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Synthesis, Characterization and Biological Evaluation of Benzylidenes and β-Lactams Bearing Aza Heterocyclic Moieties

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ABSTRACT: One of the most promising challenges faced by agriculturists is the control of crops against the attack of pathogens. To overcome this, various fungicides like Carbendazim and Mancozeb has been used due to the presence of potential nuclei, nitrogen. In the light of importance of nitrogen nuclei, present study was carried out with the synthesis of benzylidenes 1-7 by reacting different aza heterocyclic amines and iso vanillin followed by synthesis of β-lactams 8-9 using chloroacetyl chloride that carried the cyclization of CH=N moiety in benzylidenes of 1 and 5 compounds. Structural elucidation of the synthesized compounds using various spectroscopic techniques *viz.* UV-Visible, IR, ^{1}H NMR, and ^{13}C NMR along with their elemental analysis was done. Synthesized compounds were biologically evaluated as antioxidants using phosphomolybdate assay and ascorbic acid as standard. In addition to this, fungitoxicity of the compounds was evaluated against pathogenic strains *Rhizoctonia solani, Macrophomina phaseolina, Fusarium verticillioides,* and *Dreschlera maydis*. It was found that compound 1 was most effective against *Rhizoctonia solani* and *Macrophomina phaseolina* while compound 6 was effective against *Fusarium verticillioides*. Compound 8 was proved better antioxidant as compared to other derivatives. Henceforth, the present study highlighted the biological importance of aza heterocycles.

Keywords: Aza heterocycles, Benzylidenes, β-lactams, phosphomolybedate, *Rhizoctonia solani, Macrophomina phaseolina, Fusarium verticillioides,* and *Dreschlera maydis.*

INTRODUCTION

Benzylidenes, also known as Schiff bases or imines, formed via the condensation reaction of aromatic/heteroaromatic aldehydes and amines, were first reported by Hugo Schiff (Dhar and Taploo 1982). Due to the presence of active moiety (CH=N), they played a magnificent role in agricultural, pharmaceutical and medicinal research areas as they possess remarkable antifungal (Kaur *et al.,* 2019), antioxidant (Kaur *et al.,* 2021), antimicrobial (Verma *et al.,* 2020), antitubercular (Aboul-Fadl *et al.,* 2003), anticancer (Ali *et al.,* 2012), anticonvulsant (Chaubey and Pandeya 2012) and many more biological properties (Pandey *et al.,* 2012; Chinnasamy *et al*., 2010; Sondhi *et al.,* 2006). Presence of nitrogen (aza) atom in imine linkage (CH=N) played significant role as it may interact with the active centers of cell constituents via hydrogen bonding and disrupt the normal functioning of various cell processes (Vashi and Naik 2004). In the light of importance of nitrogen atom, aza heterocyclic moieties catches the eyes of many researchers to derivatize the nitrogen containing amines prior to the use of merely aromatic amines or aldehydes to synthesize benzylidene derivatives (Bakshi *et al.,* 2017). Since, many commercially available bioactive drugs (carbendazim 50 WP, mancozeb 75 WP, propiconazole etc.) marked the presence of aza heteroatom as bioactive centre, additionally supported the use of aza heterocycles. Also, as the demand of food is increasing day by day due to increase in the population of the world, continuous use of fungicides on agricultural land to eliminate the attack of diseases on crops, developed the resistance in crops against their use. So designing of novel, effective and non-toxic fungicides and bioactive products need to be done to combat the loss in the yield and quality of crops (Lange *et al.,* 2014). Keeping in view the biological importance of aza heterocycles, present work was designed with two objectives; (i) chemically derivatize nitrogen containing heterocyclic amines to yield benzylidene derivatives and β-lactams and (ii) screening of the synthesized compounds as antifungal and antioxidant agents. Four different pathogenic maize fungal strains (*Rhizoctonia solani, Fusarium verticillioides, Macrophomena phaseolina* and *Dreschlera maydis*) were used for evaluating antifungal potential of

synthesized compounds. For antioxidant evaluation, phosphomolybdate assay was used to determine total antioxidant capacity.

MATERIALS AND METHODS

The chemicals used in the present study were procured from Thermo Fischer Scientific India Pvt. Ltd., Mumbai, Central Drug House Pvt. Ltd., Mumbai, Sisco Research Laboratories, Pvt. Ltd., Mumbai, Ranbaxy Pvt. Ltd., Hyderabad, Loba Chemie Pvt., Ltd., Mumbai, and Crystal, India and used as such without further purification. For the screening of antifungal potential of compounds, PDA and Agar Agar were purchased from Hi Media laboratory Pvt. Ltd., Mumbai. Melting point of all the synthesized solid products was taken on melting point apparatus (Relitech, Haryana, India) using open end capillaries and was uncorrected. Structural characterization of all the synthesized compounds were established using various spectroscopic techniques IR, ^{1}H NMR, ^{13}C NMR and among which NMR results were scrutinized from Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh, using Proton Magnetic Resonance spectra, Bruker Avance II 500 MHz spectrophotometer with TMS (tetramethylsilane) as internal standard was used with chemical shift expressed in δ (ppm), while FTIR results were recorded on Perkin-Elmer FT-IR Spectrophotometer with υ in cm^{-1}, obtained from Central Instrument Laboratory (CIL) Panjab University, Chandigarh. UV-Visible spectroscopy was also used for structural elucidation and the data was recorded on Shimadzu (UV 1800) UV-Visible spectrophotometer with λ_{max} in nm. CHN analysis was done on the vario EI III Elementor analyzer.

A. Synthesis of Benzylidene derivatives (1-7)

5-((3H-1,2,4-Triazol-4(5H)-ylimino)methyl)-2-methoxyphenol (1)

Equimolar amount of iso vanillin (0.01 mol, 1.52 g) and 4-amino-1,2,4-triazole (0.01 mol, 0.84g) was reacted with each other in the presence of glacial acetic acid (3-4 drops) as a catalyst using methanol as solvent. The reaction mixture was stirred for 6 hours along with continuously monitoring the progress of reaction using TLC plate with silica gel G as adsorbant and ethyl acetate as adsorbate. The precipitates so formed were filtered and recrystallized using methanol which were then dried and gave solid product **1**, 53.34%, white powder, mp 130-131°C, R_f 0.73 (silica gel, ethyl acetate). UV-vis (λ_{max}): 348 nm. IR: (cm^{-1}) 1654 (CH=N). ^{1}H NMR (DMSO): δ 9.52 (s, 1H), 9.08 (s, 2H), 8.62 (s, 1H), 7.07-7.62 (m, 3H), 3.85 (s, 3H). ^{13}C NMR ($CDCl_3$) ppm: δ 157.95, 151.49, 146.89 143.96,124.68, 122.28, 113.04, 111.83, and 55.56.

Anal. Calcd for $C_{10}H_{12}N_4O_2$: C, 55.04; H, 4.62; N, 25.68. Found: C, 55.00; H, 4.65; N, 25.69.

2-Methoxy-5-((pyridin-2'-ylimino)methyl) phenol (2)

Iso vanillin (0.01 mol, 1.52 g) was taken in a beaker and dissolved in methanol (10 ml). Equimolar quantity of 2-amino pyridine (0.01 mol, 0.94 g) in methanol was added to the above mixture and stirring was done for 3 hours followed by refluxing for 6 hours using 40% NaOH (0.5 ml) as basic catalyst. The progress of the reaction was checked by TLC (silica gel/ethyl acetate). The liquid so obtained was subjected for distillation at suitable temperature to get semi solid product **2,** 16.54%, green semi solid, R_f 0.65 (silica gel, ethyl acetate). UV-vis (λ_{max}): 319 nm. IR: (cm^{-1}) 1658 (CH=N). ^{1}H NMR ($CDCl_3$): δ 9.70 (s, 1H), 9.05 (s, 1H), 6.41-7.88 (m, 7H), 3.17 (s, 3H). ^{13}C NMR ($CDCl_3$) ppm: δ 160.92, 136.26, 135.11, 134.03, 133.82, 131.42, 130.71, 129.66, 129.40, 125.17, 124.96, 112.32 and 56.06.

Anal. Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.44; H, 5.29; N, 12.25.

2-Methoxy-5-((pyridin-3'-ylimino)methyl)phenol (3)

Methanolic solution of iso vanillin (0.01, 1.52 g) and 3-amino pyridine (0.01 mol, 0.94 g) was taken in round bottom flask and subjected to reflux with stirring for 10 hours in the presence of basic catalyst (40% NaOH, 0.5 ml) till a single spot on TLC was obtained which assured the formation of semi solid product **3**, 13.00%, brown semi solid, R_f 0.58 (silica gel, ethyl acetate). UV-vis (λ_{max}): 322 nm. IR: (cm^{-1}) 1676 (CH=N). ^{1}H NMR (DMSO): δ 9.36 (s, 1H), 8.51 (s, 1H), 7.05-8.46 (m, 7H), 3.85 (s, 3H). ^{13}C NMR (DMSO) ppm: δ 162.10, 151.16, 147.40, 146.72, 146.40, 142.84, 128.82, 127.40, 123.84, 122.65, 113.62 and 111.55.

Anal. Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.39; H, 5.31; N, 12.28.

2-Methoxy-5-((pyridin-4'-ylimino)methyl)phenol (4)

Sodium hydroxide (40%, 0.5 ml) catalyzed methanolic solution of iso vanillin (0.01 mol, 1.52 g) and 4-amino pyridine (0.01 mol, 0.94 g) was refluxed for 8 hours with continuously checking the progress of reaction using TLC. The precipitates so formed were filtered and recrystallized using methanol to yield pure solid compound **4,** 25.21%, 20-21°C, chocolate solid powder, R_f 0.70 (silica gel, ethyl acetate). UV-vis (λ_{max}): 321 nm. IR: (cm^{-1}) 1685 (CH=N). ^{1}H NMR ($CDCl_3$): δ 9.83 (s, 1H), 8.83 (s, 1H), 6.99-7.50 (m, 7H), 3.68 (s, 3H). ^{13}C NMR ($CDCl_3$) ppm: δ 159.76, 139.42, 128.98, 128.79, 126.57, 124.20, 121.56, 120.56, 116.77, 112.26 and 54.49.

Anal. Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.45; H, 5.27; N, 12.26.

4-(3'-Hydroxy-4'-methoxybenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (5)

Equimolar amount of 4-amino antipyrine (0.01 mol, 2.03 g) and iso vanillin (0.01 mol, 1.52 g) was taken in beaker to which methanol (10 ml) was added along with addition of glacial acetic acid as a catalyst. The contents of the mixture were stirred for 5 hours till a single spot was observed on TLC followed by filtration

of precipitates. To get pure product, precipitates so formed were recrystallized using methanol followed by filtering and drying of precipitates to yield solid product **5**, 78.45%, 186-187°C, canary solid powder, R_f 0.71 (silica gel, ethyl acetate). UV-vis (λ_{max}): 388 nm. IR: (cm^{-1}) 1614 (CH=N). ^{1}H NMR ($CDCl_3$): δ 9.73 (s, 1H), 8.69 (s, 1H), 6.73-7.33 (m, 8H), 3.89 (s, 3H), 3.80 (s, 3H) and 3.25 (s, 3H). ^{13}C NMR ($CDCl_3$) ppm: δ 158.61, 154.66, 146.68, 134.61, 130.57, 129.79, 129.20, 125.61, 122.12, 121.72, 119.26, 117.66, 113.26, 110.66, 53.26, 35.49 and 19.66.

Anal. Calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 67.67; H, 5.67; N, 12.44.

2-Methoxy-5-((4'-phenylthiazol-2'-ylimino)methyl)phenol (6)

Condensation reaction of iso vanillin (0.01 mol, 1.52 g) and 2-amino-4-phenyl thiazole (0.01 mol, 1.76 g) was carried out using methanol as solvent and in the presence of glacial acetic acid as catalyst. The reaction mixture was refluxed for 7 hours along with continuously monitoring the progress of reaction using TLC. The mixture was then allowed to cool down at room temperature followed by storing the resulted liquid at 5°C to get a solid product which was then washed with petroleum ether to get pure product **6**, 43.00%, 36-37°C, Chocolate solid powder, R_f 0.88 (silica gel, ethyl acetate). UV-Vis (λ_{max}): 403 nm. IR: (cm^{-1}) 1693 (CH=N). ^{1}H NMR (DMSO): δ 8.92 (s, 1H), 8.29 (s, 1H), 6.61-6.98 (m, 7H), 3.31 (s, 3H). ^{13}C NMR (DMSO) ppm: δ 172.02, 167.10, 138.52, 138.33, 135.82, 135.60, 134.37, 134.27, 128.51, 128.01, 127.70, 118.22, 117.56, 111.99 and 55.28.

Anal. Calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 65.81; H, 4.54; N, 9.02.

6-(2'-(3"-Hydroxy-4"-methoxybenzylideneamino)-2'-phenylacetamido)3, 3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid (7)

Glacial acetic acid catalyzed reaction of equimolar amount of ampicillin (0.05mol, 1.74 g) and iso vanillin (0.005 mol, 0.76 g) was carried out using methanol as a solvent. The contents of the reaction mixture were stirred and refluxed for 12 hours till a single spot on TLC was observed while monitoring the progress of reaction. The precipitates so formed were filtered and recrystallized with methanol to yield desired product **7**, 32.00%, 122-123°C, Yellow color solid powder, R_f 0.55 (silica gel, ethyl acetate). UV-Vis (λ_{max}): 332 nm. IR: (cm^{-1}) 1635 (CH=N). ^{1}H NMR (DMSO): δ 11.19 (s, 1H), 9.26 (s, 1H), 8.66 (s, 1H), 6.88-7.40 (m, 8H), 5.61-5.63 (d, 1H, J = 10 Hz), 5.20-5.22 (d, 1H, J = 10 Hz) 3.86 (s, 3H), 2.18 (s, 3H) and 2.04 (s, 3H). ^{13}C NMR (DMSO) ppm: δ 176.40, 172.06, 163.90, 158.30, 141.47, 139.51, 129.75, 128.41, 127.70, 124.18, 118.05, 113.47, 111.51, 106.51, 72.12, 68.70, 62.77, 55.70, 52.50, 38.70, and 21.21.

Anal. Calcd for $C_{24}H_{25}N_3O_6S$: C, 59.61; H, 5.21; N, 8.69. Found: C, 59.66; H, 5.19; N, 8.66.

B. Synthesis of β-lactams (7-9)

3-Chloro-4-(3'-hydroxy-4'-methoxyphenyl)-1-(3"H-1",2",4"-triazol-4"-(5"H)-yl)azetidin-2-one (8)

Benzylidene derivative of iso vanillin and 4-amino-1,2,4-triazole **1** (0.001 mol)was dissolved in dichloromethane (10 ml) and kept the solution at 0°C overnight. The resulted solution was then subjected for stirring after the addition of triethylamine (0.0012 mol, 0.1 ml) followed by addition of chloroacetyl chloride (0.003 mol, 0.4 ml) after 15 minutes addition of triethylamine. The contents were then refluxed for 10 hours while continuously monitoring the progress of reaction using TLC. The mixture was than kept in ice cold water bath which resulted into the formation of precipitates which were filtered and dried to yield respective β-lactam **8**, 10.00%, 98-99°C, Grey solid powder, R_f 0.74 (silica gel, ethyl acetate). UV-Vis (λ_{max}): 353 nm. IR: (cm^{-1}) 1757 (C=O stretching), 1329 (C-N stretching), 751 (C-Cl stretching). ^{1}H NMR (DMSO): δ 9.82 (s, 1H), 9.20 (s, 1H), 7.10-7.34 (m, 3H), 5.47-5.51 (d, 1H, J = 17.05 Hz), 5.38-5.41 (d, 1H, J = 17.00 Hz), 3.34 (s, 3H). ^{13}C NMR (DMSO) ppm: δ 159.95, 146.89, 143.96, 138.79, 124.68, 122.28, 113.04, 112.00, 65.36, 59.81 and 55.56.

Anal. Calcd for $C_{12}H_{11}ClN_4O_3$: C, 48.91; H, 3.76; N, 19.01. Found: C, 48.87; H, 3.78; N, 19.03.

4-(3'-Chloro-2'-(3"-hydroxy-4"-methyloxyphenyl)-4'-oxoazetidin-1'-yl)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (9)

In round bottomed flask, dichloromethane (10 ml) and benzylidene derivative of iso vanillin and 4-amino antipyrine **5** were taken and kept in freezer overnight at 0°C. On the following day, triethylamine (0.0012 mol, 0.1 ml) and chloroacetyl chloride (0.003 mol, 0.4 ml) were added to the above freeze content with 15 minutes of interval followed by stirring and refluxing for 9 hours to get single spot in TLC plate. The mixture was then poured into ice cold water that followed the formation of precipitates which were than filtered and dried to get desired β-lactam **9**, 11.07%, 130-131°C, yellow solid product, R_f 0.84 (silica gel, ethyl acetate). UV-Vis (λ_{max}): 382 nm. IR: (cm^{-1}) 1741 (C=O stretching in β-lactam ring), 1741 (C=O stretching in β-lactam ring), 1673 (C=O stretching in antipyrine ring) 1395 (C-N stretching), 755 (C-Cl stretching). ^{1}H NMR (DMSO): δ 9.47 (s, 1H), 7.06-7.63 (m, 8H), 4.46-4.49 (d, 1H, J = 15.10 Hz), 4.30-4.33 (d, 1H, J = 15.80 Hz), 3.32 (s, 3H), 3.05 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (DMSO) ppm: δ 162.29, 164.87, 159.76, 149.85, 146.68, 130.57, 128.98, 128.79, 126.79, 126.57, 124.20, 121.72, 120.56, 116.77, 112.26, 63.50, 56.49, 22.42, and 19.99.

Anal. Calcd for $C_{21}H_{20}ClN_3O_4$: C, 60.95; H, 4.87; N, 10.15. Found: C, 60.90; H, 4.90; N, 10.17.

C. Biological evaluation

Antifungal activity

Antifungal potential of the synthesized compounds was

determined using four pathogenic maize fungal strains (*Rhizoctonia solani, Fusarium verticillioides, Macrophomena phaseolina* and *Dreschlera maydis*) which were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Five different concentrations of all the benzylidene derivatives and β-lactams were used (1000, 500, 250, 100 and 50 µg/ml) and antifungal evaluation was carried out using Poisoned food technique, already reported in literature (Grover and Moore 1962). Potato Dextrose Agar (PDA) medium was used to carry out the sub culturing of fungal strains for which slants were used. PDA media was prepared in conical flasks (250 ml) followed by its sterilization in autoclave at 121°C. To 99 ml of melted PDA, test compound **1-9** (1 ml) with different concentrations, was added and poured into the petriplates under aseptic environment to avoid the contamination of plates. After the media in the petriplates solidified, inoculation was done by placing a small disc (0.5 cm diameter) of the test fungi at the centre of petriplates upside down under aseptic conditions followed by incubation at 25±1°C in BOD. Regular checks were made on the growth of fungi in tested plates and comparison was done with control plates (without test solutions). The experiment was carried out using five replications and fungi toxicity of the synthesized derivatives of aza heterocycles **1-9** was evaluated by calculating percent growth inhibition using following formula;

$$I = \frac{100\,(C - T)}{C}$$

Where I refer to inhibition percent,
C refers to colony diameter in control (cm),
T refers to colony diameter in treatment (cm)
ED_{50} values were also calculated using SPSS version 16.

Total antioxidant capacity (TAC)

Aza heterocyclic amine derivatives **1-9** were also screened for their antioxidant potential using phosphomolydate assay (Prieto *et al.,* 1999), which involved the basic principle of reduction of Mo (+6) to Mo (+5) by test compounds at acidic pH. An aliquot of tested sample (3 ml each) was taken at different concentrations (1000, 500, 250, 100, 50, and 25 µg/ml) being prepared using methanol as a solvent, and was mixed with reagent solution (0.60 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) (1 ml). Control solution was also made that contained reagent (3 ml) and methanol as solvent (1 ml). Then the prepared test tubes were covered foil paper and subjected for incubation on a water bath at 95°C for 90 minutes followed by cooling down the solutions in test tubes at room temperature and testing the absorbance at 695 nm in a spectrophotometer. Ascorbic acid served as positive control whose equivalent was used to express the TAC of each compound from their linear equations as given below;

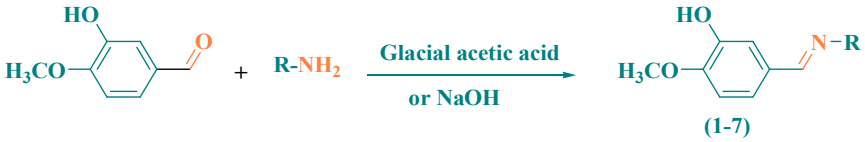
[$A = 0.002x+0.138$; $R^2 = 0.993$]

where A is the absorbance at 695 nm; C is the concentration of ascorbic acid equivalent (µg mg^{-1}).

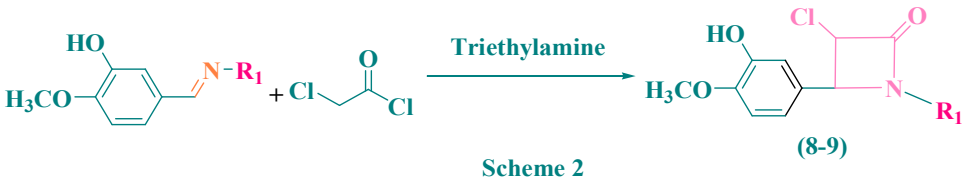
RESULTS AND DISCUSSION

The synthetic route used for the designing of benzylidene derivatives and β-lactams of aza heterocyclic amines is presented in Scheme 1 and 2. Condensation reaction of various aza heterocyclic amines (4-amino,1,2,4-triaozle, 2-aminopyridine, 3-aminopyridine, 4-aminopyridine, 4-aminoantipyrine, 2-amino-4-phenyl thiazole and ampicillin) was carried out with iso vanillin using glacial acetic acid and sodium hydroxide as acidic and basic catalysts respectively. The newly synthesized compounds were fully characterized using various spectroscopic techniques (UV-Visible, IR, ^{1}H NMR and,^{13}C NMR). On analysis of spectral data, benzylidenes synthesis was confirmed with the occurrence of CH=N signals in the range δ8.29-9.05, δ154.66-167.10 and 1614-1693 cm^{-1} for ^{1}H NMR, ^{13}C NMR and IR respectively. Additional support to the result was given with the absence of CHO and NH_2 signals in all the three spectroscopic techniques. Further, formation of β-lactams was confirmed with the absence CH=N signals and occurrence of C=O signals in ^{13}C NMR and IR in the rangeδ 159.95-162.29 and 1741-1757 cm^{-1} respectively. Additionally, peak corresponding to C-Cl in the range 751-755 cm^{-1} in IR and respective signal in ^{13}C NMR in the range 63.50-65.39 confirmed the formation of lactam ring. After this, screening of the compounds at various concentrations (1000, 500, 250, 100 and 50 µg/ml) was done to check their fungi toxicity against *R. solani, F. verticillioides, M. phaseolina* and *D. maydisvia* calculating their percent growth inhibition and ED_{50} values which are presented in Table 1 and Figure 1 respectively. Percent growth inhibition data was statistically analyzed at 5% level of significance to compute significant differences within concentrations, compounds and compounds×concentrations against four maize strains as mentioned in Table 2. It was observed that compound **1** (benzylidene derivative of triazole and iso vanillin) was more effective against *R. solani* and *M. phaseolina* with ED_{50} 15.86 and 19.22 µg/ml respectively as compared to other derivatives (Meena *et al.,* 2021) but showed more ED_{50} as compared to standard carbendazim 50 WP.Compounds **3** and **4** displayed ED_{50} more than 1000 µg/ml which suggested their inefficiency to control of growth of *R. solani* and *M. phaseolina*. Against *F. verticillioides*, compound **6 (**bearing thiazole ring) showed better result (ED_{50}- 20.55 µg/ml)as compared to other derivatives but none of the compounds gave results at par to standard carbendazim 50 WP (ED_{50}- 8.00 µg/ml) (Verma *et al* 2022a). Similar results were shown by Login *et al.,* (2019) which showed better antifungal potential of thiazolyl bearing Schiff bases. All the

synthesized compounds showed moderate to poor results against *D. maydis* except compound **1** (triazole ring),**5** (antipyrine ring) and **7** (ampicillin group) (Verma *et al.,* 2022b).

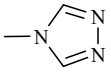
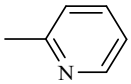
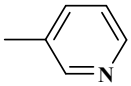
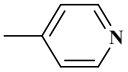
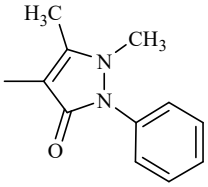
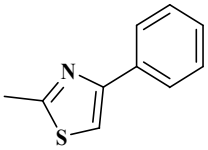
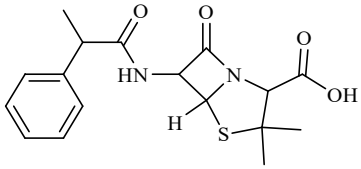


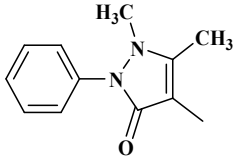
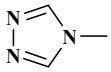
Scheme 1



Scheme 2

Better antioxidant potential with R_1 as electron releasing substituent

COMPOUNDS	R
1	
2	
3	
4	
5	
6	
7	

COMPOUNDS	R_1
8	
9	

Synthesized compounds were also evaluated for their antioxidant potential and the observed data is presented in Table 3. From the analysis of data given in Table 3, β-lactams 8-9 showed better antioxidant potential than benzylidene derivatives 1-7 which marked the effectiveness of chlorine atom present in β-lactam ring in contrast to azomethine moiety (CH=N). Among benzylidene derivatives, compounds 2, 3, and 4 gave better antioxidant results as compared to others (Arora *et al.,* 2018; Sahni *et al.,* 2022).

Table 1: Antifungal activity of benzylidene derivatives (1-7) and β lactams (8-9) on the growth of four different fungicides at different concentrations(µg/ml).

	Percent Inhibition																			
	Rhizoctonia solani					*Fusarium verticillioides*					*Dreschlera maydis*					*Macrophomena phaseolina*				
Conc. (µg/ml) Compd.	1000	500	250	100	50	1000	500	250	100	50	1000	500	250	100	50	1000	500	250	100	50
1	100.0	95.2	90.5	78.8	72.3	48.1	40.9	34.0	22.5	8.7	58.4	50.6	45.0	35.0	26.2	100.0	100.0	100.0	91.1	83.8
2	56.4	47.3	37.3	27.3	10.8	73.4	67.5	59.3	52.1	33.7	39.3	33.1	28.1	20.0	10.3	58.2	48.2	40.5	25.8	14.1
3	45.2	37.0	30.2	25.5	12.3	85.6	73.4	65.6	59.3	54.0	46.2	38.1	32.8	27.1	22.1	49.4	43.5	34.7	25.0	16.4
4	29.1	21.1	13.5	7.0	2.3	55.0	42.1	34.3	21.8	16.5	44.0	42.1	34.3	21.8	16.5	46.4	42.0	26.7	19.1	15.0
5	100.0	100.0	100.0	93.5	85.2	72.8	67.1	57.5	48.7	28.4	56.5	53.1	47.5	40.9	31.8	100.0	92.3	78.8	70.0	59.4
6	100.0	83.2	79.4	71.7	67.3	100.0	90.9	82.5	77.5	68.7	34.0	27.8	23.4	15.9	9.0	92.0	81.7	70.0	55.8	35.8
7	100.0	93.5	88.8	80.8	73.2	65.3	48.7	36.2	18.1	5.6	60.0	52.1	45.6	33.1	24.0	97.6	86.4	73.5	61.7	46.4
8	73.8	68.5	61.1	46.7	31.4	61.2	55.0	48.1	38.1	33.1	42.5	38.1	30.9	22.8	13.1	72.9	68.8	64.1	48.2	41.7
9	71.4	63.5	58.2	53.5	34.7	70.0	60.3	54.3	42.5	28.7	42.8	38.1	31.8	23.1	14.6	61.7	52.0	45.0	37.0	20.5
Carbendazim 50 WP	100.0	100.0	100.0	100.0	100	100.0	100	100.0	100	100	85.9	76.2	68.6	60.0	48.5	100.0	100.0	100.0	100.0	100.0
Mancozeb 75 WP	-	-	-	-	-	-	-	-	-	-	100.0	100.0	75.6	59.4	54.7	-	-	-	-	-

Table 2: Statistical analysis of synthesized benzylidene derivatives (1-7) and β-lactams (8-9) against four maize fungal strains.

Rhizoctonia solani

Source of variation	Degree of freedom	CD (5%)	C.V.
Compounds (A)	9	0.92	
Concentrations (B)	4	0.65	
AB	36	2.06	
Experimental error	50		1.80

Dreschlera maydis

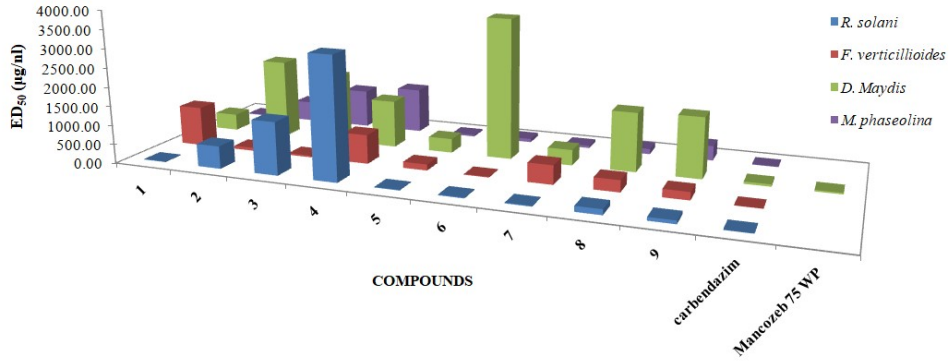
Source of variation	Degree of freedom	CD (5%)	C.V.
Compounds (A)	10	0.82	
Concentrations (B)	4	0.55	
AB	40	1.84	
Experimental error	55		2.30

Fusarium verticillioides

Source of variation	Degree of freedom	CD (5%)	C.V.
Compounds (A)	9	0.55	
Concentrations (B)	4	0.39	
AB	36	1.25	
Experimental error	50		1.24

Macrophomina phaseolina

Source of variation	Degree of freedom	CD (5%)	C.V.
Compounds (A)	9	1.41	
Concentrations (B)	4	1.00	
AB	36	3.16	
Experimental error	50		2.86



	1	2	3	4	5	6	7	8	9	carbendazim	Mancozeb 75 WP
R. solani	15.86	594.37	1392.34	3213.99	18.32	22.01	19.99	144.26	88.34	10.00	
F. verticillioides	1047.60	80.64	43.74	769.75	160.03	20.55	501.18	309.63	209.04	8.00	
D. Maydis	444.25	2091.50	1821.11	1259.43	376.14	3723.54	413.56	1553.00	1595.01	60.00	38.00
M. phaseolina	19.99	542.09	1022.42	1197.4	39.37	89.37	63.46	141.67	390.79	10.00	

Fig. 1. ED_{50} values of synthesized Benzylidene derivatives (1-7) and β-lactams (8-9) against four pathogenic maize fungal strains.

Table 3: Evaluation of antioxidant potential by Phosphomoybdate assay (ascorbic acid equivalent) of Benzylidene derivatives (1-7) and β-lactams (8-9) at different concentrations (µg/ml).

Compounds	1000	500	250	100	50	25
1	45.21±0.32	42.98±0.17	39.38±0.11	35.67±0.19	33.76±0.21	28.00±0.15
2	89.12±0.00	85.00±0.00	79.43±0.26	74.73±0.27	70.22±0.44	67.16±0.22
3	95.22±0.03	92.12±0.00	88.87±0.39	83.66±0.00	79.11±0.44	75.27±0.00
4	85.23±0.00	81.26±0.03	74.16±0.24	71.03±0.07	68.82±0.19	63.37±0.00
5	21.32±0.21	19.74±0.00	15.11±0.05	13.65±0.00	10.54±0.15	8.45±0.00
6	41.21±0.11	38.75±0.09	34.65±0.32	30.57±0.07	26.54±0.01	21.92±0.00
7	19.65±0.22	15.32±0.12	12.43±0.00	10.34±0.12	6.32±0.00	3.28±0.32
8	942.11±0.03	568.35±0.00	299.60±0.31	189.17±0.12	69.14±0.00	76.34±0.00
9	863.20±0.00	534.94±0.26	289.26±0.00	178.99±0.12	60.71±0.01	66.29±0.00
Ascorbic acid equivalent	1000.00±0.03	500.00±0.02	250.00±0.07	100.00±0.09	50.00±0.02	25.00±0.07

CONCLUSION

In conclusion, we designed various benzylidene derivatives of iso vanillin using aza heterocyclic amines followed by their characterization. Antifungal and antioxidant potential of the synthesized derivatives were also done along with their statistical analysis. It was observed that compound 1 exhibited better antifungal potential against three maize fungal strains *viz. R. solani*, *D. maydis* and *M. phaseolina* while compound 6 gave better result against *F. verticillioides*. Compounds 8 (β-lactam ring with triazole moiety) displayed better antioxidant potential at all the concentrations. Thus, it can be concluded that triazole moiety has remarkable potential to be used as antifungal and antioxidant agents.

FUTURE SCOPE

As the present study gave effective results for the control of crops against the four pathogenic fungi of maize, the more advancement or derivatization of the aza heterocyclic compounds or related nitrogen containing compounds will give remarkable potential to improve the crop yield along with protection from pathogen attack.

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Conflict of Interest. None.

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