($\mu\text{g mL}^{-1}$)									
	<i>B. subtilis</i>		<i>S. aureus</i>		<i>S. epidermidis</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of Inhibition	MIC
3	21 \pm 0.51	6.6	20 \pm 0.53	7.8	19 \pm 0.56	13.2	17 \pm 0.42	20.3	18 \pm 0.62	15.6
4	19 \pm 0.51	10.1	22 \pm 0.76	3.3	21 \pm 0.66	3.9	15 \pm 0.48	31.2	13 \pm 0.36	53.1
5	17 \pm 0.41	26.5	17 \pm 0.41	28.1	18 \pm 0.53	15.6	13 \pm 0.34	56.2	16 \pm 0.44	26.5
6	13 \pm 0.36	62.5	12 \pm 0.26	81.2	10 \pm 0.28	81.2	9 \pm 0.22	112.5	11 \pm 0.29	81.2
8a	10 \pm 0.38	112.5	NA	ND	NA	ND	7 \pm 0.12	212.5	8 \pm 0.18	162.5
8b	19 \pm 0.48	13.2	18 \pm 0.49	14.0	16 \pm 0.42	26.5	19 \pm 0.64	10.1	15 \pm 0.38	31.2
8c	20 \pm 0.61	6.6	21 \pm 0.57	3.5	18 \pm 0.46	14.0	16 \pm 0.49	28.1	20 \pm 0.53	7.0
9a	10 \pm 0.32	112.5	7 \pm 0.19	162.5	9 \pm 0.21	162.5	12 \pm 0.32	81.2	8 \pm 0.18	125.0
9b	17 \pm 0.42	20.3	16 \pm 0.31	26.5	18 \pm 0.47	15.6	19 \pm 0.56	13.2	15 \pm 0.36	28.1
9c	18 \pm 0.48	6.6	19 \pm 0.51	10.1	21 \pm 0.57	3.9	15 \pm 0.46	28.1	17 \pm 0.46	20.3
Streptomycin**	13 \pm 0.37	3.3	22 \pm 0.68	1.9	19 \pm 0.59	3.5	25 \pm 0.92	1.6	23 \pm 0.84	1.3

Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

*Compounds (50 $\mu\text{g}/\text{disk}$) were used for experiments. NA = not active. ND = not determined.

**Used as positive reference (20 $\mu\text{g}/\text{disc}$).

Experimental Section

Chemicals and solvents used were from Fluka or Merck. All the reagents were of analytical grade. Melting points were determined in open capillary tubes in a Veego (India) melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in Bruker Avance NMR 300 MHz and 75 MHz spectrometer in CDCl_3 . Chemical shifts are expressed in δ (ppm). Mass spectra were recorded on Micromass Q-ToF micro spectrometer. The reactions were monitored by TLC on aluminium sheets backed with silica gel (60 F₂₅₄, Merck) and GC (FID). All the reactions were carried out under nitrogen atmosphere.

Experimental procedure for the synthesis of Schiff bases **3** and **4**

To a solution of cinnamaldehyde (1.0 mequiv) in methanol (50 mL) was added aromatic amine derivative (1.1 mequiv) and molecular sieves at RT. The resulting reaction mixture was stirred at room temperature for about 5-6 hr, progress of reaction was monitored by TLC using 50% ethyl acetate in hexane. After completion of reaction, the reaction mixture was filtered and evaporated to dryness. Crude product was purified by column chromatography using silica gel (60-120 mesh) with DCM:MeOH (99:5 to 95:10) as an eluent to give the pure compounds in 72-79% yields.

Benzyl-(3-phenylallylidene) amine, 3: Yield: 79.0%. reddish brown viscous liquid. MS: [M^+], m/z 222.16; ^1H NMR (300 MHz, CDCl_3): δ 2.69 (s, 2H,

CH_2), 6.28 (m, 1H, CH=CH), 6.50 (d, 1H, $J = 15.6$ Hz, PhCH=CH), 7.19 (m, 5H, ArH), 7.36 (m, 5H, ArH), 8.26 (d, 1H, $J = 7.5$ Hz, CH=N); ^{13}C NMR (75 MHz, CDCl_3): δ 65.8 (CH_2), 127.0 (C_{Ar}), 127.2 (C_{Ar}), 128.0 ($2 \times \text{CH}_{\text{Ar}}$), 128.1 (CH_{Ar}), 128.2 (CH_{Ar}), 128.5 ($2 \times \text{CH}_{\text{Ar}}$), 128.8 ($2 \times \text{CH}_{\text{Ar}}$), 129.1 ($2 \times \text{CH}_{\text{Ar}}$), 139.1 (PhCH=CH), 142.0 (CH=CH), 163.4 (CH=N).

Phenyl-(3-phenylallylidene) amine, 4: Yield: 72.0%. yellow solid. m.p. 93-95°C; MS: [M^+], m/z 208.10; ^1H NMR (300 MHz, CDCl_3): δ 7.14 (m, 5H, ArH), 7.39 (m, 5H, ArH), 7.52 (d, 2H, $J = 6.3$ Hz, CH), 8.25 (dd, 1H, $J = 5.7$ Hz, 2.7 Hz, CH); ^{13}C NMR (75 MHz, CDCl_3): δ 120.8 ($2 \times \text{CH}_{\text{Ar}}$), 126.0 (C_{Ar}), 127.4 ($2 \times \text{CH}_{\text{Ar}}$), 128.5 (C_{Ar}), 128.8 ($2 \times \text{CH}_{\text{Ar}}$), 129.1 ($2 \times \text{CH}_{\text{Ar}}$), 129.5 (CH_{Ar}), 135.5 (CH_{Ar}), 143.9 (CH=CH), 151.6 (CH=CH), 161.5 (CH=N).

Experimental procedure for the synthesis of **5** and **6**

To a solution of imine derivatives (1.0 mequiv) in methanol (50 mL), were added sodium borohydride (1.0 mequiv) at 0°C. The resulting reaction mixture was stirred at RT for about 10-12 hr, progress of reaction was monitored by TLC using 50% ethyl acetate in hexane. After complete disappearance of starting compound on TLC, the reaction mixture was diluted with distilled water and extracted with DCM, combined organic layer was washed with distilled water, brine solution and dried over anhydrous sodium sulphate and concentrated in Buchi rotatory evaporator under reduced pressure, crude was purified by column chromatography using silica gel

(60-120 mesh) as adsorbent with DCM:MeOH (99:7 to 95:15) as an eluent to give the pure compounds in 89-91% yields.

Benzyl-(3-phenylallyl) amine, 5: Yield: 91.0%. yellow solid. m.p. 72-74°C; MS: $[M^{+}]$, m/z 224.23; 1H NMR (300 MHz, $CDCl_3$): δ 3.24 (s, 1H, NH), 3.40 (d, 2H, $J = 6.3$ Hz, CH_2), 4.17 (s, 2H, CH_2), 6.23 (m, 1H, PhCH=CH), 6.50 (d, 1H, $J = 15.6$ Hz, PhCH=CH), 7.26 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.9 (CH_2), 51.9 (CH_2), 125.4 (CH_{Ar}), 126.1 ($2 \times CH_{Ar}$), 127.3 (C_{Ar}), 127.4 ($2 \times CH_{Ar}$), 127.9 (C_{Ar}), 128.3 ($2 \times CH_{Ar}$), 128.4 ($2 \times CH_{Ar}$), 133.0 ($2 \times CH_{Ar}$), 136.3 (PhCH=CH), 137.3 (CH=CH- CH_2).

Phenyl-(3-phenylallyl) amine, 6: Yield: 89.0%. reddish brown viscous liquid. MS: $[M^{+}]$, m/z 210.29; 1H NMR (300 MHz, $CDCl_3$): δ 3.84 (br s, 1H, NH), 3.92 (dd, 2H, $J = 5.7$ Hz, 1.2 Hz, N- CH_2), 6.30 (m, 1H, CH=CH), 6.58 (s, 1H, CH), 6.69 (m, 3H, ArH), 7.20 (m, 3H, ArH), 7.32 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.17 (CH_2), 113.01 ($2 \times CH_{Ar}$), 117.59 (CH_{Ar}), 126.29 ($2 \times CH_{Ar}$), 127.0 (C_{Ar}), 127.49 (C_{Ar}), 128.54 ($2 \times CH_{Ar}$), 129.24 ($2 \times CH_{Ar}$), 131.47 (C_{Ar}), 136.81 (CH=CH), 148.0 (CH=CH).

Experimental procedure for the synthesis of 8a-c and 9a-c

To a solution of **5** or **6** (1.0 mequiv) in acetonitrile (20 mL), were added powdered potassium carbonate (5.0 mequiv) at 0°C, and stirred the resulting solution for 15 min at 0°C then *p*-substituted phenacyl bromide derivatives **7a-c** (1.0 mequiv) were added at 0°C. The reaction mixture was stirred at RT for about 8-10 hr, progress of reaction was monitored by TLC using 5% methanol in DCM. After complete disappearance of starting compound on TLC, the reaction mixture was diluted with distilled water and extracted with chloroform, combined chloroform layer was washed with aqueous solution of sodium bicarbonate, distilled water, brine solution and dried over anhydrous sodium sulphate, and concentrated in Buchi rotatory evaporator under reduced pressure, crude product was purified by column chromatography using silica gel (60-120 mesh) as adsorbent with DCM:MeOH (99:1 to 95:5) as eluent to give the pure compounds in 53-69% yields.

2-[Benzyl-(3-phenylallyl)-amino]-1-*p*-tolylethanone, 8a: Yield: 69.0%. reddish brown viscous liquid. MS: $[M^{+}]$, m/z 356.47; 1H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.88 (d, 2H, $J = 19.5$ Hz, CH_2), 5.22 (s, 4H, CH_2), 6.42 (m, 2H, CH=CH), 7.24 (m, 12H, ArH),

7.79 (d, 1H, $J = 8.1$ Hz, ArH), 7.94 (d, 1H, $J = 8.1$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4 (CH_3), 56.3 (CH_2), 58.2 (CH_2), 58.6 (CH_2), 123.2 (C_{Ar}), 126.21 ($2 \times CH_{Ar}$), 126.4 (CH_{Ar}), 127.1 (C_{Ar}), 127.3 (C_{Ar}), 128.1 ($2 \times CH_{Ar}$), 128.3 ($2 \times CH_{Ar}$), 128.7 ($2 \times CH_{Ar}$), 129.1 ($2 \times CH_{Ar}$), 129.8 ($2 \times CH_{Ar}$), 133.2 (C_{Ar}), 133.5 (C_{Ar}), 136.7 (CH=CH), 142.8 (CH=CH), 197.8 (C=O).

2-[Benzyl-(3-phenylallyl)-amino]-1-(4-bromophenyl) ethanone, 8b: Yield: 61.0%. reddish brown viscous liquid. MS: $[M^{+}]$ and $[M^{+}+2]$, m/z 420.34 and 422.34; 1H NMR (300 MHz, $CDCl_3$): δ 3.88 (d, 2H, $J = 19.5$ Hz, CH_2), 5.22 (s, 4H, CH_2), 6.44 (m, 2H, CH=CH), 7.28 (m, 12H, ArH), 7.79 (d, 1H, $J = 8.1$ Hz, ArH), 7.95 (d, 1H, $J = 8.1$ Hz, ArH); ^{13}C -NMR (75 MHz, $CDCl_3$): δ 48.1 (CH_2), 49.9 (CH_2), 53.3 (CH_2), 123.9 (CH_{Ar}), 126.4 (C_{Ar}), 126.6 (C_{Ar}), 127.6 (C_{Ar}), 127.8 ($2 \times CH_{Ar}$), 128.1 ($2 \times CH_{Ar}$), 128.4 ($2 \times CH_{Ar}$), 128.5 ($2 \times CH_{Ar}$), 128.8 ($2 \times CH_{Ar}$), 129.6 (C_{Ar}), 131.3 ($2 \times CH_{Ar}$), 137.7 (CH_{Ar}), 135.6 (CH=CH), 136.9 (CH=CH), 196.5 (C=O).

2-[Benzyl-(3-phenylallyl)-amino]-1-(4-methoxyphenyl) ethanone, 8c: Yield: 59.0%. orange solid; m.p. 78-80°C. MS: $[M^{+}]$, m/z 372.47; 1H NMR (300 MHz, $CDCl_3$): δ 3.43 (d, 2H, $J = 6.3$ Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 5.15 (br s, 2H, CH_2), 6.20 (m, 1H, CH=CH), 6.55 (d, 1H, $J = 15.3$ Hz, CH=CH), 6.88 (t, 2H, $J = 8.1$ Hz, ArH), 7.33 (m, 10H ArH), 7.91 (d, 1H, $J = 8.7$ Hz, ArH), 8.02 (d, 1H, $J = 8.7$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.3 (CH_2), 56.5 (CH_2), 58.4 (CH_2), 59.0 (OCH_3), 113.5 ($2 \times CH_{Ar}$), 126.0 ($2 \times CH_{Ar}$), 126.9 (C_{Ar}), 127.1 (C_{Ar}), 127.4 (C_{Ar}), 128.2 ($2 \times CH_{Ar}$), 128.4 ($2 \times CH_{Ar}$), 129.2 ($2 \times CH_{Ar}$), 130.5 ($2 \times CH_{Ar}$), 131.9 (CH_{Ar}), 133.2 (CH_{Ar}), 136.9 (CH=CH), 138.4 (CH=CH), 163.1 (C_{Ar}), 197.0 (C=O).

2-[Phenyl-(3-phenylallyl)-amino]-1-*p*-tolylethanone, 9a: Yield: 67.0%. off white solid. m.p. 92-94°C. MS: $[M^{+}]$, m/z 342.45; 1H NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3), 4.22 (d, 2H, $J = 5.1$ Hz, CH_2), 4.76 (s, 2H, CH_2), 6.28 (m, 1H, CH=CH), 6.56 (d, 1H, $J = 15.9$ Hz, CH=CH), 6.69 (m, 3H, ArH), 7.19 (m, 2H, ArH), 7.23 (d, 1H, $J = 3.6$ Hz, ArH), 7.29 (m, 4H, ArH), 7.35 (d, 2H, $J = 7.2$ Hz, ArH), 7.90 (d, 2H, $J = 8.1$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.7 (CH_3), 53.9 (CH_2), 56.2 (CH_2), 112.5 ($2 \times CH_{Ar}$), 117.2 (CH_{Ar}), 125.9 (CH_{Ar}), 126.3 ($2 \times CH_{Ar}$), 127.4 (C_{Ar}), 127.9 ($2 \times CH_{Ar}$), 128.5 ($2 \times CH_{Ar}$), 129.2 ($2 \times CH_{Ar}$), 129.4 ($2 \times CH_{Ar}$), 131.3 (C_{Ar}), 132.9 (C_{Ar}), 136.8 (C_{Ar}), 144.4 (CH=CH), 148.6 (CH=CH), 195.9 (C=O).

1-(4-Bromophenyl)-2-[phenyl-(3-phenylallyl)-amino] ethanone, 9b: Yield: 53.0%. off white solid. m.p. 84-

86°C; MS: $[M^{+1}]$ and $[M^{+1}+2]$, m/z 406.07 and 408.07; ^1H NMR (300 MHz, CDCl_3): δ 4.21 (d, 2H, $J = 4.8$ Hz, CH_2), 4.74 (s, 2H, CH_2), 6.29 (m, 1H, $\text{CH}=\text{CH}$), 6.56 (d, 1H, $J = 15.9$ Hz, $\text{CH}=\text{CH}$), 6.67 (d, 2H, $J = 8.4$ Hz, ArH), 6.73 (t, 1H, $J = 7.2$ Hz, ArH), 7.25 (m, 5H, ArH), 7.61 (m, 4H, ArH), 7.88 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3): δ 53.9 (CH_2), 56.4 (CH_2), 112.7 ($2 \times \text{CH}_{\text{Ar}}$), 117.5 (CH_{Ar}), 125.7 (CH_{Ar}), 126.3 ($2 \times \text{CH}_{\text{Ar}}$), 127.5 (C_{Ar}), 128.5 ($2 \times \text{CH}_{\text{Ar}}$), 129.2 ($2 \times \text{CH}_{\text{Ar}}$), 129.5 ($2 \times \text{CH}_{\text{Ar}}$), 130.2 (C_{Ar}), 131.6 (C_{Ar}), 132.1 ($2 \times \text{CH}_{\text{Ar}}$), 134.9 (C_{Ar}), 136.6 ($\text{CH}=\text{CH}$), 148.4 ($\text{CH}=\text{CH}$), 195.6 ($\text{C}=\text{O}$).

1-(4-Methoxyphenyl)-2-[phenyl-(3-phenylallyl)-amino] ethanone, 9c: Yield: 55%. off white solid. m.p. 76-78°C. MS: $[M^{+1}]$, m/z 358.17; ^1H NMR (300 MHz, CDCl_3): δ 3.86 (s, 3H, OCH_3), 4.21 (d, 2H, $J = 4.8$ Hz, CH_2), 4.74 (s, 2H, CH_2), 6.29 (m, 1H, $\text{CH}=\text{CH}$), 6.55 (d, 1H, $J = 15.9$ Hz, $\text{CH}=\text{CH}$), 6.69 (m, 3H, ArH), 6.94 (d, 2H, $J = 8.7$ Hz, ArH), 7.19 (m, 3H, ArH), 7.31 (m, 4H, ArH), 7.99 (d, 2H, $J = 8.7$ Hz, ArH); ^{13}C NMR (75 MHz, CDCl_3): δ 53.9 (OCH_3), 55.4 (CH_2), 55.9 (CH_2), 112.4 ($2 \times \text{CH}_{\text{Ar}}$), 113.9 ($2 \times \text{CH}_{\text{Ar}}$), 117.1 (CH_{Ar}), 125.9 (CH_{Ar}), 126.3 ($2 \times \text{CH}_{\text{Ar}}$), 127.4 (C_{Ar}), 128.3 (C_{Ar}), 128.5 ($2 \times \text{CH}_{\text{Ar}}$), 129.1 ($2 \times \text{CH}_{\text{Ar}}$), 130.1 ($2 \times \text{CH}_{\text{Ar}}$), 131.2 (C_{Ar}), 136.7 (C_{Ar}), 148.6 ($\text{CH}=\text{CH}$), 163.7 ($\text{CH}=\text{CH}$), 194.7 ($\text{C}=\text{O}$).

Antibacterial activity

The antibacterial activity of synthesized cinnamyl derivatives were determined by screening against the Gram positive bacteria *B. subtilis* (MTCC-121), *S. aureus* (MTCC-96) and *S. epidermidis* (MTCC-435) and Gram negative bacteria *E. coli* (MTCC-723) and *P. aeruginosa* (MTCC 741).

Paper disc diffusion method

The paper disc diffusion method was performed in sterilized (autoclaved at 120°C for 1 hr) petri-dish. Discs impregnated with 50 $\mu\text{g}/\text{disc}$ test samples were placed on the surface of agar plates already inoculated with pathogenic bacteria. The plates were incubated at 37°C and examined after 24 hr for zone of inhibition. Streptomycin was used as a standard. An additional control disc with an equivalent amount of solvent (DMSO) was also used in the assay. The result showed some of the compounds to possess large zone of inhibition comparable with streptomycin (**Table I**).

Minimum inhibitory concentration

The lowest concentration of compound giving complete inhibition of visible growth, were

determined by micro titer plate broth dilution method. Various concentrations of compounds were prepared in by two-fold dilution method. The last well of micro titer plate, having no test compound, was considered as control. The inoculum was prepared using a 16 hr broth culture of each bacterial strain adjusted to a turbidity equivalent to a 0.5 Mc Farland standard (3×10^6 cfu mL^{-1} bacteria). The micro titer plates were incubated for 24 hr at 37°C.

Conclusion

Among the synthetic cinnamyl derivatives under investigation, **3**, **4**, **8c** and **9c** showed significant activity. The Schiff base derivatives **3**, **4** and cinnamyl-amine derivatives **8c** and **9c** viz. benzyl-(3-phenylallylidene)amine **3**, phenyl-(3-phenylallylidene)amine **4**, 2-[benzyl-(3-phenylallyl)-amino]-1-(4-methoxyphenyl)ethanone **8c** and 1-(4-methoxyphenyl)-2-[phenyl-(3-phenylallyl)-amino]ethanone **9c**, in particular, showed remarkable antibacterial activity even at low concentration against *B. subtilis*, *S. aureus*, and *S. epidermidis* which were closer to streptomycin (MIC 3.3 to 7.0 $\mu\text{g mL}^{-1}$). Furthermore, presence of methoxy group at *para* position of phenacyl nucleus enhanced the antibacterial activity as compared to the methyl and bromo substituent under identical conditions and might be of interest for developing new antibacterial molecules.

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References

- 1 Yu B S, Lai S G & Tan QL, *Chem Pharm Bull*, 54, **2006**, 114.
- 2 Yanaga A, Goto H, Nakagawa T, Hikiami H, Shibahara N & Shimada Y, *Biol Pharm Bull*, 29, **2006**, 2415.
- 3 Bickers D, Calow P, Greim H, Hanifin J M, Rogers A E, Saurat J H, Sipes I G, Smith R L & Tagami H, *Food Chem Toxicol*, 43, **2005**, 799.
- 4 Kwon B M, Cho Y K, Lee S H, Nam J Y, Bok S H, Chun S K, Kim J A & Lee I R, *Planta Med*, 62, **1996**, 183.
- 5 Lee C W, Hong D H, Han S B, Park S H, Kim H K, Kwon B M & Kim H M, *Planta Med*, 65, **1999**, 263.
- 6 Wang S Y, Chen P F & Chang S T, *Bioresour Technol*, 96, **2005**, 813.
- 7 Cheng S S, Liu J Y, Hsui Y R & Chang S T, *Bioresour Technol*, 97, **2006**, 306.
- 8 Tarafder M T, Kasbollah A, Saravan N, Crouse K A, Ali A M & Tin O K, *J Biochem Mol Bio Biophys*, 6, **2002**, 85.

- 9 Küçükgülzel İ, Küçükgülzel Ş G, Rollas S, Ötük-Sarış G, Özdemir O, Bayrak İ, Altuğ T & Stables J P, *Il Farmaco*, 59, **2004**, 839.
- 10 Pignatello R, Panico A, Mazzone P, Pinizzotto M R, Garozzo A & Fumeri P M, *Eur J Med Chem*, 29, **1994**, 781.
- 11 Felton L C & Brewer J H, *Science*, 105, **1947**, 409.
- 12 Shi L, Ge H M, Tan S H, Li H Q, Song Y C, Zhu H L & Tan R X, *Eur J Med Chem*, 42, **2007**, 558.
- 13 Joshi M C, Bisht G S & Rawat D S, *Bio Med Chem Lett*, 17, **2007**, 3226.
- 14 Singh K K, Joshi S C & Mathela C S, *Indian J Chem*, 50B, **2011**, 196.
- 15 Swamy N S, Sarala B G, Priya B S, Gaonkar S L, Prasad J S & Rangappa K S, *Bioorg Med Chem Lett*, 16, **2006**, 999.
- 16 Foroumadi A, Emami S, Mansouri S, Javidnia A, Adeli N S, Shirazi F H & Shafiee A, *Eur J Med Chem*, 42, **2007**, 985.
- 17 Song K S, Lee S H, Chun H J, Kim J Y, Jung M E, Ahn K, Kim S U, Kim J & Lee J, *Bioorg Med Chem*, 16, **2008**, 4035.
- 18 Bauer A W, Kirby W M M, Sherris J C & Turk M, *Amer J Clin Pathol*, 45, **1966**, 493.
- 19 Baker F J, Breach M R, Leighton I & Taylor P, *Medical Microbiological Techniques*, 1st Edn, (Butterworth & Co. Ltd., London), **1980**, pp. 343.