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Synthesis, characterization and antimicrobial studies of imine derivatives of amoxicillin

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ABSTRACT

Some novel imine derivatives (1-14) of a broad spectrum antibiotic amoxicillin were prepared by condensation with different carbonyl compounds. The amoxicillin imine derivatives were characterized using elemental analysis and spectroscopic techniques such as FT-IR and ^{1}H NMR. The prepared imine derivatives were evaluated for antimicrobial activities against some pathogens using disc diffusion method. The results of present studies demonstrate enhanced antimicrobial activity of the novel imine derivatives of amoxicillin as compared to the parent drug.

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1. Introduction

Antibiotics of natural or synthetic origin have an enormous role in the treatment of infectious disease of clinical importance caused by microorganisms [1,2]. Amoxicillin, which belongs to the class of penicillin is well-known *β*-lactam antibiotic and offer some benefits over the other due to superior absorptive nature (Figure 1) [3]. However the drug efficacy get diminish due to the development of bacterial resistance. To overcome such resistance minor chemical modifications were performed with the drug to convert them into pro-drugs. Major chemical modifications can possibly alter or weaken the entire drug activity. *β*-lactam drugs are usually obtained from fungi or molds [4]. Though most of the β-lactam antibiotics are active against gram positive bacteria, yet there are some recently developed drug like cephalosporin [5] *etc.* which serve as broad spectrum antibiotics both against gram positive and gram negative bacteria [6,7]. Prominence of amoxicillin in research studies associated to pharmaceutical and biologically active zones has been increased in current years [8,9].

Literature showed that imine derivatives of drugs exhibit the same or more potency, but have advantages to overcome the issues of drug resistance in medicinal chemistry. The pro-drugs encompassing imine linkage in their structural framework have been described as antibacterial, antifungal and antiviral *etc*. [10,11].

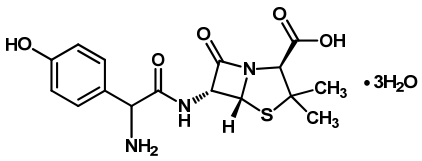

Figure 1. Amoxicillin trihydrate, I.

As the extensive use of antibiotics lead to severe drug resistant complications, so to minimize such problems drug modification has gain much attention from chemists and pharmacologists. Tomi *et al.* synthesized some Schiff base type compounds derived from amoxicillin and reported their boosted biological activity in the fresh studies [12]. Joshi, S. *et al.* synthesized certain new Schiff bases derived from amoxicillin trihydrate with cinnamaldehyde and *p*-chloro benzaldehyde and described their antibacterial activity [13].

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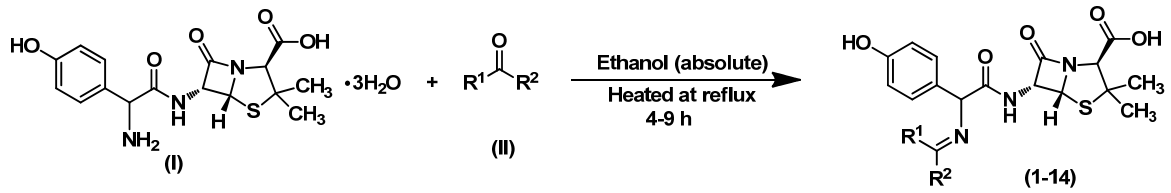


Scheme 1

Metal complexes with the amoxicillin derivatives were also synthesized [14,15]. Copper, Cobalt and Zinc chloride salts were used for metal complexation. It was found that novel Schiff bases were found to be having better antibacterial activity as compared to the parent amoxicillin. Antibacterial potential has also been investigated using chalcone based heterocycles [16] while some xantnone derivatives have been reported as antitumor, antimicrobial, anti-oxidant, anti-allergic agents *etc*. [17]. Very recently, we have reported the synthesis of novel xanthene based imine derivatives showing excellent antibacterial activity [18]. Inspired with biological activities possessed by imines [19-23] and amoxicillin, an endeavor was made to manufacture novel imine derivatives (**1**-**14**) of amoxicillin by condensing it with different aldehydes and ketones. The novel amoxicillin imine derivatives were characterized well by different spectroscopic techniques and screened for antimicrobial activities.

2. Experimental

2.1. Materials and methods

Amoxicillin trihydrate was obtained from a company based in china. Aldehydes and ketones were purchased from Sigma Aldrich. Absolute ethanol was purchased from Merck. Thin Layer Chromatography (TLC) to monitor reaction progress was performed with silica gel 60, 0.063-0.200 mm as the stationary phase with analytical grad solvents. Melting points of compounds were obtained from Electro thermal apparatus. For elemental analysis, Euro-Vector model EA 3000 instrument was used. Shimadzu model FT-IR-8400S was used for FT-IR spectra. BRUKER model Ultra shield 300 MHz spectrophotometer was used for ^{1}H NMR spectra in DMSO-d_6 solution with TMS taken as internal standard.

2.2. General synthetic layout for amoxicillin imine derivatives (1-14)

The novel imine derivatives were synthesized by condensing the amoxicillin trihydrate and different carbonyl compounds. In typical reaction, the corresponding aldehydes of ketones (1.0 mmol) were mixed with amoxicillin trihydrate (0.419 g, 1.0 mmol) in absolute ethanol. The resulting mixture was heated at reflux for 6 to 14 h. The solution obtained after reaction completion was transferred into cold water (60 mL) which resulted in immediate precipitation of the corresponding imine derivative. The precipitates were collected, washed several times with cold water, dried under vacuum and kept in a desiccators [12,13] (Scheme 1, Table 1).

(2S,5R,6R)-6-(2-((Z)-(4-Acetamidobenzylidene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicycle[3.2.0]heptane-2-carboxylic acid (**1**): Color: Yellow powder. Yield: 78%. M.p.: 260-262 °C. R_f = 0.26 (Petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1662. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.01 (brs, 1H, COOH), 10.09 (s, 1H, CH=N), 9.09 (s, 1H, OH), 8.1 (s, 1H, CO-NH-Ar), 8.61 (d, 1H, NH), 7.71 (m, 2H, Ar-H), 7.34 (d, *J* = 7.2 Hz, 2H, Ar-Hb), 6.9 (m, 2H, Ar-H), 6.72 (d, *J* = 7.2 Hz, 2H, Ar-Ha), 5.55 (brs, 1H, CH-S), 5.28 (d, 1H, N-CH-CO), 4.89 (s, 1H, CH-N=C), 3.99 (s, 1H, CH-COOH), 2.01 (s, 3H, CH_3CON), 1.48 (s, 3H, B-CH_3), 1.34 (s, 3H, A-CH_3). Anal. calcd. for $C_{25}H_{26}N_4O_6S$: C, 58.81; H, 5.13; N, 10.97. Found: C, 58.52; H, 5.06; N, 10.75%.

(2S,5R,6R)-6-(2-(((E)-2-Bromo-1-(4-nitrophenyl)ethylidene) amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**2**): Color: Dark brown powder. Yield: 86%. M.p.: 248-250 °C. R_f = 0.31 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1664. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.08 (brs, 1H, COOH), 9.01 (d, s, 1H, OH), 8.74 (d, 1H, NH), 8.32 (m, 2H, Ar-H), 7.81 (m, 2H, Ar-H), 7.33 (d, *J*= 7.2 Hz, 2H, Ar-Hb), 6.73 (d, *J*= 7.2 Hz, 2H, Ar-Ha), 5.7 (s, 2H, CH_2Br), 5.52 (brs, 1H, CH-S), 5.29 (d, 1H, N-CH-CO), 4.8 (s, 1H, CH-N=C), 3.95 (s, 1H, CH-COOH), 1.47 (s, 3H, B-CH_3), 1.32 (s, 3H, A-CH_3). Anal. calcd. for $C_{24}H_{23}N_4O_7BrS$: C, 48.74; H, 3.92; N, 9.47. Found: C, 48.65; H, 4.02; N, 9.39%.

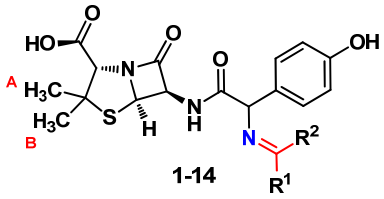
(2S, 5R, 6R)-6-(2-(((E)-1-(2-Hydroxyphenyl)ethylidene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**3**): Color: Yellow powder. Yield: 74%. M.p.: 258-260 °C. R_f = 0.30 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1663. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.06 (brs, 1H, COOH), 9.08 (s, 1H, OH), 9.04 (s, 1H, OH), 8.63 (d, 1H, NH), 7.32-7.21 (m, 4H, Ar-H, Ar-Hb), 6.91 (dd, *J* = 7.8 Hz, 1.89 Hz, 1H, Ar-H), 6.77-6.73 (m, 3H Ar-H, Ar-Ha), 5.57 (brs, 1H, CH-S), 5.31 (d, 1H, N-CH-CO), 4.86 (s, 1H, CH-N=C), 3.97 (s, 1H, CH-COOH), 2.3 (s, 3H, CH_3-C=N), 1.53 (s, 3H, B-CH_3), 1.39 (s, 3H, A-CH_3). Anal. calcd. for $C_{24}H_{25}N_3O_6S$: C, 59.61; H, 5.21; N, 8.69. Found: C, 59.73; H, 5.33; N, 8.81%.

(2S,5R,6R)-6-(2-(((E)-5, 6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)amino)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**4**): Color: Yellow powder. Yield: 69%. M.p.: 255-257 °C. R_f = 0.34 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1662. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.01 (brs, 1H, COOH), 9.10 (s, 1H, OH), 8.69 (d, 1H, NH), 7.33 (d, *J* = 7.2 Hz, 2H, Ar-Hb), 6.73 (d, *J* = 7.2 Hz, 2H, Ar-Ha), 6.63 (s, 1H, Ar-H), 6.52 (s, 1H, Ar-H), 5.48 (brs, 1H, CH-S), 5.31 (d, 1H, N-CH-CO), 4.82 (s, 1H, CH-N=C), 3.93 (s, 1H, CH-COOH), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 2.78 (t, 2H, *J* = 4.5 Hz, CH_2-C=N), 1.75 (t, 2H, *J* = 4.5 Hz, CH_2-cyclopentyl), 1.46 (s, 3H, B-CH_3), 1.37 (s, 3H, A-CH_3). Anal. calcd. for $C_{27}H_{29}N_3O_7S$: C, 60.10; H, 5.42; N, 7.79. Found: C, 60.22; H, 5.65; N, 7.93%.

(2S, 5R, 6R)-6-(2-(((E)-(1H-Indol-3-yl)methylene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**5**): Color: Red powder. Yield: 88%. M.p.: 281-283 °C. R_f = 0.29 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1662. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.86 (brs, 1H, indole-NH), 11.08 (brs, 1H, COOH), 9.35 (s, 1H, CH=N), 9.05 (s, 1H, OH), 8.67 (d, 1H, NH), 8.44 (dd, *J* = 7.5 Hz, 1.8 Hz, 1H, indole-H), 8.15 (d, *J* = 7.9 Hz, 1H, indole-H), 7.36 (d, *J* = 7.2 Hz, 2H, Ar-Hb), 7.29-7.10 (m, 3H, indole-H), 6.77 (d, *J* = 7.2 Hz, 2H, Ar-Ha), 5.56 (brs, 1H, CH-S), 5.30 (d, 1H, N-CH-CO), 4.87 (s, 1H, CH-N=C), 3.94 (s, 1H, CH-COOH), 1.48 (s, 3H, B-CH_3), 1.33 (s, 3H, A-CH_3). Anal. calcd. for $C_{25}H_{24}N_4O_5S$: C, 60.96; H, 4.91; N, 11.37. Found: C, 61.09; H, 5.15; N, 11.59%.

(2S,5R,6R)-6-(2-(((E)-1-(Benzofuran-2-yl)ethylidene)amino) -2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (**6**): Color: Yellow

Table 1. Amoxicillin imine derivatives **1-14**.



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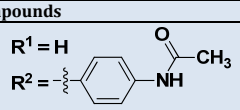
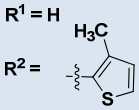
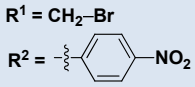
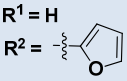
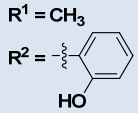
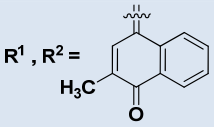
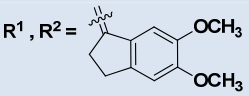
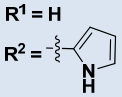
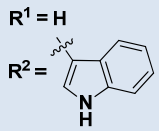
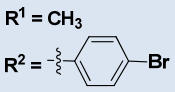
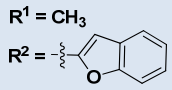
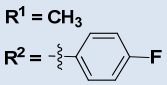
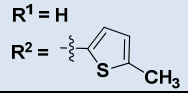
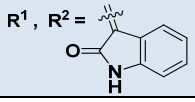
1		78	8		68
2		86	9		62
3		74	10		87
4		69	11		90
5		88	12		75
6		71	13		83
7		82	14		76

powder. Yield: 71%. M.p.: 271-273 °C. R_f = 0.31 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1665. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.09 (brs, 1H, COOH), 9.09 (s, 1H, OH), 8.72 (d, 1H, NH), 8.41 (dd, *J* = 7.3 Hz, 1.9 Hz, 1H, benzofuran-H), 8.16 (d, *J* = 7.8 Hz, 1H, benzofuran -H), 7.36 (d, *J* = 7.2 Hz, 2H, Ar-Hb), 7.29-7.1 (m, 3H, benzofuran -H), 6.75 (d, *J* = 7.2 Hz, 2H, Ar-Ha), 5.46 (brs, 1H, CH-S), 5.27 (d, 1H, N-CH-CO), 4.85 (s, 1H, CH-N=C), 3.97 (s, 1H, CH-COOH), 2.7 (s, 3H, CH_3-C=N), 1.50 (s, 3H, B-CH_3), 1.38 (s, 3H, A-CH_3). Anal. calcd. for $C_{26}H_{25}N_3O_6S$: C, 61.53; H, 4.96; N, 8.28. Found: C, 61.37; H, 5.12; N, 8.50%.

(2S, 5R, 6R)-6-(2-(4-Hydroxyphenyl)-2-(((E)-(5-methylthiophen-2-yl)methylene)amino)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**7**): Color: Brown powder. Yield: 82%. M.p.: 272-274 °C. R_f = 0.30 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1663. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.05 (brs, 1H, COOH), 10.03 (s, 1H, CH=N), 9.08 (s, 1H, OH), 8.74 (d, 1H, NH), 6.91 (d, *J* = 8.2 Hz, 1H, thiophene-H), 6.72 (d, *J* = 8.2 Hz, 1H, thiophene-H), 7.32 (d, *J* = 7.4 Hz, 2H, Ar-Hb), 6.70 (d, *J* = 7.4 Hz, 2H, Ar-Ha), 5.50 (brs, 1H, CH-S), 5.36 (d, 1H, N-CH-CO), 4.80 (s, 1H, CH-N=C), 3.96 (s, 1H, CH-COOH), 2.55 (s, 3H, CH_3-thiophene), 1.47 (s, 3H, B-CH_3), 1.36 (s, 3H, A-CH_3). Anal. calcd. for $C_{22}H_{23}N_3O_5S_2$: C, 55.80; H, 4.90; N, 8.87. Found: C, 55.98; H, 5.09; N, 9.07%.

(2S, 5R, 6R)-6-(2-(4-Hydroxyphenyl)-2-(((E)-(3-methylthiophen-2-yl)methylene)amino)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**8**): Color: Green powder. Yield: 68%. M.p.: 257-259 °C. R_f = 0.29 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1664. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.08 (brs, 1H, COOH), 10.11 (s, 1H, CH=N), 9.06 (s, 1H, OH), 8.65 (d, 1H, NH), 7.23 (d, *J* = 7.5 Hz, 1H, thiophene-H), 6.72 (d, *J* = 7.5 Hz, 1H, thiophene-H), 7.31 (d, *J* = 7.3 Hz, 2H, Ar-Hb), 6.71 (d, *J* = 7.3 Hz, 2H, Ar-Ha), 5.52 (brs, 1H, CH-S), 5.35 (d, 1H, N-CH-CO), 4.83 (s, 1H, CH-N=C), 3.90 (s, 1H, CH-COOH), 2.54 (s, 3H, CH_3-thiophene), 1.51 (s, 3H, B-CH_3), 1.33 (s, 3H, A-CH_3). Anal. calcd. for $C_{22}H_{23}N_3O_5S_2$: C, 55.80; H, 4.90; N, 8.87. Found: C, 55.96; H, 5.11; N, 9.05%.

(2S, 5R, 6R)-6-(2-(((E)-Furan-2-ylmethylene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**9**): Color: Dark grey powder. Yield: 62%. M.p.: 262-264 °C. R_f = 0.37 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1661. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.09 (brs, 1H, COOH), 10.10 (s, 1H, CH=N), 9.04 (s, 1H, OH), 8.66 (d, 1H, NH), 7.31 (d, *J* = 7.31 Hz, 2H, Ar-Hb), 7.21 (dd, *J* = 8.5 Hz, 2.1 Hz, 1H, furan-H), 6.76 (d, *J* = 7.3 Hz, 2H, Ar-Ha), 6.2-6.01 (m, 2H, furan-H), 5.59 (brs, 1H, CH-S), 5.32 (d, 1H, N-CH-CO), 4.85 (s, 1H, CH-N=C), 3.90 (s, 1H, CH-COOH), 1.49 (s, 3H, B-CH_3), 1.35 (s, 3H, A-CH_3). Anal. calcd. for $C_{21}H_{21}N_3O_6S$: C, 56.87; H, 4.77; N, 9.48. Found: C, 56.79; H, 4.62; N, 9.64%.

(2S, 5R, 6R)-6-(2-(4-Hydroxyphenyl)-2-(((E)-3-methyl-4-oxo naphthalen-1(4H)-ylidene)amino)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**10**): Color: Brown powder. Yield: 87%. M.p: 290-292 °C. R_f = 0.33

(petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1666. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.07 (brs, 1H, COOH), 9.05 (s, 1H, OH), 8.69 (d, 1H, NH), 8.05-7.77 (m, 4H, Ar-H), 7.33 (d, *J* = 7.51 Hz, 2H, Ar-Hb), 6.8 (s, 1H, CH-C=N), 6.72 (d, *J* = 7.5 Hz, 2H, Ar-Ha), 5.51 (brs, 1H, CH-S), 5.29 (d, 1H, N-CH-CO), 4.88 (s, 1H, CH-N=C), 4.01 (s, 1H, CH-COOH), 2.20 (s, 3H, CH_3-C=C), 1.43 (s, 3H, B-CH_3), 1.40 (s, 3H, A-CH_3). Anal. calcd. for $C_{27}H_{25}N_3O_6S$: C, 62.41; H, 4.85; N, 8.09. Found: C, 62.59; H, 5.01; N, 8.32%.

(2S,5R, 6R)-6-(2-(((E)-(1H-Pyrrol-2-yl)methylene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-aza bicyclo[3.2.0] heptane-2-carboxylic acid (**11**): Color: Silvery powder. Yield: 90%. M.p.: 243-245 °C. R_f = 0.38 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1662. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.09 (brs, 1H, COOH), 10.04 (s, 1H, CH=N), 9.08 (s, 1H, OH), 8.70 (d, 1H, NH), 7.81 (s, 1H, NH-pyrrole), 7.30 (d, *J* = 7.51 Hz, 2H, Ar-Hb), 7.25 (dd, *J* = 8.1 Hz, 2.0 Hz, 1H, pyrrole-H), 6.71 (d, *J* = 7.5 Hz, 2H, Ar-Ha), 6.5-6.21 (m, 2H, pyrrole-H), 5.55 (brs, 1H, CH-S), 5.36 (d, 1H, N-CH-CO), 4.89 (s, 1H, CH-N=C), 3.98 (s, 1H, CH-COOH), 1.45 (s, 3H, B-CH_3), 1.36 (s, 3H, A-CH_3). Anal. calcd. for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.66. Found: C, 57.28; H, 5.40; N, 12.77%.

(2S,5R,6R)-6-(2-(((E)-1-(4-Bromophenyl)ethylidene) amino) -2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**12**): Color: Bright Yellow powder. Yield: 75%. M.p.: 270-272 °C. R_f = 0.32 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1665. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.06 (brs, 1H, COOH), 9.12 (s, 1H, OH), 8.72 (d, 1H, NH), 8.02 (m, 2H, Ar-H), 7.71 (m, 2H, Ar-H), 7.28 (d, *J* = 7.9 Hz, 2H, Ar-Hb), 6.71 (d, *J* = 7.9 Hz, 2H, Ar-Ha), 5.52 (brs, 1H, CH-S), 5.31 (d, 1H, N-CH-CO), 4.81 (s, 1H, CH-N=C), 3.96 (s, 1H, CH-COOH), 2.5 (s, 3H, CH_3-C=N), 1.46 (s, 3H, B-CH_3), 1.37 (s, 3H, A-CH_3). Anal. calcd. for $C_{24}H_{24}BrN_3O_5S$: C, 52.75; H, 4.43; N, 7.69. Found: C, 52.96; H, 4.45; N, 7.89%.

(2S,5R,6R)-6-(2-(((E)-1-(4-Fluorophenyl)ethylidene)amino)-2-(4-hydroxyphenyl)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**13**): Color: Cream yellow powder. Yield: 83%. M.p.: 258-260 °C. R_f = 0.29 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1665. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.05 (brs, 1H, COOH), 9.04 (s, 1H, OH), 8.69 (d, 1H, NH), 8.12 (m, 2H, Ar-H), 7.91 (m, 2H, Ar-H), 7.29 (d, *J* = 7.5 Hz, 2H, Ar-Hb), 6.66 (d, *J* = 7.5 Hz, 2H, Ar-Ha), 5.48 (brs, 1H, CH-S), 5.35 (d, 1H, N-CH-CO), 4.90 (s, 1H, CH-N=C), 3.99 (s, 1H, CH-COOH), 2.47 (s, 3H, CH_3-C=N), 1.54 (s, 3H, B-CH_3), 1.34 (s, 3H, A-CH_3). Anal. calcd. for $C_{24}H_{24}FN_3O_5S$: C, 59.37; H, 4.98; N, 8.65. Found: C, 59.53; H, 5.09; N, 8.42%.

(2S,5R,6R)-6-(2-(4-Hydroxyphenyl)-2-(((Z)-2-oxoindolin-3-ylidene)amino)acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-aza bicyclo[3.2.0]heptane-2-carboxylic acid (**14**): Color: Dark grey powder. Yield: 76%. M.p.: 279-281 °C. R_f = 0.30 (petroleum ether: CH_2Cl_2, 2:1). FT-IR (KBr, ν, cm^{-1}): 1666. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.07 (brs, 1H, COOH), 11.0 (s, 1H, NH-isatin), 9.08 (s, 1H, OH), 8.71 (d, 1H, NH), 8.44 (dd, *J* = 7.9 Hz, 1.8 Hz, 1H, isatin-H), 8.15 (d, *J* = 7.89 Hz, 1H, isatin-H), 7.39 (d, *J* = 7.8 Hz, 2H, Ar-Hb), 7.28 (m, 2H, isatin-H), 6.68 (d, *J* = 7.8 Hz, 2H, Ar-Ha), 5.49 (brs, 1H, CH-S), 5.33 (d, 1H, N-CH-CO), 4.87 (s, 1H, CH-N=C), 3.92 (s, 1H, CH-COOH), 1.52 (s, 3H, B-CH_3), 1.41 (s, 3H, A-CH_3). Anal. calcd. for $C_{24}H_{22}N_4O_6S$: C, 58.29; H, 4.48; N, 11.33. Found: C, 58.52; H, 4.60; N, 11.53%.

2.3. In vitro antimicrobial studies

Amoxicillin was used in different concentrations (1×10^{-1}, 1×10^{-3}, 1×10^{-5} and 1×10^{-7} M) as a standard antimicrobial. All the synthetic derivatives (**1**-**14**) were screened for antimicrobial activities using disc diffusion method by measuring the inhibition zone in comparison with the standard drug, amoxicillin. Antimicrobial activity of the compounds was determined against bacterial strains including *Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli* which were isolated from urinary tract infections, surgical theaters, nose swab and infected wounds respectively in Muller Hinton agar. These sterilized agar media were transferred to petri-dishes and allowed to solidify. Microbial suspensions were spread on the surface of the media with sterilized triangular loop. A pre-sterilized stainless cylinder having 12 mm diameter was used to bore the cavities. The amoxicillin imine derivatives of various concentration (1×10^{-1}, 1×10^{-3}, 1×10^{-5} and 1×10^{-7} M) were placed in the cavities using a micropipette and allowed to de diffused for 1 h. DMSO was used as a solvent for all the synthetic imine derivatives while sterile distilled water was used for pure amoxicillin. These plates were incubated at 370 °C for 48 h. The inhibition zone was measured in mm around the cups after respective incubation.

3. Results and discussion

3.1. Chemistry

The synthetic layout of the amoxicillin imine derivatives has been presented in the Scheme 1. Commercially available amoxicillin trihydrate was coupled with different carbonyl compounds in absolute ethanol as solvent. The reaction mixtures were poured in cold water to afford precipitates of the imine derivative (**1**-**14**). The progress of the reaction was monitored by thin layer chromatography until the complete consumption of starting material. All the novel compounds were characterized by different spectroscopic techniques. Physical parameters including color, solubility, melting point, elemental analysis were also determined. Elemental analysis data confirms the expected molar mass and formation of new compounds. FT-IR spectroscopic data in which the bands (1661-1666 cm^{-1}) indicate clearly the presence of CH=N function. Conversion of NH_2 group of amoxicillin to imine linkage CH=N is confirmed by the disappearance of two bands at (3332 and 3163 cm^{-1}) which could be attributed to symmetric and asymmetric stretching of NH_2 group of amoxicillin. ^1H NMR data of amoxicillin derivatives in DMSO-d_6 solvent has been provided in the experimental section.

3.2. Bioactivity

Novel amoxicillin imine compounds (**1**-**14**) have been screened for *in vitro* antimicrobial or antibacterial activities (Table 2). Examining the data of inhibition zone against *Pseudomonas aeruginosa*, it is observed that almost all the new compounds showed antibacterial activities more than the parent drug amoxicillin. The bioactivity increases with increasing the concentration of the compounds. Imine derivatives of amoxicillin with carbonyl compounds **5**, **6**, **9**, **10**, **11**, **12** and **13** showed remarkable activity against *Staphylococcus epidermis* (Table 2). The novel Schiff base compounds of amoxicillin with compounds **1**, **6**, **7**, **9**, **10**, **11** and **13** found to be possessing extra-ordinary bioactivity against *Pseudomonas aeruginosa* (Table 2). Derivatives of amoxicillin with carbonyl compounds **2**, **4**, **5**, **7**, **9**, **10**, and **11** found to be having more bioactivity against *E. coli* (Table 2).

4. Conclusion

Imine derivative compounds derived from amoxicillin trihydrate were synthesized and structurally characterized using spectroscopic techniques. The synthetic route started with reaction between amoxicillin and different carbonyl compounds; aldehydes and ketones in ethanol as solvent. The imine derivatives containing amoxicillin moiety have been evaluated *in vitro* for their antimicrobial activities against bacterial strains (*Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli*) microorganisms at different concentrations (1×10^{-1}, 1×10^{-3}, 1×10^{-5} and 1×10^{-7} M).

Table 2. Antimicrobial activities of the prepared compounds *.

Compound	*S. Epidermis*				*P. aeruginosa*				*E. coli*			
	Concentration				Concentration				Concentration			
	1×10^{-1} mg/mL	1×10^{-3} mg/mL	1×10^{-5} mg/mL	1×10^{-7} mg/mL	1×10^{-1} mg/mL	1×10^{-3} mg/mL	1×10^{-5} mg/mL	1×10^{-7} mg/mL	1×10^{-1} mg/mL	1×10^{-3} mg/mL	1×10^{-5} mg/mL	1×10^{-7} mg/mL
1	27	23	20	17	25	22	17	13	22	17	13	8
2	22	17	14	10	21	17	14	10	25	20	16	13
3	27	22	18	15	22	17	11	6	19	15	12	9
4	21	17	14	9	23	16	10	5	24	18	15	11
5	28	24	19	15	21	16	12	7	25	22	17	14
6	31	28	23	19	27	19	12	6	21	17	14	10
7	24	20	17	13	28	23	17	11	24	21	16	12
8	23	18	11	7	20	15	11	7	17	13	10	6
9	35	31	27	24	35	29	26	22	31	28	23	17
10	34	29	26	23	34	30	25	23	34	29	25	21
11	28	25	21	16	30	26	23	20	31	28	23	19
12	30	27	23	20	18	15	11	6	29	26	24	20
13	29	24	20	17	26	23	19	14	21	18	13	7
14	23	20	16	13	22	17	13	10	22	17	14	9
Amoxicillin	22	17	13	8	20	16	11	6	15	14	11	7

* Method used: Disc diffusion method, Culture media used: Muller Hinton agar media, Incubation time: 20 hr, Incubation temperature: 37±1°C.

The results showed that majority of the novel imine derivatives have good antibacterial activity compared to the parent drug.

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