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Synthesis, spectral characterization and antimicrobial studies of novel series of allylidene based multifaceted chalcones analogues

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Abstract: Following the Claisen Schmidt condensation a series of chalcone, their allylidene derivatives and metallic complexes were produced and subsequently screened for antibacterial assay. The precursors were simple acetophenone and different substituted aryl benzaldehydes; which were made to react in basic ethanolic conditions. The structure of synthesized targets was established by IR, ^{1}H-NMR and EIMS data. The antibacterial statistics showed that most of the bacterial strains particularly *S. typhi* and *E. coli* were potently inhibited by majority of the compounds like 3c, 5c, 7a & 7c. This structure activity relationship studies showed that these molecules possessed *p*-methoxy substituents in their framework and found active in rupturing the cell wall. These molecules might serve as potential drug candidates for future drug discovery and design. The presented manuscript highlights the pharmacological diversity of chalcones holding allylidene moiety and Zn^{+2} complexes.

Keywords: Chalcones, allylidene, ^{1}H-NMR, EIMS, metallation.

INTRODUCTION

Plants served as the basis for the traditional medicine systems that have been in use for the several years in many countries owing to the presence of a vast range of secondary metabolites. The polyphenolic compounds, flavonoids and tannins form one of the major groups of phytochemicals (Rao and Ravishankar, 2002). Flavan is the basic structure of flavonoid, which comprises of fifteen carbon skeleton organized in three rings.

Chalcones belong to natural product family and designed from shikimate pathway (Motta *et al.,* 2006; Awasthi *et al*., 2009; Lim *et al*., 2007). They comprised of flavonoids and iso-flavonoids (open chain) and isolated from edible plants. The two phenyl rings of unsubstituted chalcone are linked by an electrophilic centre of α, β-unsaturated enol system; it assumes the linear or planar geometry. They are colored compounds owing to the presence of the chromophore ketoethylenic group (-CO-CH=CH-). They have crystal structure and 3D orientation (Wu *et al*., 2006).Chalcones showed variety of addition and substitution reactions predominantly diels elder reactions, epoxidation, alkylation and halogenation. Their derivatives with nicotinohydrazide and 1,3,5-trisubstituted pyrazoline showing potent anti-malarial potential. Chalcones are important in producing many heterocyclic compounds (Yoneto *et al*., 1996). They yielded reagents of great biological importance like pyrazolines (Ezhilarasi *et al*., 2015), cyanopyridines, cyanopyrans, cyanopyridones, pyrimidines, isoxazoles and indazoles (Gaede and McDermott, 1993; Shibatai *et al*., 1993; EI-Hamouly *et al*., 2011; Abonic *et al*., 2008). The liquoric chalcone are in practice for treatment of stomach cancer and gastritis, thrombotic diseases and for the ailments causes by parasitic infection (Samoszuk *et al*., 2005; Lee *et al*., 2004). The biological applications includes antioxidant (Vasil *et al*., 2010; Sivakumar *et al*., 2011; Vogel *et al*., 2008), antimalarial (Motta *et al*., 2006; Awasthi *et al*., 2009; Lim *et al*., 2007), anticancer (Achanta *et al*., 2006; Echeverria *et al*., 2009; Ilango *et al*., 2010), anti-inflammatory (Yadav *et al*., 2011; Zhang *et al*., 2010), antimicrobial (Hamdi *et al*., 2011; Bhatia *et al*., 2009; Awasthi *et al*., 2009; Bag *et al*., 2008; Lahtchev *et al*., 2008; Yadav *et al*., 2012) anticonvulsant (Kaushik *et al*., 2010; Nawakocoska 2007), xanthine oxidase inhibitor, aldol reductase inhibitor, epoxide hydrolase inhibitor (Najafian *et al*., 2010; Zarghi *et al*., 2006; Chimenti *et al*., 2009).

Chalcone derivatives are used as sensing material in analytical chemistry due to the binding of metal vacations at their specific sites. When a guest species binds to the receptor site, a certain property such as fluorescent intensity or wavelength changes. Such a change serve as an indicator of guest binding (Day *et al.*, 2008; Kim *et al*, 2007), like the binding of the M^+ causes a quenching of the fluorescence emission (Qi *et al*., 2006). We here in report the synthesis of different substituted chalcones and their allylidene derivatives with *O*-amino phenol. The subsequent metallation of allylidene targets and screening against various microbial strains.

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MATERIALS AND METHODS

The chemical reagents and solvents used in the synthesis were purchased from Merck, Sigma-Aldrich and Alfa Aesar. For thin-layer chromatography (TLC), silica gel plates G-25-UV_{254} were used and observed by using ultra violet spectroscopy. The dichloromethane and *n*-hexane were employed as gradient solvent system. The melting points were determined by Griffin and George M.P apparatus. On a Jasco-320-A spectrophotometer the IR spectra were recorded. KBr pallet method was employed and functional group stretching was reported in wave number (cm^{-1}). NMR spectra were recorded on Bruker spectrometer. The deutrated chloroform was used to run spectra at operating frequency of 400 MHz. The chemical shifts reported in ppm keeping TMS as standard. The mass spectra (EIMS) were traced at JMS-HX-110 spectrometer.

Synthesis

General Procedure for the Synthesis of Chalcones (3a-e)

1 equiv (0.01M) of acetophenone (1) was dispensed in 100mL of round bottom flask containing 20mL of ethanol. Then 0.01M of different substituted benzaldehydes (2a-e) was added slowly in the reaction medium. The contents of reaction were stirred at room temperature for 2-3h. The pH of reaction mixture was maintained between 9-10 by adding aq. KOH (20% w/v aqueous solution). Thin layer chromatography was employed to monitor the progress of reaction. On completion of condensation reaction; the mixture was cooled to 0^oC (ice-water bath) and acidified with HCl (10% v/v aqueous solution). In most of cases the product precipitates were formed. These were filtered and washed with 10% aqueous HCl solution. The products from oily solution were extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and on evaporating the solvent different substituted chalcones (3a-e) were acquired as a solid.

Characterization of synthesized compounds

The synthesized compounds were characterized by employing spectral techniques like EIMS, IR and ^{1}H-NMR. The ^{1}H-NMR data of all compounds is provided in table 3.

(2E)-1,3-Diphenylprop-2-en-1-one (3a)

HR-MS: $[M]^+$ 208.2656 (calculated for $C_{15}H_{12}O$; 208.26); IR (KBr, cm^{-1}): ν_{max}: 3164 (C-H str of Ar ring), 1674 (C=O str), 1635 (C=C str) 1456 (C=C str of Ar ring); EIMS: *m/z* 208 $[M^+]$, 132 $[C_9H_8O]^+$, 106 $[C_7H_6]^+$, 104 $[C_8H_8]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$, 27 $[C_2H_3]^+$.

3-(2-Methoxyphenyl)-1-phenylprop-2-en-1-one (3b)

HR-MS: $[M]^+$ 238.1098 (calculated for $C_{16}H_{14}O_2$; 238.10); IR (KBr, cm^{-1}): ν_{max}: 3089 (C-H str of Ar ring), 1687 (C=O str), 1614 (C=C str), 1487 (C=C str of Ar ring), 1437 (CH_3 str), 1152 (C-O-C str of ether); EIMS: *m/z* 238 $[M]^+$, 207 $[C_{15}H_{11}O]^+$, 134 $[C_9H_{10}O]^+$, 132 $[C_9H_8O]^+$, 106 $[C_7H_6O]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$, 27 $[C_2H_3]^+$.

3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (3c)

HR-MS: $[M]^+$ 238.1098 (calculated for $C_{16}H_{14}O_2$; 238.10); IR (KBr, cm^{-1}): ν_{max}: 3127 (C-H str of Ar ring), 1691 (C=O str), 1636 (C=C str), 1479 (C=C str of Ar ring), 1425 (CH_3 str), 1163 (C-O-C str of ether); EIMS: *m/z* 238 $[M]^+$, 207 $[C_{15}H_{11}O]^+$, 134 $[C_9H_{10}O]$, 132 $[C_9H_8O]^+$, 106 $[C_7H_6O]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$, 27 $[C_2H_3]^+$.

3-(3,4-Dimethoxyphenyl)-1-phenylprop-2-en-1-one (3d)

HR-MS: $[M]^+$ 268.3124 (calculated for $C_{17}H_{16}O_3$; 268.31); IR (KBr, cm^{-1}): ν_{max}: 3098 (C-H str of Ar ring), 1686 (C=O str), 1624 (C=C str), 1459 (C=C str of Ar ring), 1431 (CH_3 str), 1171 (C-O-C str of ether); EIMS: *m/z* 268 $[M]^+$, 237 $[C_{16}H_{13}O_2]^+$, 132 $[C_9H_8O]^+$, 106 $[C_7H_6O]^+$, 104 $[C_8H_8]^+$, 77 $[C_7H_5]^+$, 51 $[C_4H_3]^+$, 27 $[C_2H_3]^+$.

3-(3-Nitrophenyl)-1-phenylprop-2-en-1-one (3e)

HR-MS: $[M]^+$ 253.2617 (calculated for $C_{15}H_{11}NO_3$; 253.26); IR (KBr, cm^{-1}): ν_{max}: 3122 (C-H str of Ar ring), 1679 (C=O str), 1632 (C=C str), 1556 (N=O str of nitro group); EIMS: *m/z* 253 $[M]^+$, 207 $[C_{15}H_{11}O]^+$,132 $[C_9H_8O]^+$, 122 $[C_6H_4NO_2]^+$, 106 $[C_7H_6O]^+$, 104 $[C_8H_8]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$, 27 $[C_2H_3]^+$.

Synthesis of allylidene derivative of chalcones from O-aminophenol (5a-e)

To 200mg of substituted chalcones (3a-e) contained in 15mL of methanol in RB flask; 1 eqv of ortho-amino phenol (4) was added slowly into reaction contents. The mixture was heated for 15 minutes at 60^0C. 3-5 drops of acetic acid were added laterally. This acidified methanolic reaction mixture was refluxed for 2 hours. Then it was cooled down at room temperature after asserting from TLC. Later on 3mL of aqueous sodium carbonate solution was added with crushed ice. The vigorous hand shaking resulted in precipitation of target compounds from reaction mass. The sticky products were acquired from solvent extraction. The products were collected, washed with distilled water and weighed.

2-[1,3 Diphenylallylidene]aminophenol (5a)

HR-MS: $[M]^+$ 299.3730 (calculated for $C_{21}H_{17}NO$; 299.37); IR (KBr, cm^{-1}): ν_{max}: cm^{-1}; 3400 (OH str), 2940 (C-H str of Ar ring), 1674 (C=C str of R group), 1660 (C=N str), 1480 (C=C str of Ar ring); EIMS: *m/z* 299 $[M]^+$, 282 $[C_{21}H_{16}N]$, 105 $[C_7H_7N]$, 104 $[C_8H_8]$, 77 $[C_6H_5]$, 51 $[C_4H_3]$, 27 $[C_2H_3]$.

2-(((1Z,2E)-3-(2-Methoxyphenyl)-1-phenylallylidene) amino)phenol (5b)

HR-MS: $[M]^+$ 329.3990 (calculated for $C_{22}H_{19}NO_2$; 329.39); (KBr, cm^{-1}): ν_{max}: cm^{-1}; 3448 (OH str), 3123 (C-H str of Ar ring), 1656 (C=N str), 1621 (C=C str), 1512 (C=C str of Ar ring), 1440 (CH_3 str), 1123 (C-O-C str of ether);

EIMS: *m/z* 329 $[M]^+$, 282 $[C_{21}H_{16}N]$, 134 $[C_9H_{10}O]$, 105 $[C_7H_7N]$, 103 $[C_8H_7]$, 77 $[C_6H_5]$, 51 $[C_4H_3]$, 27 $[C_2H_3]$.

2-(((1Z,2E)-3-(4-Methoxyphenyl)-1-phenylallylidene) amino)phenol (5c)

HR-MS: $[M]^+$ 329.3990 (calculated for $C_{22}H_{19}NO_2$; 329. 39); IR (KBr, cm^{-1}): ν_{max}: 3378 (OH str), 3092 (C-H str of aromatic ring), 1634 (C=N str), 1626 (C=C str), 1521 (C=C str of aromatic ring), 1451 (CH_3 str), 1144 (C-O-C str of ether); EIMS: *m/z* 329 $[M]^+$, 282 $[C_{21}H_{16}N]$, 134 $[C_9H_{10}O]$, 105 $[C_7H_7N]$, 103 $[C_8H_7]$, 77 $[C_6H_5]$, 51 $[C_4H_3]$, 27 $[C_2H_3]$.

2-(((1Z,2E)-3-(3,4-Dimethoxyphenyl)-1-phenylallylidene) amino)phenol (5d)

HR-MS: $[M]^+$ 359.4250 (calculated for $C_{23}H_{21}NO_3$; 359. 42); IR (KBr, cm^{-1}): ν_{max}: 3385 (OH str), 3111 (C-H str of Ar ring), 1645 (C=N str), 1632 (C=C str), 1544 (C=C str of aromatic ring), 1446 (CH_3 str), 1099 (C-O-C str of ether); EIMS: *m/z* 359 $[M]^+$, 342 $[C_{23}H_{20}NO_2]$, 164 $[C_{10}H_{12}O_2]$, 133 $[C_9H_9O]$, 105 $[C_7H_7N]$, 103 $[C_8H_7]$, 77 $[C_6H_5]$, 51 $[C_4H_3]$, 27 $[C_2H_3]$.

2-(((1Z,2E)-3-(3-Nitrophenyl)-1-phenylallylidene) amino)phenol (5e)

HR-MS: $[M]^+$ 344.1278 (calculated for $C_{21}H_{16}N_2O_3$; 344. 12); IR (KBr, cm^{-1}): ν_{max}: 3410 (OH stretching), 2933 (C-H str of Ar ring), 1681 (C=C R group str), 1638 (C=N str), 1561 (N=O str of nitro group), 1477 (C=C str of Ar ring); EIMS: *m/z* 344 $[M]^+$, 327 $[C_{21}H_{14}N_2O_2]$, 122 $[C_6H_4NO_2]^+$, 105 $[C_7H_7N]$, 103 $[C_8H_7]$, 77 $[C_6H_5]$, 51 $[C_4H_3]$, 27 $[C_2H_3]$.

^{1}H-NMR of all compounds (3a-e) and (5a-e) was conducted at 400MHz in deutrated chloroform ($CDCl_3$). The chemical shift value (δ) is reported in ppm. The data is given in table 3.

Mettallation of substituted allylidene amino phenol derivatives (7a-e)

For complex formation 15mL of methanol was taken in RB flask along with 200mg of compounds (5a-e). The mixture was heated and stirred for 5 minutes with subsequent addition of 5-6 drops of acetic acid. The refluxing was done at 60^{o}C for 30 minutes. 100 mg of zinc chloride (6) dissolved in 15mL of CH_3OH of was added in reaction mixture. Again the refluxing of mixture continued for 1:20 hours. It was cooled to room temperature after keeping for 10 minutes. During workup 10mL of 1M methanolic solution of sodium hydroxide was added. Again mixture was refluxed for 20-30 minutes. It was cooled down to room temperature and added crushed ice with vigorous hand shaking. The product precipitates were filtered after keeping it at room temperature for 24 hrs. The target molecules were collected, weighed and analyzed by performing TLC.

The infra-red spectrum of all the synthesized ligands (5a-e) displayed a profound high intensity band about 1630 cm^{-1}, which was attributed due to the presence of (C=N) τ stretching frequency and a comparatively low & broad band near 3400cm^{-1} owing to the presence of phenolic hydroxyl group (Ar-OH) τ.

The resultant complexes (7a-e) showed C=N band at slightly lower frequency than the ligands (5a-e); about 40-45 cm^{-1} low frequency shifts was observed. Therefore the C=N band shifted from 1630 τ to 1580 τ which indicated the incorporation of M^{+2} into the ligand through the C=N co-ordination; whereas the frequency band around 3400 cm^{-1} (Ar-OH stretching) was not observed in the complexes; it provided clear indication of ligand complexes to the metal through phenolic oxygen atom *via* deprotonation. The two new bonds appeared 529-544cm^{-1}& 382-418cm^{-1} assigned to the M-N & M-O bond co-ordination respectively (Atlam *et al*., 2018).

Antibacterial assay

The antibacterial activity was conducted under aseptic conditions in sterile 96-wells microplates. The principle beneath the studied method lies that the microbial growth increment in a medium is directly related to the increase in absorbance of broth medium as depicted in a log growth phase (Kaspady *et al.,* 2009; Yang *et al*., 2006). The synthesized ligands and complexes were screened against four gram-negative (*Pseudomonas aeroginosa*, *Salmonella typhi, Kelbsiella pneumonae* and *Escherichia coli*) and two gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*). The bacterial cells were maintained in agar medium. The samples were dissolved in MeOH and 20µg/ well of diluted test solution was pipette out into wells. In fresh nutrient broth kept in wells; 180µL of the fresh bacterial culture with appropriate dilutions was poured. Therefore 200µL of total volume was kept in each well and incubated at 37^{0}C for 16-24hrs. The initial absorbance was maintained between 0.12-0.19. It was measured at 540nm by micro plate reader; the difference of absorbance was recorded as an index of bacterial growth. The % inhibition was computed as:

$$\text{Inhibition (\%)} = 100 \times \frac{(X - Y)}{X}$$

X denotes absorbance of control with bacterial culture and Y represents absorbance due to test sample. The ciprofloxacin was taken as standard.

Measurement of MIC

The MIC (minimum inhibitory concentration) was stated as lowest concentration of sample that has capability to inhibit complete growth of bacterial strain being tested. It was calculated graphically as an extraplotation of linear relationship to zero value.

Calculation of IC_{50} values

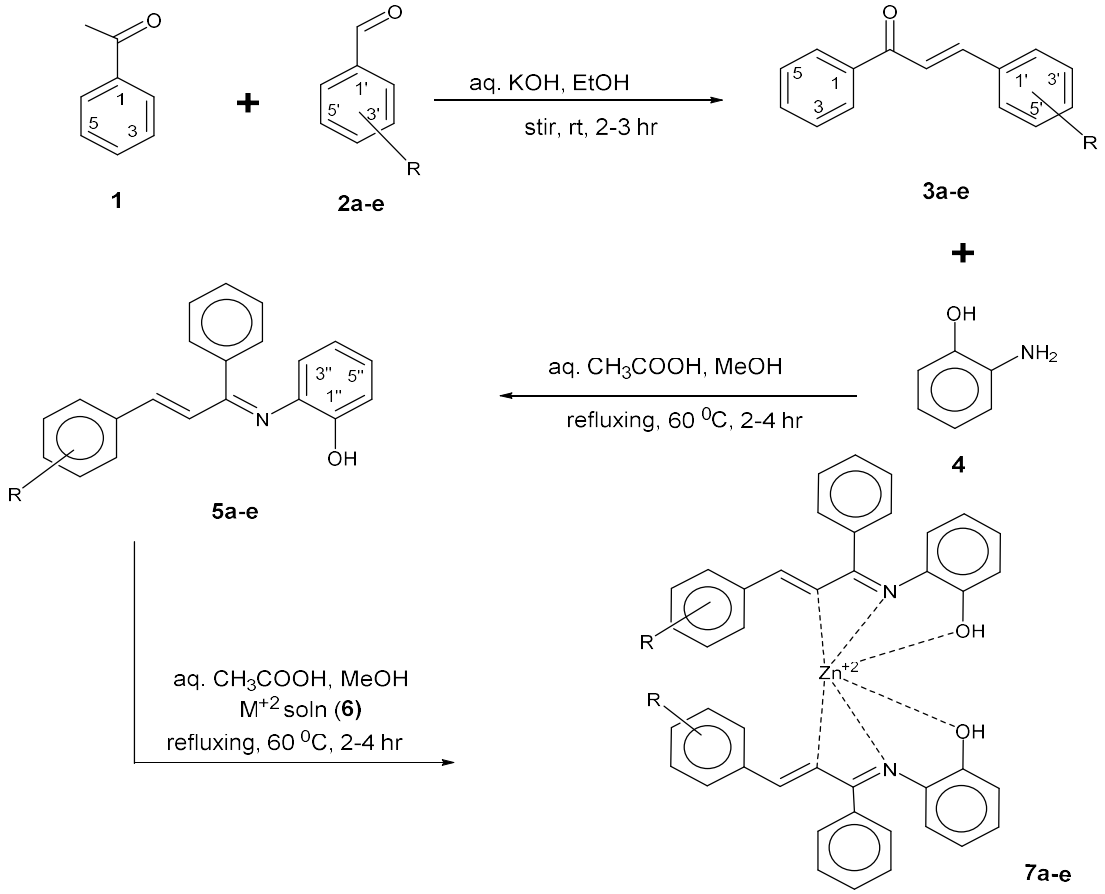
IC_{50} values (concentration that cause 50% inhibition of the enzyme) of samples were measured by using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

STATISTICAL ANALYSIS

All the experiments were performed in triplicate and Microsoft Excel 2010 was used to for statistical analysis.

RESULTS

The different substituted benzaldehydes, acetophenone and ortho amino phenols functionalities have been successfully introduced through a series of different methods by following the protocol outlined in Scheme-1. First the new parent compounds 3-(substitutedphenyl)-1-phenylprop-2-en-1-one (3a-e) were synthesized by the reaction of acetophenone (1) with various substituted aryl aldehydes (2a-e). The reaction of 3a-e with ortho amino phenol (4) in MeOH and dilute acetic acid yielded 2-(((1Z,2E)-3-(substiyutedphenyl)-1-phenylallylidene) amino)phenol (5a-e). The complete conversion was achieved within few hours on refluxing. The metallation of all allylidene derivatives 5a-e were also achieved in acidic methanolic medium. The products were isolated by filtration or solvent extraction method depending upon the nature of the compound. The structures of all the synthesized compounds were confirmed by spectroscopic techniques like HR-MS, IR, ^{1}H-NMR and EIMS.



Scheme 1: Synthesis of allylidene based chalcones analogues and their metal derivatives

Table 1: Different -R groups of synthesized chalcones analogues

Sr. No.	Compound	Substituent (R)	Position
1	3a	H	---
2	3b	OCH_3	2
3	3c	OCH_3	4
4	3d	OCH_3	3,4
5	3e	NO_2	3

Table 2: Physical characteristics of chalcones analogues and their metal derivatives

Entry	Physical state	Color	Mol. formula	Mol. Wt.	M.P (°C)	% yield
3a	Solid	White	$C_{15}H_{12}O$	208	126	85
3b	Solid	White	$C_{16}H_{14}O_2$	238	134	82
3c	Solid	White	$C_{16}H_{14}O_2$	238	158	75
3d	Solid	White	$C_{17}H_{16}O_3$	268	164	62
3e	Solid	White	$C_{15}H_{11}NO_3$	253	176	73
5a	Crystalline solid	black	$C_{21}H_{17}NO$	299	189	85
5b	Solid	Grayish black	$C_{22}H_{19}NO_2$	329	195	86
5c	Solid	Grayish black	$C_{22}H_{19}NO_2$	329	199	87
5d	Solid	Grayish black	$C_{23}H_{21}NO_3$	359	187	79
5e	Solid	Grayish black	$C_{21}H_{16}N_2O_3$	344	179	89
7a	Solid	grayish	-	-	298	-
7b	Solid	grayish	-	-	287	-
7c	Pallets	grayish	-	-	276	-
7d	Pallets	grayish	-	-	269	-
7e	Solid	grayish	-	-	293	-

Table 3: ^{1}H-NMR ($CDCl_3$, 400 MHz) data of chalcones & their allylidene derivatives

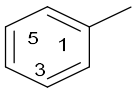
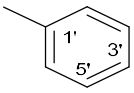
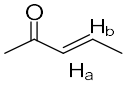
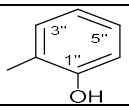
No				
3a	7.87 (d, *J* = 7.8 Hz, 2H, H-2 & H-6), 7.49 (t, *J* = 7.2 Hz, 1H, H-4), 7.43 (d, *J* = 7.4 Hz, 2H, H-3 & H-5)	7.52 (d, *J* = 7.6 Hz, 1H, H-6′ & H-2′),7.39 (t, *J* = 7.8 Hz, 1H, H-4′), 7.26 (d, *J* = 7.6 Hz, 2H, H-3′ & H-5′)	7.98 (d, 1H, *J* = 16 Hz, H_a), 7.59 (d, 1H, *J* = 16 Hz, H_b)	-
3b	7.78 (d, *J* = 7.8 Hz, 2H, H-2 & H-6), 7.52 (d, *J* = 7.4 Hz, 2H, H-3 & H-5), 7.60 (t, *J* = 7.2 Hz, 1H, H-4)	7.65 (d, *J* = 7.6 Hz, 1H, H-6′), 7.30 (t, *J* = 7.8 Hz, 1H, H-4′), 7.10 (d, *J* = 7.6 Hz, 2H, H-3′ & H-5′), 3.71 (s, 3H, OCH_3)	7.90 (d, 1H, *J* = 16 Hz, H_a), 7.35 (d, 1H, *J* = 16 Hz, H_b)	-
3c	7.80 (d, *J* = 7.6 Hz, 2H, H-2 & H-6), 7.47 (t, *J* = 7.1 Hz, 1H, H-4), 7.56 (d, *J* = 7.2 Hz, 2H, H-3 & H-5)	7.71 (d, *J* = 7.2 Hz, 2H, H-2′& H-6′), 6.98 (d, *J* = 7.6 Hz, 2H, H-3′ & H-5′), 3.42 (s, 3H, OCH_3)	7.89 (d, 1H, *J* = 16 Hz, H_a), 7.32 (d, 1H, *J* = 16 Hz, H_b)	-
3d	7.76 (d, *J* = 7.4 Hz, 2H, H-2 & H-6), 7.66 (t, *J* = 7.2 Hz, 1H, H-4),7.58 (d, *J* = 7.6 Hz, 1H, H-3 & H-5)	7.67 (brd s, 1H, H-2′), 7.21-7.37 (m, 2H, H-5′ & H-6′), 3.20 (s, 3H, OCH_3), 3.17 (s, 3H, OCH_3)	7.85 (d, 1H, *J* = 16 Hz, H_a), 7.46 (d, 1H, *J* = 16 Hz, H_b)	-
3e	7.81 (d *J* = 7.4 Hz, 2H, H-2 & H-6), 7.27-7.45 (m, 3H, H-3 to H-5)	8.40 (s, 1H, H-2'), 7.86 (brd d, 1H, H-4'), 7.69 (t, *J* = 7.6 Hz, 1H, H-5'), 7.67 (d, *J* = 7.6 Hz, 1H, H-6')	8.07 (d, 1H, *J* = 16 Hz, H_a), 7.46 (d, 1H, *J* = 16 Hz, H_b)	-
5a	7.51 (d *J* = 7.4 Hz, 2H, H-2 & H-6), 7.48-7.36 (m, 3H, H-3 to H-5)	7.85 (d, *J* = 7.8 Hz, 2H, H-2' & H-6'), 7.60-7.54 (m, 3H, H-3' to H-5')	4.67 (d, 1H, *J* = 16 Hz, H_a), 5.34 (d, 1H, *J* = 16 Hz, H_b)	6.62 to 6.51 (m, 2H, H-5" & H-6"), 7.21 (d, *J* = 7.8 Hz, 1H, H-4"), 6.68 (d, *J* = 7.4 Hz, 1H, H-3")
5b	7.79 (d, *J* = 7.6 Hz, 2H, H-2 & H-6), 7.62-7.57 (m, 3H, H-3 to H-5)	7.53 (d, *J* = 7.8 Hz, 1H, H-6'), 7.48 (t, *J* = 7.6 Hz, 1H, H-4'), 7.39 (d, *J* = 7.8 Hz, 1H, H-3'), 7.24 (d, *J* = 7.4 Hz, 1H, H-5'), 3.59 (s, 3H, OCH_3)	5.34 (d, 1H, *J* = 16 Hz, H_a), 4.67 (d, 1H, *J* = 16 Hz, H_b)	6.62 to 6.58 (m, 2H, H-5" & H-6"), 6.49 (d, *J* = 7.6 Hz, 1H, H-4"), 6.43 (d, *J* = 7.8 Hz, 1H, H-3")
5c	7.86 (d, *J* = 7.6, 2H, H-2 & H-6), 7.72 to 7.67 (m, 3H, H-3 to H-5)	7.59 (d, *J* = 8.4 Hz, 2H, H-2′ to H-6′), 7.54 (d, *J* = 8.0 Hz, 2H, H-3′ to H-5′), 3.63 (s, 3H, OCH_3)	5.58 (d, 1H, *J* = 16 Hz, H_a), 5.42 (d, 1H, *J* = 16 Hz, H_b)	7.27 (d, *J* = 7.8 Hz, 1H, H-6"), 7.02 (t, *J* = 7.2 Hz, 1H, H-5"), 6.99 (t, *J* = 7.4 Hz, 1H, H-4"), 6.92 (d, *J* = 7.8 Hz, 1H, H-3")
5d	7.83 (d, *J* = 7.8 Hz, 2H, H-2 & H-6), 7.69 to 7.65 (m, 3H, H-3 to H-5)	7.38 (d, *J* = 8.4 Hz, 1H, H-2′), 7.29 (d, *J* = 7.8 Hz, 1H, H-5′), 7.15 (d, *J* = 8.0 Hz, 1H, H-6′), 3.61 (s, 6H, 2OCH_3)	5.54 (d, 1H, *J* = 16 Hz, H_a), 5.49 (s, 1H, H_b)	7.12 (d, *J* = 7.8 Hz, 1H, H-6″), 7.09 (t, *J* = 7.6 Hz, 1H, H-5″), 6.89 (t, *J* = 7.2 Hz, 1H, H-4″), 6.77 (d, *J* = 7.4 Hz, 1H, H-3″)
5e	7.99 (d, *J* = 8.4 Hz, 2H, H-2 & H-6), 7.81 to 7.76 (m, 3H, H-3 to H-5)	7.96 (s, 1H, H-2′), 7.92 (d, H = 8.4 Hz, 1H, H-4′),7.86 (t, *J* = 7.2 Hz, 1H, H-5′), 7.81 (d, *J* = 7.6 Hz, 1H, H-6′)	5.23 (d, 1H, *J* = 16 Hz, H_a), 4.92 (d, 1H, *J* = 16 Hz, H_b)	7.01 (t, *J* = 7.4 Hz, 1H, H-6″), 6.71 to 6.68 (m, 2H, H-4 & H-5), 6.62 (d, *J* = 7.8 Hz, 1H, H-3″)

Table 4: *MIC_{50}* of chalcones and their derivatives against different bacterial strains

Micro-organisms	MIC_{50} (μM)					
Codes	*S. typhi* (-) (Mean ± S.D)	*E.coli* (-) (Mean ± S.D)	*K. pneumonae* (-) (Mean ± S.D)	*B. subtilis* (+) (Mean ± S.D)	*P. aeroginosa* (-) (Mean ± S.D)	*S. aerus* (+) (Mean ± S.D)
3a	12.21 ±0.32	11.87±0.26	15.41±0.32	-	-	10.21±0.32
3b	10.41±0.34	9.23±0.32	12.11±0.47	10.43±0.67	9.12±0.33	12.04±0.78
3c	9.21±0.11	8.47±0.98	10.97±0.89	11.81±0.47	8.53±0.67	11.10±0.45
3d	9.48±0.45	12.78±0.92	-	-	-	-
3e	-	-	-	-	-	-
5a	-	10.12±0.18	-	-	-	-
5b	9.62±0.34	-	11.55±0.48	9.56±0.35	8.41±0.43	12.68±0.67
5c	8.10±0.21	-	13.84±0.65	10.10±0.34	-	-
5d	9.11±0.17	-	-	-	-	10.43±0.28
5e	-	14.22±0.55	-	-	-	-
7a	8.51±0.99	-	12.29±0.66	12.38±0.67	-	-
7b	8.88±0.45	-	-	-	-	-
7c	8.34±0.69	10.67±0.34	7.32±0.44	-	9.45±0.37	11.57±0.78
7d	7.33±0.67	9.95±0.78	-	-	-	9.82±0.12
7e	11.39±0.87	13.80±0.66	-	-	11.6±0.38	11.94±0.67
Ciprofloxacin	8.12 ± 0.21	8.22 ± 0.12	10.03 ± 0.1	8.96 ± 0.02	8.09± 0.0.2	8.12 ± 0.21

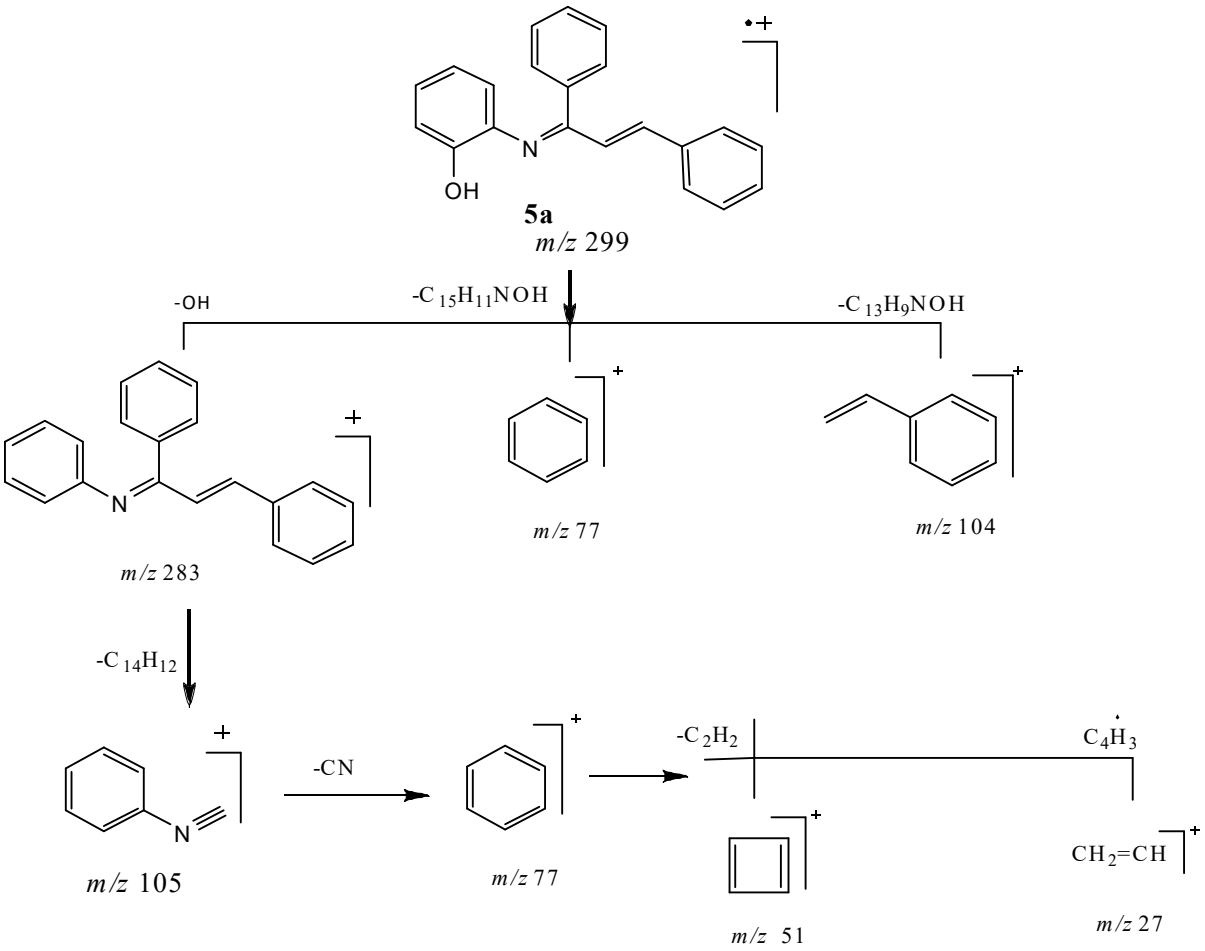


Fig. 1: The mass fragmentation pattern of 2-[1,3 Diphenylallylidene]aminophenol (5a)

Antibacterial assay

The synthesized molecules were evaluated for antibacterial activities (table 4) and found to be valuable against different bacterial strains as evident from their MIC_{50} values.

DISCUSSION

In the undertaken research, a series of organic compounds containing chalcones and their allylidene derivatives were synthesized. The parent compounds 3(alkyl-(substitutedphenyl)-1-phenylprop-2-en-1-one (3a-e), were prepared by a condensation reaction between acetophenone (1) and different substituted benzaldehydes (2a-e); yielding α-β unsaturated ketonic linkage. The subsequent reactions of 3a-e with ortho amino phenol 4 provided a series of 2-(1Z-2E)3-(alkylsustitutedphenyl)-1-phenylallylidene)aminophenol (5a-e) as depicted from Scheme 1. Further metal complexes (7a-e) of ligands 5a-e was accomplished with zinc chloride 6 in methanolic solution. Synthesis of all derivatives 5a-e and 7a-e was performed in methanol using dilute acidic conditions. Complete conversion was achieved within 3h by stirring on 60-70^{o}C. On reaction completion the workup was done by adding dilute base and cold distilled water; on vigorous shaking and standing for 20mins the solid product precipitates were emerged in the reaction mixture. On filtration the target compound was obtained. The compound 5a was synthesized as black crystalline solid with yield of 85% and melting point of 189^{o}C. The molecular formula $C_{21}H_{17}NO$ was determined by HR-MS showing molecular ion peak at *m/z* 299 and also by doing the count of the protons in ^{1}H-NMR spectrum. The IR spectrum showed absorption bands at 3400, 2940, 1674, 1660 and 1480cm^{-1} which confirmed the presence of O-H of phenolic group, C-H moiety of aromatic rings, C=C of α-β unsaturated system in the vicinity imine unit, C=N of imine linkage, C=C of aromatic ring respectively. The EI-MS gave distinguished peaks at *m/z* 104 and 77 which have confirmed the formation of $C_6H_5C_2H_3^+$ and $C_6H_5^+$ cations respectively. In the aromatic region of the ^{1}H-NMR spectrum signals appeared at δ 7.85 due to the doublet of magnetically equivalent two protons H-2' & H-6', the multiplet at 7.60-7.54 having integration of three protons provided clue for the presence of H-3', 4' & 5' protons. These were assigned to the phenyl ring pertained to the benzaldehyde moiety; whereas two aromatic signals at δ 7.51 due to the doublet and multiplet at chemical shift of 7.48-7.36 provided authentication for the phenyl ring of acetophenone. The four protons of ortho amino phenol were displayed at 7.21 and 6.68 in the form of doublets and a multiplet appeared at 6.62 to 6.51. In the upfield/shielded region of the spectrum, the two doublets of higher coupling constants were appeared at 5.34 and 4.67. The coupling constants of 16 Hz indicated the presence of two methine protons of allylidene functionality, which appeared as the trans spatial orientation about doublet bond. On the basis of above summative confirmations, the structure of 5a was assigned as 2-[1,3diphenylallylidene]aminophenol. On the same grounds the structure of other compounds was characterized by above illustrated spectral techniques as mentioned in the experimental section. The comprehensive data of all molecules has been provided in table 2 and 3. The mass fragmentation pattern of 5a is given in fig. 1.

Antibacterial activity

Against *S. typhi* the excellent activity potential was shown by 5c, 7b & 7d as depicted from MIC_{50}. Whereas against *E. coli* 3c exhibiting good inhibiting trend. It was showing 8.47±0.98mm zone of inhibition as MIC_{50} comparable to ciprofloxacin. 7c was found more active (7.32±0.44mm zone of inhibition as MIC_{50}) than the standard in inhibiting the *K. pneumonae* bacterial strain. For *B. subtilis* and *S. aureus* the targets displayed moderate activity and some were even found inactive like 3d, 3e, 5e and 7b. While antibacterial data against *P. aeroginosa* showed excellent potential of 5b (8.41±0.43mm zone of inhibition as MIC_{50}). It was observed that the compounds containing electron releasing groups such as methoxy and hydroxyl exhibited higher antibacterial activity than electron withdrawing groups i.e NO_2. In complexes the lone pairs of electrons became engaged with the metal cation in building the secondary binding forces, therefore they inhibited the bacterial cells to an insignificant extent.

CONCLUSION

The ligands and complexes containing electron releasing groups such as hydroxyl showed good antibacterial activity. The results suggested that the chalcones & allylidene derivatives on incorporation of 2-amino phenol have resulted in extended conjugation system. The α and β unsaturated system of chalcone along with the allylidene moiety, made the targets more liphophilic in nature and interact with the DNA of bacterial strains, resulting in the cells disruption. Therefore it can be concluded that synthesized compounds can exhibit excellent scope in drug chemistry & provide a milestone in the investigation & designing of new antibacterial drugs.

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