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Design, synthesis and characterization of novel substituted pyrazol-azetidin-2-one derivatives for their antimicrobial activity

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A novel method is elucidated herein, describing the synthesis of novel 3-chloro-4-(2-substituted phenyl)-1-(4'-((1-(5-(4-substituted phenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl) ethylidene) amino)-[1,1'-biphenyl]-4-yl) azetidin-2-one, consisting of a pyrazol motif (prepared from chalcone) and a lactam ring (synthesized from Schiff base of aromatic aldehyde) as a potential antimicrobial agent. The structural elucidation of the synthesized compounds have been confirmed from the elemental analysis, UV-Vis absorption spectroscopy, IR, ^{1}H NMR and mass spectral studies. The novel compounds have been subjected to *in vitro* antimicrobial screening against certain gram positive (*S. aureus*, *B. subtilis*) and gram negative (*P. aeruginosa*, *E. coli*) bacterial species. Compounds **Vb**, **Vd** and **Ve** are the most potent amongst all the synthesized compounds against the tested microbes.

Keywords: Antimicrobial, Aromatic aldehyde, Antimicrobial activity, Chalcone, *S. aureus*, *B. subtilis*, β-Lactam, Elemental analysis

The propensity of the β-lactam compounds as targeted drug molecule has been extensively explored and is well documented. β-lactams moieties are primarily characterized for their efficient potencies as chemotherapeutic agents. They were among the first possible antibiotic molecules to be accidentally discovered by Alexander Fleming in 1928. Since then the molecules have been used in diseases like malaria[1,2] tuberculosis[3,4] brucella[5] tumor, cancer[6,7] HIV[8], hypersensitivity and parkinsonia[9,10].

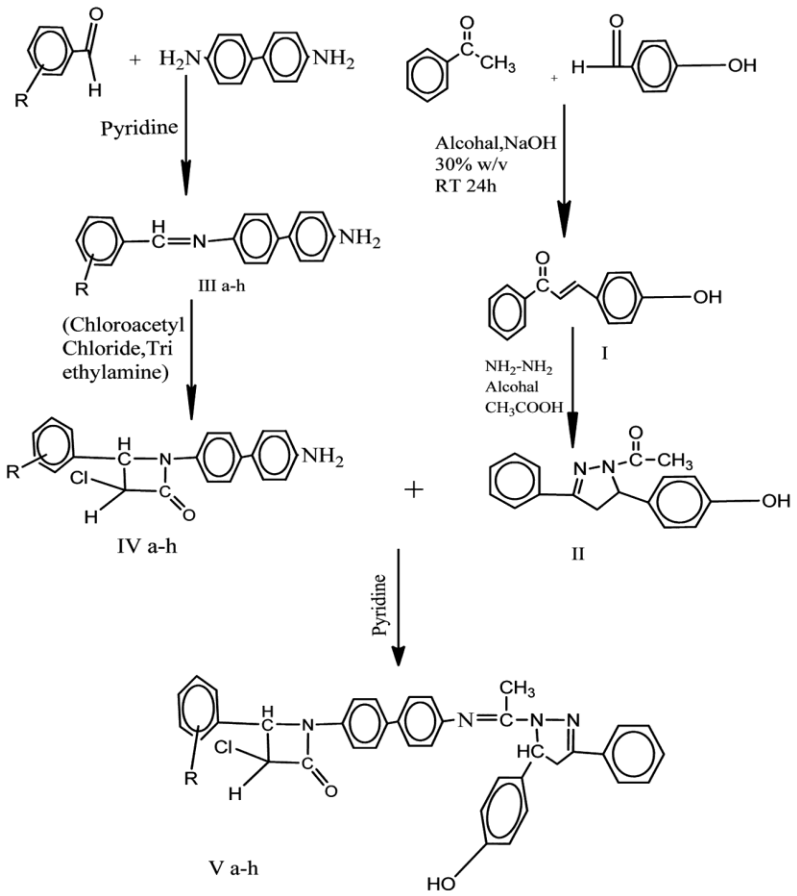
The chalcone bonding with the heterocyclic moieties[11] like pyrazole,1,2,4 triazoles, triazines, and β-lactams have gathered considerable attention and allows a large number of derivatives with promising biological activities to be synthesized in lieu of their targeted effectiveness against various diseases like gout, inflammation, obesity anti-oxidant, hypnotic and anti-spasmodic activities[12,13] and is hugely successful as an antimicrobial[14] and antitubercular[15-18] agent. Chalcones have pitched in as cardinal building blocks in the preparation of many diverse, biologically significant compounds, polymers as well as in potential drug target molecules[19,23]. In view of these observations, it was thought wise to undertake the designing and synthesis of some new chalcone-lactam derivative compounds (Scheme 1).

Experimental Details

The melting points (m.p.) of all the compounds were determined in open capillaries using Jindal melting point apparatus and are uncorrected. Routine analysis of the homogeneity of all the compounds was assessed through Thin layer Chromatography (TLC) using silica gel G (Merck). The spectral data of ^{1}H and ^{13}C NMR were recorded in $CDCl_3$ and DMSO-d_6 on Bruker NMR spectrometer, at 300 MHz. Tetramethyalsilane (TMS) was employed as an internal reference and chemical shift value (δ) were given in part per million (ppm). Jasco FTIR-470 spectrometer (KBr) instrument with diffuse reflectance method was employed along with MS-JEOL SX102 Mass spectrometer, with Argon/Xenon (6 KV, 10 mA) as the FAB gas and *m*-nitro benzyl alcohol (NBA) as the matrix. UV-visible spectra of the samples were recorded on double beam UV-Vis spectrophotometer.

Synthesis of (E)-3-(*p*-hydroxy phenyl)-1-phenylprop-2-en-1-one, I

(0.1 mol) Acetophenone (0.1 mol) and *p*-hydroxy benzaldehyde (0.1 mol) was stirred in ethanol (50 mL). Aqueous potassium hydroxide solution (15 mL, 30%) was then added to the reactants. The above mixture was then refluxed on a water bath for 8 h. Thin layer chromatography (TLC) with



Scheme 1 — Synthesis of chalcone-lactam derivatives

methanol-chloroform, was used to monitor the reaction. After completion, the reaction mixture was subjected to crushed ice followed by acidification with dilute hydrochloric acid. The precipitated solid chalcone was then filtered and crystallized from ethanol. Yield 87%. m.p. 132-133°C. Anal. Calcd for $C_{15}H_{24}O_2$ (Mol. Wt. 236.35): C, 76.23; O, 13.54. Found: C, 76.18; O, 13.47%. IR (KBr): 1752 (C=O), 1626 (CH=CH), 3122 cm^{-1} (OH).

Synthesis of 1-(5-(*p*-hydroxy phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl) ethanone, II

A (0.02 mol) of (E)-3-(*p*-hydroxy phenyl)-1-phenylprop-2-en-1-one in acetic acid (20 mL) and hydrazine hydrate (0.02 mol) in absolute ethyl alcohol (30 mL) was refluxed for 10-12 h on a water bath. After completion of the reaction, the solution was concentrated by distillation under reduced pressure. After cooling, the obtained solid was dried and purified by recrystallization from methanol. Yield 76%. m.p.138-139°C. Anal. Calcd for $C_{17}H_{16}N_2O_2$ (Mol. Wt. 280.32): C, 72.84; O, 11.42; N, 9.99. Found: C, 72.78; O, 11.32; N, 9.91%. IR (KBr): 1610 (C=N), 1623 (C=C-H), 2354 (C-N), 3010 cm^{-1} (OH).

Synthesis of N^4-(substituted benzylidene)-[1,1'-biphenyl]-4,4'-diamine, IIIa-h

Benzidene (0.01 mol) and aryl aldehyde (0.01 mol) in absolute ethyl alcohol (30 mL) were taken in the presence of glacial acetic acid (1 mL). The mixture was then refluxed for 8-10 h on water bath. After removing the excess solvent under reduced pressure, the obtained solid was subjected to washing, with cold water. The washing was repeated several times, the compounds obtained were purified further by recrystallization from methanol. Characterization data of the compounds thus synthesized, are given here.

5-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-2-methoxy-phenol, IIIa: Yield 73%. m.p. 130-132°C. Anal. Calcd for $C_{20}H_{18}N_2O_2$ (Mol. Wt. 318.37): C, 75.45; O, 10.05; N, 8.80. Found: C, 75.41; O, 10.00; N, 8.78%.

N4-(4-Chlorobenzylidene)-[1,1'-biphenyl]-4,4'-diamine, IIIb: Yield 81%. m.p. 184°C. Anal. Calcd for $C_{19}H_{15}N_2Cl$

(Mol. Wt. 306.79): C, 74.38; N, 9.13. Found: C, 74.41; N, 9.09%.

4-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl) phenol, IIIc: Yield 78%. m.p. 95-96°C. Anal. Calcd for $C_{19}H_{16}N_2O$ (Mol. Wt. 288.34): C, 79.14; N, 9.72; O, 5.55. Found: C, 79.11; O, 05.43; N, 9.63%.

N4-(3-Nitrobenzylidene)-[1,1'-biphenyl]-4,4'-diamine, IIId: Yield 72%. m.p. 155°C. Anal. Calcd for $C_{19}H_{15}N_3O_2$ (Mol. Wt. 317.37): C, 71.91; N, 13.24; O, 10.08. Found: C, 71.83; N, 13.19; O, 10.01%.

3-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)phenol, IIIe: Yield 62%. m.p. 131-130°C. Anal. Calcd for $C_{19}H_{16}N_2O$ (Mol. Wt. 288.34): C, 79.14; N, 9.72; O, 5.55. Found: C, 79.09; O, 05.48; N, 9.67%.

4-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-2-methoxyphenol, IIIf: Yield 76%. m.p. 114-115°C. Anal. Calcd for $C_{20}H_{18}N_2O_2$ (Mol. Wt. 318.37): C, 75.45; O, 10.05; N, 8.80. Found: C, 75.39; O, 10.00; N, 8.73%.

N4-(4-Bromobenzylidene)-[1,1'-biphenyl]-4,4'-diamine, IIIg: Yield 72%. m.p. 133-134°C. Anal. Calcd for $C_{19}H_{15}N_2Br$ (Mol. Wt. 351.24): C, 64.97; N, 7.98. Found: C, 64.91; N, 7.90%.

4-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)benzaldehyde, IIIh: Yield 68%. m.p. 142-143°C. Anal. Calcd for $C_{20}H_{16}N_2O$(Mol. Wt. 300.35): C, 79.98; O, 05.22; N, 9.33. Found: C, 79.93; O, 05.29; N, 9.27%.

Synthesis of 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-substituted phenyl)azetidin-2-one, IVa-h

Chloroacetyl chloride (0.01 mol) and triethylamine (0.01 mol) at 0°C were added to (0.01) mol of compound **IIIa-e** and (N^4-(substituted benzylidene)-[1,1'-biphenyl]-4,4'-diamine)in dixon (50 mL), with constant stirring. The reaction mixture was kept at RT for 3 h and was then refluxed for 10 h. The excess solvent was distilled off. The residue was poured into crushed ice and recrystallized from diluted ethyl alcohol. Characterization data of the compounds synthesized are illustrated here.

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one, IVa: Yield 67%. m.p.153°C. Anal. Calcd for $C_{22}H_{19}N_2O_3Cl$ (Mol. Wt. 394.85): C, 66.92; N, 7.09; O, 12.16. Found: C, 66.88; N, 7.03; O, 12.09%. IR (KBr): 3036 (Ar-H), 1756 (C=O,monocyclic β-lactam), 3420 (3-OH, 3-hydroxyphenyl), 1675 (OCH_3, *p*-methoxyphenyl), 780 (C-Cl, β-lactam), 1527 cm^{-1} (C=C,Ar); ^1H NMR ($CDCl_3$): δ 4.86 (s, 1H, Ar-OH), 6.73-7.60 (m, 11H, Ar_H), 5.19 (d, 1H, Cl-CH), 4.91(d,1H,N-CH-R of β-lactam) 3.34 (s,3H,Ar-OCH_3).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(4-chlorophenyl)azetidin-2-one, IVb: Yield 63%. m.p.147°C. Anal. Calcd for $C_{21}H_{16}N_2OCl_2$ (Mol. Wt. 383.27): C, 65.81; N, 7.31; O, 4.17. Found: C, 65.78; N, 7.27; O, 4.1%. IR (KBr): 562 (C-Cl), 3036 (Ar-H), 1745 (C=O, monocyclic β-lactam), 1280 (C-N), 3390 (N-H), 786 (C-Cl, β-lactam), 1520 cm^{-1} (C=C, Ar); ^1H NMR ($CDCl_3$): δ 6.94-7.71 (m, 12H, Ar-H), 4.98 (d, 1H, Cl-CH), 4.91(d,1H,N-CH-R of β-lactam).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one, IVc: Yield 69%. m.p.129-130°C. Anal. Calcd for $C_{21}H_{17}N_2O_2Cl$ (Mol. Wt. 364.82): C, 69.14; N, 7.68; O, 8.77. Found: C, 69.09; N, 7.63; O, 8.71%. IR (KBr): 3040 (Ar-H), 1764 (C=O, monocyclic β-lactam), 3435 (4-OH, 4-hydroxyphenyl), 1180 (C-O str, *p*-hydroxyphenyl), 3350 (Ar-OH), 765 (C-Cl, β-lactam), 1535 cm^{-1} (C=C, Ar); ^1H NMR ($CDCl_3$): δ 4.65 (s, 1H, Ar-OH), 6.95-7.60 (m, 12H, Ar_H), 5.21 (d, 1H, Cl-CH), 4.98 (d,1H,N-CH-R of β-lactam).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-nitrophenyl)azetidin-2-one, IVd: Yield 71%. m.p.152-153°C. Anal. Calcd for $C_{21}H_{16}N_3O_3Cl$ (Mol. Wt. 393.82): C, 64.05; N, 10.67; O, 12.19. Found: C, 64.01; N, 10.58; O, 12.11%. IR (KBr): 1510.36 (N=O str.asym), 1335(N=O str,sym), 3055 (Ar-H), 1744 (C=O, monocyclic β-lactam), 784 (C-Cl, β-lactam), 1529 cm^{-1} (C=C,Ar); ^1H NMR ($CDCl_3$): δ 695-7.63 (m, 12H, Ar_H), 5.19 (d, 1H, Cl-CH), 4.98(d,1H,N-CH-R of β-lactam).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-hydroxyphenyl)azetidin-2-one, IVe: Yield 61%. m.p.122°C. Anal. Calcd for $C_{21}H_{17}N_2O_2Cl$ (Mol. Wt. 364.82): C, 69.14; N, 7.68; O, 8.77. Found: C, 69.11; N, 7.66; O, 8.75. IR (KBr): 1170 (C-O str, *o*-hydroxyphenyl), 3345 (Ar-OH) 3028 3036 (Ar-H), 1756 (C=O, monocyclic β-lactam), 3420, 780 (C-Cl,β-lactam), 1530 cm^{-1} (C=C,Ar); ^1H NMR ($CDCl_3$): δ 6.78 7.65 (m, 12H, Ar_H), 5.19 (d, 1H, Cl-CH), 4.91(d,1H,N-CH-R of β-lactam)4.96 (s, 1H, Ar-OH).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(4-hydroxy-3-methoxyphenyl)azetidin-2-one, IVf: Yield 58%. m.p.137°C. Anal. Calcd for $C_{22}H_{19}N_2O_3Cl$ (Mol. Wt. 394.85): C, 66.92; N, 7.09; O, 12.16. Found: C, 66.86; IR (KBr): 3052 (Ar-H), 1736 (C=O, monocyclic β-lactam), 3440 (4-OH, *p*-hydroxyphenyl), 1665 (OCH_3 *m*-methoxyphenyl), 765 (C-Cl, β-lactam), 1534 cm^{-1}

(C=C,Ar); ^{1}H NMR ($CDCl_3$): δ 4.83 (s, 1H, Ar-OH), 6.85-7.78 (m, 11H, Ar_H), 5.19 (d, 1H, Cl-CH), 4.84 (d, 1H, N-CH-R of β-lactam), 3.37 (s, 3H, Ar-OCH_3).

1-(4'-Amino-[1,1'-biphenyl]-4-yl)-4-(4-bromophenyl)-3-chloroazetidin-2-one, IVg: Yield 70%. m.p. 119-120°C. Anal. Calcd for $C_{21}H_{16}N_2OClBr$ (Mol. Wt. 427.72): C, 58.97; O, 03.74; N, 06.55. Found: C, 58.92; O, 03.69; N, 06.51%. IR (KBr): 3052 (Ar-H), 1736 (C=O, monocyclic β-lactam), 765(C-Cl, β-lactam), 1530 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 6.65-7.76 (m, 12H, Ar_H), 5.15 (d, 1H, Cl-CH), 4.87 (d, 1H, N-CH-R of β-lactam).

4-(1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-oxoazetidin-2-yl)benzaldehyde, IVh: Yield 65%. m.p. 102-103°C. Anal. Calcd for $C_{22}H_{17}N_2O_2Cl$ (Mol. Wt. 376.84): C, 70.12; O, 08.495; N, 07.43. Found: C, 70.08; O, 08.44; N, 07.38. IR (KBr): 3045 (Ar-H), 1740 (C=O, monocyclic β-lactam), 3440, 765 (C-Cl, β-lactam), 1534 cm^{-1} (C=C,Ar); ^{1}H NMR ($CDCl_3$): δ 6.66-7.66 (m, 12H, Ar_H), 5.26 (d, 1H, Cl-CH), 4.80 (d, 1H, N-CH-R of β-lactam).

Synthesis of 3-chloro-4-(2-substitutedphenyl)-1-(4'-((1-(5-(4-substitu tedphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl) ethylidene)amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Va-h

1-(5-(*p*-Hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl) ethanone **II** (0.02 mole) along with

(1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-substitutedphenyl) azetidin-2-one) **IV(a-f)** (0.02 mole) was taken in pyridine (30 mL). The mixture was then subjected to heating under reflux, on a heating mantle for 6 h. Subsequently, the reaction mixture was added to ice cold water (100 mL). A solid mass started to surface out which was then allowed to settle down for 1 h. The solid mass so obtained was then filtered and washed repeatedly with water. The solid mass was then dried in a vacuum desiccator, to obtain the final clean compound. Characterization data of the final compounds thus synthesized, are given here.

3-Chloro-4-(3-hydroxy-4-methoxyphenyl)-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Va: Yield 58%. m.p.129°C. Anal. Calcd for $C_{39}H_{33}N_4O_4Cl$ (Mol. Wt. 657.16): C 71.28; N, 8.53; O, 9.74. Found: C, 71.23; N, 8.49; O, 9.67%. IR (KBr): 1628 (C=N), 1136 (C-N str.) 3036 (Ar-H), 1755 (C=O, monocyclic β-lactam), 3430 (3-OH, 3-hydroxyphenyl), 1677 (OCH_3, *p*-methoxyphenyl), 784 (C-Cl, β-lactam), 1527 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 5.86 (s, 2H, Ar-OH), 6.73-7.70 (m, 20H, Ar_H), 2.86 (s, 3H, N=C-CH_3), 3.25 (dd, 1H, CH_2), 5.10 (d, 1H, Cl-CH), 4.91 (d, 1H, N-CH-R of β-lactam), 2.86 (s, 3H, Ar-OCH_3). MS: M^+ 656, 393, 279, 563, 579, 487, 533, 214, 95.

3-Chloro-4-(4-chlorophenyl)-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)-amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Vb: Yield 67%. m.p.163-164°C. Anal. Calcd for $C_{38}H_{30}N_4O_2Cl_2$ (Mol. Wt. 645.58): C, 70.70; N, 8.68; O, 4.96. Found: C, 70.66; N, 8.61; O, 04.89%. IR (KBr): 1140 (C-N str.) 3036 (Ar-H), 565 (C-Cl) 1755 (C=O, monocyclic β-lactam), 1633 (C=N), 780 (C-Cl, β-lactam), 1523 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 3.24 (dd, 1H, CH_2), 6.63-7.79 (m, 21H, Ar-H), 2.80 (s, 3H, C=NCH_3), 5.08 (d, 1H, Cl-CH), 4.91 (d, 1H, N-CH-R of β-lactam). MS: M^+ 644, 552, 558, 534, 214, 111, 91, 77, 27.

3-Chloro-4-(4-hydroxyphenyl)-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-ethylidene)-amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Vc: Yield 71%. m.p.147°C. Anal. Calcd for $C_{38}H_{31}N_4O_3$ Cl (Mol. Wt. 627.13): C, 72.78; N, 8.93; O, 07.65. Found: C, 72.75; N, 08.88; O, 7.61%. IR (KBr): 3034 (Ar-H), 1764 (C=O, monocyclic β-lactam), 3432 (4-OH, 4-hydroxyphenyl), 1186 (C-O str, *p*-hydroxyphenyl), 3360 (Ar-OH), 755 (C-Cl,β-lactam), 1545 (C=C,Ar), 1150 (C-N str.), 1644 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 4.69 (s, 1H, Ar-OH), 6.75-7.70 (m, 21H, Ar-H), 5.27 (d, 1H, Cl-CH), 4.88 (d, 1H, N-CH-R of β-lactam), 2.90 (s, 3H, C=NCH_3). 2.78 (s, 3H, N=C-CH_3), 3.30 (dd, 1H, CH_2); MS: M^+ 627,534,551,535, 108,93,77,14.

3-Chloro-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)amino)-[1,1'-biphenyl]-4-yl)-4-(2-nitrophenyl)azetidin-2-one, Vd: Yield 73%. m.p.181°C. Anal. Calcd for $C_{38}H_{30}N_5O_4Cl$ (Mol. Wt. 656.13): C, 69.56; N, 10.67; O, 09.75. Found: C, 69.47; N, 10.55; O, 09.67. IR (KBr): 1155 (C-N str.), 1648 (C=N), 1510 (N=O str. asym), 1325 (N=O str, sym), 3065 (Ar-H), 1721 (C=O, monocyclic β-lactam), 774 (C-Cl, β-lactam), 1534 cm^{-1} (C=C,Ar); ^{1}H NMR ($CDCl_3$): δ 2.88 (s, 3H, C=N-CH_3), 2.69 (s, 3H, N=C-CH_3), 3.31 (dd, 1H, CH_2), 6.70-7.60 (m, 21H, Ar_H), 5.17 (d, 1H, Cl-CH), 4.90 (d, 1H, N-CH-R of β-lactam); MS: M^+ 655, 564, 580, 535, 122, 93, 17.

3-Chloro-4-(2-hydroxyphenyl)-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)-amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Ve: Yield 67%. m.p.137-138°C. Anal. Calcd for $C_{38}H_{31}N_4O_3$ Cl (Mol. Wt. 627.13): C, 72.78; N, 8.93; O, 07.65. Found: C, 72.69; N, 08.91; O, 7.59. IR (KBr): 1180

(C-O str, *o*-hydroxyphenyl), 3352 (Ar-OH), 3028, 3040 (Ar-H), 1766 (C=O, monocyclic β-lactam), 3435, 765 (C-Cl, β-lactam), 1530 (C=C, Ar), 1160 (C-N str.), 1650 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.33 (dd,1H, CH_2), 6.66-7.60 (m, 21H, Ar_H), 5.24 (d, 1H, Cl-CH), 4.96(d,1H,N-CH-R of β-lactam), 4.91 (s, 1H, Ar-OH), 2.80 (s, 3H, C=NCH_3); MS: M^+ 627, 534, 551, 534, 93, 77, 14.

3-Chloro-4-(4-hydroxy-3-methoxyphenyl)-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Vf: Yield 61%. m.p.147-148°C. Anal. Calcd for $C_{39}H_{33}N_4O_4Cl$ (Mol. Wt. 657.16): C, 71.28; N, 8.53; O, 9.74. Found: C, 71.21; N, 8.47; O, 9.69. IR (KBr): 1150 (C-N str.), 1660 (C=N), 3037 (Ar-H), 1730 (C=O, monocyclic β-lactam), 3430 (4-OH, *p*-hydroxyphenyl), 1675 (OCH_3 *m*-methoxyphenyl), 775 (C-Cl, β-lactam), 1540 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 4.95 (s, 1H, Ar-OH), 2.83 (s, 3H, N=C-CH_3), 3.29 (dd, 1H, CH_2), 6.66-7.68 (m, 20H, Ar-H), 5.19 (d, 1H, Cl-CH), 4.84 (d, 1H, N-CH-R of β-lactam), 3.25 (s, 3H, Ar-OCH_3); MS: M^+ 256, 564, 580, 534, 123, 108, 93.

4-(4-Bromophenyl)-3-chloro-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)amino)-[1,1'-biphenyl]-4-yl)azetidin-2-one, Vg: Yield 58%. m.p. 160-161°C. Anal. Calcd for $C_{38}H_{30}N_4O_2Cl$,Br (Mol. Wt. 690.03): C, 66.14; O, 04.64; N, 08.12. Found: C, 66.10; O, 04.58; N, 08.08. IR (KBr): 1160 (C-N str.), 1658 (C=N), 3037 (Ar-H), 1740 (C=O, monocyclic β-lactam), 778C-Cl, β-lactam), 1548 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 2.81 (s, 3H, N=C-CH_3), 3.21 (dd, 1H, CH_2), 6.66-7.68 (m, 21H, Ar_H), 5.19 (d, 1H, Cl-CH), 4.81 (d, 1H, N-CH-R of β-lactam); MS: M^+ 689, 613, 597, 522, 155, 105.

4-(3-Chloro-1-(4'-((1-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethylidene)amino)-[1,1'-biphenyl]-4-yl)-4-oxoazetidin-2-yl)benzaldehyde, Vh: Yield 70%. m.p. 168-169°C. Anal. Calcd for $C_{39}H_{31}N_4O_3Cl$ (Mol. Wt. 638.14): C, 73.29; O, 07.51; N, 08.77. Found: C, 73.21; O, 07.44; N, 08.68. IR (KBr): 1168 (C-N str.), 1666 (C=N), 3041 (Ar-H), 1733 (C=O, monocyclic β-lactam), 775C-Cl, β-lactam), 1553 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 2.79 (s, 3H, N=C-CH_3), 3.30 (dd, 1H, CH_2), 6.60-7.69 (m, 21H, Ar_H), 5.26 (d, 1H, Cl-CH), 4.88 (d, 1H, N-CH-R of β-lactam); MS: M^+ 637, 545, 469, 562, 106, 105, 70.

***In vitro* anti-microbial activity**

The Gram +ve *S. aureus*, *B. subtilis*, Gram –ve *E. coli*, *Pseudomonas aeruginosa* are customary to be grown in separately arranged identical sets of LB (Lysogeny broth is most common media used to grow microbes) media culture having variable concentration of compounds. The conical flask filled with 10 mL of LB media Gram +ve S. *aureus*, *B.subtills* Gram –ve *E. coli*, *Pseudomonas aeruginosa* were incubated for 10 h at 37°C and the growth of mico-organisms were evaluated using optical density (OD) of the solution at 600 nm. Then 100 µL suspension were transferred into each conical containing the test compounds of final concentrations with 10 mL of LB media.

Antimicrobial activity

The compounds **Vb** and **Vd** showed profound activity (12.5-50 µg/mL) against the tested Gram positive bacterial strains (*S. aureus* and *B. subtilis*), whereas compound **Vd** and **Ve** were most potent against *E. coli* (12.5 µg/mL) as compared with Ampicillin. All the other compounds showed moderate to good activity against the test bacterial strains (Table 1).

Table 1 — Antimicrobial activity of synthesized compounds against Gram positive and Gram negative bacteria

S. No.	R	Gram positive		Gram negative	
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E.coli*
1	3-OH,4-OCH_3-benzaldehyde	50	50	>100	>100
2	*p*-Chloro benzaldehyde	25	12.5	50	50
3	*p*-Hydroxy benzaldehyde	>100	>100	>100	25
4	*o*-nitro benzaldehyde	12.5	50	50	12.5
5	*o*-Hydroxy benzaldehyde	>100	50	>100	12.5
6	4-OH,3-OCH_3-Benzaldehyde	25	25	50	>100
7	*p*-Bromo benzaldehyde	100	25	12.5	100
8	Benzaldehyde	100	100	50	50

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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