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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In vitro and in silico evaluation of 2-(substituted phenyl) oxazolo [4,5-b]pyridine derivatives as potential antibacterial agents

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Abstract Increasing resistance to antibiotics is a major problem worldwide and stimulates the development of new bacterial inhibitors. Series of oxazolo[4,5-b]pyridine synthesized in our lab was examined to screen them as potential antimicrobial agents. The antimicrobial analyses of the synthesized compound were based on minimum inhibitory concentration determination, against four strains of bacteria. The results showed that the synthesized compounds have elicited good activity profile against methicillin-resistant *Staphylococcus aureus* (*S. aureus*), the bacterium that is largely responsible for hospital-acquired infections. Moreover, synthesized compounds were also docked against enterotoxin protein of *S. aureus* which belongs to *Staphylococcal* enterotoxin type A(SEA). In vitro and in silico studies revealed that compounds **3d**, **3g**, and **3h** have demonstrated significant antibacterial activity in comparison to the standard control drug ampicillin and streptomycin.

Keywords Antibacterial · Docking · Oxazolo[4,5-b] pyridine · *Staphylococcus* enterotoxin A

Introduction

The increasing incidence of bacterial resistance towards a large number of antibacterial agents *viz*., glycopeptides, sulfonamides, β-lactams, nitroimidazoles, quinolones, tetracyclins, chloramphenicol, and macrolides, is becoming a major threat of concern now a days (Davies 1996; Chu et al. 1996). One of the most important mechanisms of the resistance against third generation antibiotics is the production of extended spectrum β-lactamases (Murthy et al. 2012). These enzymes are responsible for hydrolyzing and inactivating the β-lactam rings in the antibiotics like *penicillins*, *cephalosporins*, and *aztreonam*. Strategies to overcome such resistance require rigorous efforts towards recategorization of existing classes of antibiotics, modification of the target, and the innovative approaches to development of new antimicrobial agents (Vondenhoff and Aerschot 2011). In this regard nitrogen containing heterocycles, especially those containing pyridine rings have received a great deal of attention in the literature due to their role as active pharmacophores, which are found to be associated with a number of diverse pharmacological properties such as antimicrobial (Patel et al. 2010), antiviral (Bernardino et al. 2007), anticonvulsant (Paronikyan et al. 2002), antifungal (Duchowicz et al. 2007), and anticancer activities (Mohamed et al. 2012).

In particular oxazolo[4,5-*b*]pyridines, are well known to the chemists, owing to their inherent wide spectrum of antimicrobial potential, herbicidal, antihelmintic, antioxidant and antitumoral properties (Mikko et al. 2007; Arisoy et al. 2008; Ertan et al. 2009; Ouyang et al. 2012; Khizhan et al. 2011).

Hence, encouraged by these findings it was thought worthwhile to develop more powerful antibacterial agents comprising of pyridine skeleton in their core

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armamentarium. Therefore, in continuation of our work on the synthesis of a variety of heterocycles and evaluation of their biological potential (Sharma et al. 2004, 2009, 2010, 2012), we have tried to rationalize the investigation of antibacterial potential of the compounds synthesized in our lab (Sharma et al. 2015). The screening was carried out against a representative panel of two Gram-positive and two Gram-negative bacterial strains by estimating their potential in terms of minimum inhibitory concentration (MIC).

Furthermore, in view to gain an insight about the structure–activity relationship (SAR) of the docked compound at the active site of the enzyme, a docking simulation of synthesized oxazolopyridines was also performed considering SEA as the protein receptor of *S. aureus* belonging to the family of *Staphylococcal* enterotoxins (SEs) (Pinchuk et al. 2010). However, out of a number of protein receptors derived from twenty distinct SEs, SEA is the most common one related to the food poisoning. SEs are potent gastro intestinal toxins that can resist heat treatment, low pH and proteolytic enzymes, and thereby retained in the GIT after ingestion (Argudin et al. 2010).

Experimental

Materials and methods

All reagents were purchased from Sigma Aldrich, India, and used without further purification. The reactions were performed in an aerobic atmosphere without any specific precautions. Melting points were determined in open capillary tubes on a Veego melting-point apparatus and are uncorrected. Fourier transform infrared (FTIR) spectra were recorded within the range of 4000–400 cm^{-1} using FTIR STD10 Perkin-Elmer. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the synthesized compounds were recorded at 400 and 100 MHz respectively, using Bruker Avance II 400 NMR spectrometer in dimethylsulfoxide (DMSO) solvent, and the chemical shifts were expressed in parts per million. Spin multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass analysis was performed on quadruple-time-of-flight mass spectrometer (MICROMASS) using electrospray ionization (ESI) in positive mode. Thin-layer chromatography (TLC) is performed using precoated aluminum sheets with silica gel 60 F254.

General procedure for synthesis of 2-(substituted phenyl) oxazolo[4,5-b]pyridine

To the mixture of benzoic acid (2 mmol) and 2-amino-3-hydroxypyridine (1 mmol) in 3 mL methanol, $HClO_4{\cdot}SiO_2$ nanoparticles (5 mol%) were added under stirring under ambient conditions. Progress of the reaction was continuously monitored by TLC (n-hexane and ethyl acetate in 2:1). After the completion of process as marked by TLC, the solid was recovered by rotatory evaporator. The beauty of the process lies in the easy recovery of the catalyst by adding excess of chloroform. The product was washed with dichloromethane (2 × 5 mL) and then recrystallized from acetonitrile.

2-Phenyloxazolo[4,5-b] pyridine (**3a**) This compound was obtained from 2-amino-3-hydroxypyridine and benzoic acid (**2a**) from the previously described procedure as off-white solid (90%); M.P.: 132–134 °C; IR ($\bar{\upsilon}$, cm^{-1}): 3052, 1678, 1595, 1554, 1490, 1260, 1065, 662; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93–8.03 (m, 2H, Ar–H), 7.68 (d, 1H, J = 7.2 Hz, Pyr–H), 7.51–7.60 (m, 2H, Ar–H), 7.41–7.52 (m, 1H, Ar–H), 7.02 (d, 1H, J = 7.2 Hz, Pyr–H), 6.45 (t, 1H, J = 6.9 Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.5 (C-2), 156.8 (C-3a), 143.2 (C-5), 138.4 (C-7a), 136.1 (C-10,12), 127.6 (C-11), 122.6 (C-9,13), 119.3 (C-8), 116.2 (C-6), 114.5 (C-7); HRESIMS m/z $[M + H]^+$: 197.067 (calcd. 196.205); anal. calcd. for $C_{21}H_{15}N$: C, 73.51, H, 4.34, N, 13.98; found C, 73.58, H, 4.45, N, 13.84 (Sharma et al. 2015).

2-(2-Chlorophenyl)oxazolo[4,5-b]pyridine (**3b**) This compound was obtained from 2-amino-3-hydroxypyridine and 2-chlorobenzoic acid (**2b**) from the previously described procedure as off white solid (89%); M.P. 98–103 °C; IR ($\bar{\upsilon}$, cm^{-1}): 3061, 1670, 1627, 1579, 1540, 1258, 1050, 1030, 756; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, 1H, J = 7.2 Hz, Pyr–H), 7.33–7.56 (m, 4H, Ar–H), 7.09 (d, 1H, J = 7.2 Hz, Pyr–H), 6.54 (t, 1H, J = 6.8 Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2 (C-2), 154.3 (C-3a), 148.4 (C-5), 142.2 (C-7a), 132.5 (C-8), 130.5 (C-9), 129.1 (C-10), 128.3 (C-13), 125.8 (C-11,12), 120.8 (C-6), 118.6 (C-7); HRESIMS m/z $[M + H]^+$: 231.029 (calcd. 230.650); anal. calcd. for $C_{12}H_7ClN_2O$: C, 62.49, H, 3.06, N, 12.15; found C, 62.42, H, 3.08, N, 12.17.

2-o-Tolyloxazolo[4,5-b]pyridine (**3c**) This compound was obtained from 2-amino-3-hydroxypyridine and 2-methylbenzoic acid (**2c**) from the previously described procedure as white solid (90%); M.P.: 66–69 °C [Lit. 64–66 °C] (Clark et al. 1978); IR ($\bar{\upsilon}$, cm^{-1}): 3046, 2969, 2854, 1685, 1600, 1510, 1490, 1264, 1080, 755; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (dd, 1H, J = 5.0, 1.6 Hz, Pyr–H), 7.83 (dd, 1H, J = 8.2, 1.6 Hz, Pyr–H), 7.44 (t, 1H, J = 7.9 Hz, Pyr–H), 7.29–7.18 (m, 4H, Ar–H), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3 (C-2), 155.4 (C-3a), 148.9 (C-5), 143.4 (C-7a), 137.4 (C-8), 136.5 (C-9), 129.9 (C-10), 128.5 (C-11), 127.2 (C-13), 126.1 (C-12), 122.4 (C-6), 117.8 (C-7), 18.1 (C, CH_3); HRESIMS

m/z $[M + H]^+$: 211.079 (calcd. 210.231); anal. calcd. for $C_{13}H_{10}N_2O$: C, 74.27, H, 4.79, N, 13.33; found C, 74.29, H, 4.82, N, 13.35.

2-(2-Methoxyphenyl)oxazolo[4,5-b]pyridine (**3d**) This compound was obtained from 2-amino-3-hydroxypyridine and 2-methoxybenzoic acid (2d) from the previously described procedure as off white solid (92%): M.P.: 110–112 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3050, 2962, 2845, 1680, 1596, 1518, 1490, 1244, 1085, 760; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, 1H, $J = 6.4$ Hz, Pyr–H), 7.69 (d, 1H, $J = 6.4$ Hz, Pyr–H), 7.357–7.562 (m, 4H, Ar–H), 6.483 (t, 1H, $J =$ 6.2 Hz, Pyr–H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.3 (C-2), 157.3 (C-9), 154.8 (C-3a), 148.5 (C-5), 142.6 (C-7a), 129.4 (C-11,13), 123.2 (C-6), 121.2 (C-12), 118.9 (C-7), 115.2 (C-10), 111.6 (C-8), 62.2 (C, OCH_3); HRESIMS *m/z* $[M + H]^+$: 227.077 (calcd. 226.231); anal. calcd. for $C_{13}H_{10}N_2O_2$: C, 69.02, H, 4.46, N, 12.38, found C, 69.05, H, 4.50, N, 12.35.

2-(3-Bromophenyl)oxazolo[4,5-b]pyridine (**3e**) This compound was obtained from 2-amino-3-hydroxypyridine and 3-bromobenzoic acid (**2e**) from the previously described procedure as pale yellow solid (88%); M.P.: 156–160 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3047, 1693, 1595, 1522, 1486, 1225, 1062, 965, 755; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (s, 1H, Ar–H), 7.58 (d, 1H, $J = 6.8$ Hz, Pyr–H), 7.39 (d, 1H, $J =$ 6.8 Hz, Pyr–H), 7.082–7.196 (m, 3H, Ar–H) 6.58 (t, 1H, J = 7 Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C-2), 154.3 (C-3a), 148.3 (C-5), 142.4 (C-7a), 133.8 (C-9), 131.6 (C-11,12), 128.3 (C-8), 126.3 (C-13), 122.7 (C-6), 119.9 (C-10), 118.8 (C-7); HRESIMS *m/z* $[M + H]^+$: 276.092 (calcd. 275.101); anal. calcd. for $C_{12}H_7BrN_2O$: C, 52.39, H, 2.56, N, 10.18; Found C, 52.42, H, 2.58, N, 10.20.

2-(3-Chlorophenyl)oxazolo[4,5-b]pyridine (**3f**) This compound was obtained from 2-amino-3-hydroxypyridine and 3-chlorobenzoic acid (**2f**) from the previously described procedure as white solid (90%); M.P.: 148–151 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3045, 1684, 1591, 1517, 1492, 1263, 1082, 1055, 772; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.561–7.642 (m, 3H, Ar–H), 7.42 (s, 1H, Ar–H), 7.36 (d, 1H, $J = 8.4$ Hz, Pyr–H), 7.11 (d, 1H, $J = 8.4$ Hz, Pyr–H), 6.65 (t, 1H, J = 6.2 Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C-2), 154.5 (C-3a), 148.7 (C-5), 142.4 (C-7a), 134.5 (C-9), 129.1 (C-11,12), 128.2 (C-8), 127.8 (C-13), 125.1 (C-6), 119.4 (C-10), 118.6 (C-7); HRESIMS *m/z* $[M + H]^+$: 231.024 (calcd. 230.65); anal. calcd. for $C_{12}H_7ClN_2O$, C, 62.49, H, 3.06, N, 12.15; Found C, 62.45, H, 3.10, N, 12.14.

2-(3-Nitrophenyl)oxazolo[4,5-b]pyridine (**3g**) This compound was obtained from 2-amino-3-hydroxypyridine and 3-nitrobenzoic acid (**2g**) from the previously described procedure as white solid (86%); M.P.: 200–203 °C [Lit. 199–201 °C] (Clark et al. 1978); IR ($\overline{\upsilon}$, cm^{-1}): 3039, 1690, 1592, 1519, 1484, 1360, 1348, 1260, 1077, 768; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (dd, 1H, $J = 4.8$, 1.4 Hz, Pyr–H), 8.41(s, 1H, Ar–H), 8.09–7.98 (m, 3H, Ar–H), 7.78 (dd, 1H, $J = 7.8$, 1.4 Hz, Pyr–H), 7.42 (t, 1H, $J = 7.6$ Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.4 (C-2), 154.6 (C-3a), 149.2 (C-10), 148.4 (C-5), 143.2 (C-7a), 134.1 (C-13), 129.8 (C-12), 127.3 (C-8), 122.3 (C-6,9), 121.5 (C-11), 118.6 (C-7); HRESIMS *m/z* $[M + H]^+$: 242.028 (calcd. 241.202); anal. calcd. for $C_{12}H_7N_3O_3$: C, 59.75, H, 2.93, N, 17.42; found C, 59.79, H, 2.95, N, 17.45.

2-(3-Methoxyphenyl)oxazolo[4,5-b]pyridine (**3h**) This compound was obtained from 2-amino-3-hydroxypyridine and 3-methoxybenzoic acid (**2h**) from the previously described procedure as white solid (92%); M.P.: 115–118 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3038, 2958, 2855, 1684, 1590, 1517, 1497, 1260, 1082, 755; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, 1H, $J = 8.4$ Hz, Pyr–H), 7.56 (d, 1H, $J = 8.4$ Hz, Pyr–H), 7.29–7.36 (m, 3H, Ar–H), 7.16 (t, 1H, $J = 9.2$ Hz, Pyr–H), 6.59 (s, 1H, Ar–H), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.8 (C-10), 161.5 (C-2), 154.8 (C-3a), 148.1 (C-5), 142.8 (C-7a), 130.7 (C-12), 127.1 (C-8), 119.2 (C-6), 118.8 (C-7,13), 114.5 (C-11), 111.3 (C-9), 62.2 (C, OCH_3); HRESIMS *m/z* $[M + H]^+$: 227.074 (calcd. 226.231); anal. calcd. for $C_{13}H_{10}N_2O_2$, C, 69.02, H, 4.46, N, 12.38; Found C, 69.01, H, 4.48, N, 12.38.

2-(4-Bromophenyl)oxazolo[4,5-b]pyridine (**3i**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-bromobenzoic acid (**2i**) from the previously described procedure as light yellow solid (90%); M.P.: 172–175 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3061, 1684, 1582, 1516, 1485, 1268, 1086, 780; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (dd, 1H, $J =$ 5.2, 1.5 Hz, Pyr–H), 7.82 (dd, 1H, $J = 8.1$, 1.5 Hz, Pyr–H), 7.42 (t, 1H, $J = 7.9$ Hz, Pyr–H), 7.24–7.36 (m, 4H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3 (C-2), 155.2 (C-3a), 147.8 (C-5), 143.2 (C-7a), 131.8 (C-10,12), 129.4 (C-9,13), 124.8 (C-8), 123.5 (C-11), 122.2 (C-6), 117.8 (C-7); HRESIMS *m/z* $[M + H]^+$: 276.098 (calcd. 275.101); anal. calcd. for $C_{12}H_7BrN_2O$, C, 52.39, H, 2.56, N, 10.18; found C, 52.42, H, 2.54, N, 10.21.

2-(4-(Trifluoromethyl)phenyl)oxazolo[4,5-b]pyridine (**3j**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-trifluoromethylbenzoic acid (**2j**) from the previously described procedure as white solid (86%); M.P. 162–164 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3055, 1693, 1596, 1509, 1490, 1265, 1150, 1082, 776; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 7.96 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.88 (d, 1H, $J = 9.6$ Hz, Pyr–H), 7.37 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.15 (d, 1H, $J = 9.6$ Hz, Pyr–H), 6.72 (t, 1H, $J = 7.2$ Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6 (C-2), 154.6 (C-3a), 148.8 (C-5), 142.4 (C-7a), 133.5 (C-11), 131.9 (C-8), 127.7 (C-9,13), 125.5 (C-10,12), 124.7 (C, CF_3), 119.6 (6C), 118.1 (7C); HRESIMS *m/z* $[M + H]^+$: 265.059 (calcd. 264.203); anal. calcd. for $C_{13}H_7F_3N_2O$, C, 59.10, H, 2.67, N, 10.60; found C, 59.09, H, 2.69, N, 10.55.

2-(4-Chlorophenyl)oxazolo[4,5-b]pyridine (**3k**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-chlorobenzoic acid (**2k**) from the previously described procedure as off white solid (87%): M.P.: 165–168 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3057, 1689, 1585, 1519, 1488, 1270, 1090, 1056, 768; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, 1H, $J = 8.4$ Hz, Pyr–H), 7.58 (d, 2H, $J = 9.6$ Hz, Ar–H), 7.39 (d, 2H, $J = 9.6$ Hz, Ar–H), 7.22 (d, 1H, $J = 8.4$ Hz, Pyr–H), 6.68 (t, 1H, $J = 7$ Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.9 (C-2), 154.6 (C-3a), 148.5 (C-5), 142.1 (C-7a), 134.3 (C-11), 129.7 (C-10,12), 128.1 (C-9,13), 119.9 (C-6), 118.3 (C-7a); HRESIMS *m/z* $[M + H]^+$: 231.030 (calcd. 230.65); anal. calcd. for $C_{12}H_7ClN_2O$: C, 62.49, H, 3.06, N, 12.15; found C, 62.48, H, 3.11, N, 12.17.

2-(4-Methylphenyl)oxazolo[4,5-b]pyridine (**3l**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-methylbenzoic acid (**2l**) from the previously described procedure as off white solid; yield: 96%; M.P. 138–140 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3043, 2974, 2857, 1689, 1597, 1508, 1493, 1267, 1078, 759; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87–7.90 (m, 3H, Pyr–H), 7.21–7.34 (m, 4H, Ar–H), 2.24 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5 (C-2), 154.3 (C-3a), 148.8 (C-5), 142.5 (C-7a), 131.4 (C-11), 127.5 (C-10,12), 126.3 (C-9,13), 119.7 (C-6), 118.7 (C-7), 29.3 (C, CH_3); HRESIMS *m/z* $[M + H]^+$: 211.085 (calcd. 210.231); anal. calcd. for $C_{13}H_{10}N_2O$: C, 74.27, H, 4.79, N, 13.33; found C, 74.25, H, 4.83, N, 4.77.

2-(4-Nitrophenyl)oxazolo[4,5-b]pyridine (**3m**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-nitrobenzoic acid (**2m**) from the previously described procedure as off white solid (88%); M.P.: 217–220 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3042, 1680, 1596, 1512, 1482, 1365, 1352, 1271, 1074, 789; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (dd, 1H, $J = 5.0$, 1.3 Hz, Pyr–H), 8.11–7.98 (m, 4H, Ar–H), 7.81(dd, 1H, $J = 8.2$, 1.3 Hz, Pyr–H), 7.45 (t, 1H, $J = 7.8$ Hz, Pyr–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6 (C-2), 154.5 (C-3a), 148.3 (C-11), 147.6 (C-5), 143.2 (C-7a), 133.1 (C-8), 128.2 (C-9,13), 122.4 (C-6), 121.8 (C-10,12), 118.6 (C-7); HRESIMS *m/z* $[M + H]^+$: 242.012 (calcd. 241.202); anal. calcd. for $C_{12}H_7N_3O_3$: C, 59.75, H, 2.93, N, 17.42; found C, 59.72, H, 2.90, N, 17.46.

2-(4-Methoxyphenyl)oxazolo[4,5-b]pyridine (**3n**) This compound was obtained from 2-amino-3-hydroxypyridine and 4-methoxybenzoic acid (**2n**) from the previously described procedure as white solid (94%); M.P.: 178–180 °C; IR ($\overline{\upsilon}$, cm^{-1}): 3047, 2968, 2850, 1691, 1588, 1503, 1492, 1267, 1060, 750; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.83 (d, 1H, $J = 7.2$ Hz, Pyr–H), 7.31 (d, 1H, $J = 7.2$ Hz, Pyr–H), 7.05 (d, 2H, $J = 8.8$ Hz, Ar–H), 6.613–6.645 (t, 1H, $J = 6.4$ Hz, Pyr–H), 3.86 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6 (C-11), 160.5 (C-2), 154.5 (C-3a), 148.1 (C-5), 142.8 (C-7a), 122.3 (C-9,13), 119.2 (C-6), 118.6 (C-8), 115.7 (C-7), 114.5 (C-10,12), 63.2 (C, OCH_3); HRESIMS *m/z* $[M + H]^+$: 227.082 (calcd. 226.231); anal. calcd. for $C_{13}H_{10}N_2O_2$: C, 69.02, H, 4.46, N, 12.38; found C, 68.99, H, 4.51, N, 12.35.

In vitro antibacterial procedure

All the test compounds were assayed in vitro for antibacterial activity against methicillin-resistant *S. aureus*, *B. subtilis*, *E. coli*, and *P. diminuta* using nutrient agar as growth medium. The MIC was determined by using two-fold serial dilution method. Stock solutions of test compounds were prepared in dimethyl formamide (DMF) at a concentration of 800 µg/mL, suspension containing 10^7 CFU/mL of bacteria was prepared from broth culture. Bacterial plates were prepared in triplicate and incubated at 37 °C within 16–24 h. Ampicillin and streptomycin were tested under the similar conditions as reference drugs. In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing broth supplemented with only DMF at the same dilution used in our experiment. The MIC values were obtained from the lowest concentration of the test compound which was required to inhibit the bacterial growth. The MIC values were expressed in µg/mL.

Molecular docking studies

The X-ray crystal structure of SEA protein of methicillin-resistant *S. aureus* (PDB ID: 1esf) was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/). The protein was imported in MVD, and missing bond orders, hybridization states, and angles were then assigned. To obtain better potential binding sites in the protein, a maximum of five cavities were detected using parameters such as molecular surface (expanded van der Waals), maximum number of cavities ($n = 5$), minimum cavity volume (10), probe size (1.20), maximum number of ray checks ($n = 16$), minimum number of ray hits ($n = 12$), and grid resolution (0.80).

All docking calculations were carried out using the grid-based Moldock score (GRID) function with a grid resolution of 0.30 Å. The binding site on the receptor was defined by cavity of volume 50.688 $Å^3$ and surface area of 200.96 $Å^2$ with a radius of 15 Å. The Moldock optimization search algorithm with a maximum of ten runs was used through the calculations, with all other parameters kept as defaults. Each time five poses were obtained and the best pose was selected based on the scoring function such as the Rerank score, Moldock score and interaction energy (Thomsen and Christensen 2006). This pose was then converted to ligand and the ligand energy inspector was employed to determine ligand–protein interaction.

Results and discussion

Chemistry

2-(Substituted phenyl) oxazolo[4,5-b]pyridine derivatives were synthesized as per the procedural follow up developed in our lab (Sharma et al. 2015). Syntheses and characterization details of some of the new derivatives (**3c**, **3g**, **3i**, and **3m**) are being reported here. The compounds were prepared using a one pot synthetic strategy (Scheme 1) in quantitative yield by the reaction between 2-amino-3-hydroxy pyridine (**1**) and substituted benzoic acids (**2**) in the presence of silica-supported perchloric acid. The reaction was performed under ambient conditions using methanol as the solvent. The silica-supported catalyst has high mechanical and thermal stability, low toxicity, good recyclability, and ease of handling (Khan et al. 2006). Structures of the synthesized compounds were confirmed by elemental, infrared (IR), ^{1}H NMR, ^{13}C NMR, and mass spectral analyzes.

Biology

All the synthesized derivatives were screened *in vitro* for their antibacterial activities against two Gram-positive bacterial strains, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 2943, *Bacillus subtilis* ATCC 6633 and two Gram-negative bacterial strains, *Escherichia coli* ATCC 13067 and *Pseudomonas diminuta* MTCC 3361.

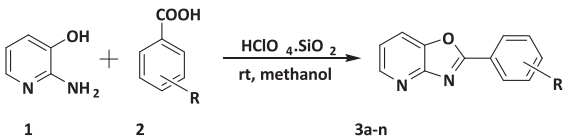
In the present investigation, 2-(phenyl)oxazolo[4,5-b]-pyridine derivatives were tested against the chosen bacterial strains using standard two-fold serial dilution method and reported in terms of MIC (Table 1). The in vitro antibacterial activity results revealed that the synthesized compounds **3d**, **3g**, and **3h** were found to be highly active (MIC: 1.56–25 μg/mL), whereas **3b**, **3c**, **3e**, **3f**, and **3m** were moderately active (MIC: 6.25–50 μg/mL) as compared to the standard drug ampicillin and streptomycin. A systematic perusal of the data depicted in Table 1, reveals that in comparison to Gram-negative bacteria, Gram-positive bacteria are more sensitive to synthesized oxazolo[4,5-b] pyridine analogs. Compounds **3d**, **3g**, and **3h** showed strong

Table 1 MIC values of oxazolo[4,5-b]pyridine derivatives

MIC in μg/mL				
Compounds	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus ATCC 2943	*B. subtilis* ATCC 6633	*E. coli* ATCC 13067	*P. diminuta* MTCC 3361
3a	100	100	100	>100
3b	6.25	25	50	25
3c	6.25	12.5	25	25
3d	3.125	6.25	25	12.5
3e	12.5	12.5	50	25
3f	6.25	25	50	50
3g	1.56	3.125	12.5	6.25
3h	3.125	6.25	12.5	12.5
3i	50	50	100	100
3j	50	50	50	50
3k	25	100	100	50
3l	50	50	>100	>100
3m	12.5	25	50	25
3n	25	50	50	100
A	6.25	12.5	50	25
S	12.5	25	50	25

A ampicillin, *S* streptomycin

Scheme 1 Synthesis of 2-(phenyl)oxazolo[4,5-b]pyridine derivatives using $HClO_4{\cdot}SiO_2$ as supported catalyst



1 2 3a-n

3	a	b	c	d	e	f	g	h	i	j	k	l	m	n
R	H	2-Cl	2-Me	2-OMe	3-Br	3-Cl	3-NO2	3-OMe	4-Br	4-CF3	4-Cl	4-Me	4-NO2	4-OMe

activity against methicillin-resistant *S. aureus* (MIC: 1.56–3.125 µg/mL) as compared to control drugs (MIC: 6.25 and 12.5 µg/mL). These compounds are also active against other strains of bacteria but their MIC values are more in comparison to methicillin-resistant *S. aureus*. Antibacterial profile of the tested compounds elicits the following progression (Fig. 1):

P. diminuta < *E. coli* < *B. subtilis* < *S. aureus* (MRSA)

In Fig. 1, MIC values greater than 100 have not been incorporated for compounds **3a** and **3l** against gram-negative bacteria.

Further the close inspection of screening results revealed that substitution in the aromatic ring attached at C-2 position of oxazolo[4,5-b]pyridine nucleus exerted significant influence on the investigated biological activity. Interestingly, it was noticed that in vitro antibacterial activity of the compounds **3a**-**n** is found to be increased when groups such as $-NO_2$, –OMe and –Cl and –Br are present in *meta* and *ortho* position (Fig. 2).

Molecular docking studies

Molecular modeling, vis-à-vis molecular docking involves the prediction of ligand confirmation and orientation in the binding pocket of receptor. In the present study, docking simulations were carried out using Molegro Virtual Docker (MVD) 4.0. All fourteen derivatives along with the standard drug (ampicillin) were docked with protein receptor SEA of most sensitive bacteria, *S. aureus* (MRSA) in order to rationalize the obtained biological data and explain the possible molecular interactions that might take place. Furthermore, to comprehend the deepness of the ligand–protein molecular interaction, the ligand energy inspector was employed. The molecular docking results of test compounds and reference drug with the protein receptor in terms of Moldock score, Rerank score, interaction and hydrogen bonding energies are depicted in Table 2. The docking studies have revealed that out of fourteen derivatives three were found to have favorable Rerank score and docking

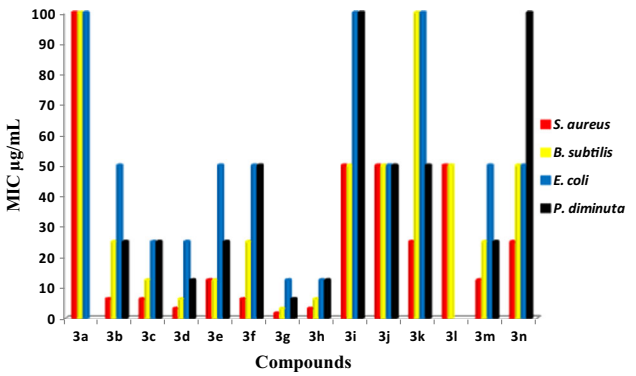


Fig. 1 Graphical representation depicting antibacterial activity of the synthesized moieties

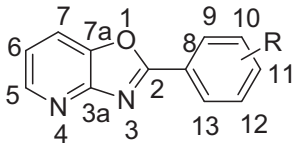


Fig. 2 Compounds **3a–n**

Table 2 Docking scores of the synthesized derivatives and control drug

Compounds	MolDock score[a]	Rerank Score	Interaction[b]	H bond[c]
3a	−109.06	−91.16	−107.76	−10.54
3b	−116.73	−96.50	−115.05	−10.58
3c	−116.90	−97.61	−114.38	−10.58
3d	−117.31	−98.66	−118.43	−9.72
3e	−114.69	−95.42	−112.35	−10.59
3f	−115.06	−95.67	−112.40	−10.61
3g	−122.65	−103.20	−121.43	−8.17
3h	−119.10	−101.02	−117.10	−10.13
3i	−113.30	−92.90	−110.99	−10.55
3j	−115.34	−93.54	−114.32	−5.30
3k	−113.41	−93.06	−111.14	−10.55
3l	−113.51	−93.89	−111.21	−10.55
3m	−116.99	−94.38	−115.34	−10.01
3n	−113.47	−92.33	−114.01	−9.11
Ampicillin	−121.98	−100.23	−122.00	−5.79

[a] Moldock score is derived from the PLP scoring functions with a new hydrogen bonding term and new charge schemes (Pathak et al. 2013)

[b] The total interaction energy between the best pose and the protein (kJ/mol)

[c] Hydrogen bonding energy (kJ/mol)

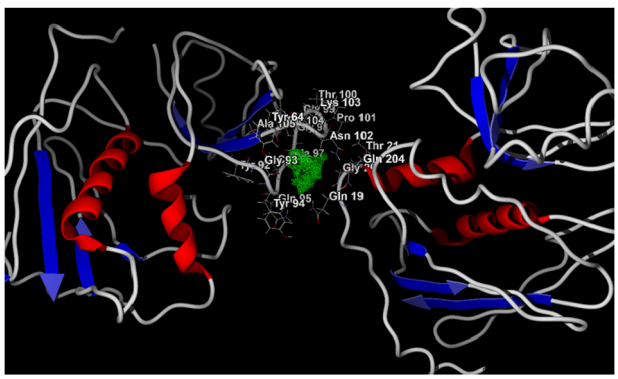


Fig. 3 Secondary structure of protein along with the binding cavity (*green* wireframe) (color figure online)

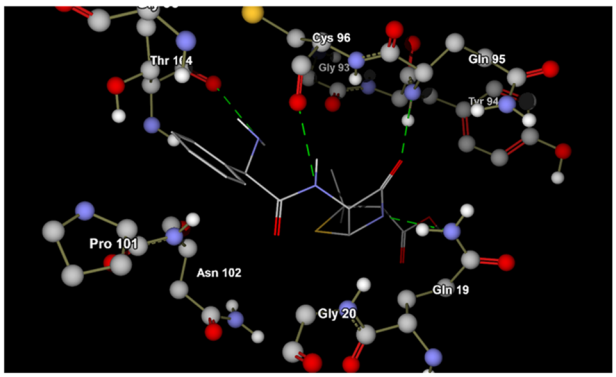


Fig. 4 Binding interactions of ampicillin with the protein (PDB ID: 1esf)

score compared to ampicillin. The ligand–protein interaction is considered based on the Rerank score, which is defined as a linear combination of E-inter which is of van der Waals, steric, electrostatic, hydrogen bonding, energy between the ligand and the protein, and E-intra which is of van der Waals, hydrogen bonding, torsional, electrostatic, sp^2–sp^2 energy of the ligand weighted by pre-defined coefficients (Pathak et al. 2013).

Out of these three compounds **3g** has highest affinity with Rerank score of −103.20 kJ/mol followed by **3h** and **3d** exhibiting significant binding affinities. In compound **3a** with least binding affinity (−91.16 kJ/mol) there is no substitution in the aromatic ring, suggesting that the presence of substitutions on aromatic ring enhances the ligand–protein interactions, which can be further enhanced by the presence of aromatic ring with substituents at *ortho* and *meta* positions. Moreover, the docking results were in good correlation with in vitro antibacterial activity of these compounds. The most important amino acid components present in the close proximity to the binding cavity of size 50.688 $Å^3$ are depicted in Fig. 3, where arrows represent β sheets and α helices are represented by helical lines.

The snapshots of the ligand–protein molecular interaction illustrating the binding mode of the reference drug, compounds **3d**, **3g**, and **3h** are depicted in Figs. 4, 5, respectively. Ampicillin showed molecular interaction with Cys 96, Thr104, Gln95, and Gln19 (Fig. 4).

Compound **3d** interacted with Asn102, Thr104, Gly98, and Gly99 (Fig. 5a), **3g** showed interaction with Asn102, Thr104, Gln95, and Gln19 (Fig. 5b) and **3h** showed molecular interaction with Gly98, Gly99, Thr104, and Asn102 (Fig. 5c). Green colored dashed lines represent hydrogen bonds. Furthermore, ligand-protein interaction analyzes for **3d**, **3g**, **3h**, and reference drug, which represents the interacting residues, interaction energy and interaction distances are shown in Table 3.

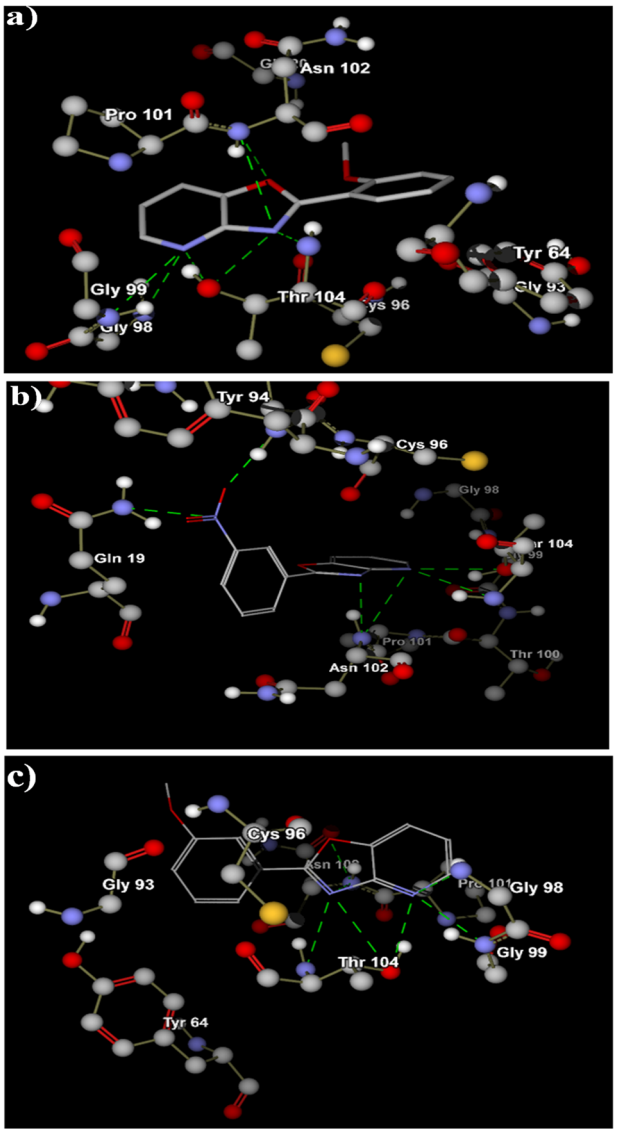


Fig. 5 Ligand–protein (PDB ID: 1esf) interactions of compounds **3d** (**a**), **3g** (**b**) and **3h** (**c**) at the binding cavity

Conclusion

In the present studies, synthesized oxazolo pyridine derivatives were evaluated for their antibacterial potency against four different strains of bacteria. Three derivatives (**3d**, **3g**, and **3h**) possessed strong antibacterial activity against all the four strains with best activity profile against *S. aureus* (MRSA) and five compounds (**3b**, **3c**, **3e**, **3f**, and **3m**) were found to be moderately active against all the tested strains of bacteria. The compounds were further screened for molecular docking simulation against the *S. aureus* (MRSA) protein (PDB ID: 1esf) and observed that three compounds were found to exhibit favorable ligand–protein binding affinities compared to standard drug (ampicillin and streptomycin).

Table 3 Molecular interaction analysis

Compound	Interaction	Interaction energy	Interaction distance (Å)
Ampicillin	Cys96(O)–N(8)	−0.53	3.28
	Thr104(O)–N(11)	−1.59	3.14
	Gln95(N)–O(15)	−2.31	2.58
	Gln19(N)–N(13)	−1.35	3.22
3g	Asn102(N)–N(2)	−0.74	3.33
	Thr104(N)–N(2)	−0.22	3.50
	Thr104(O)–N(2)	−2.05	3.10
	Asn102(N)–N(6)	−1.26	3.15
	Gln95(N)–O(17)	−2.50	2.66
	Gln19(N)–N(15)	−1.40	2.91
3h	Gly98(N)–N(2)	−1.41	3.17
	Gly99(N)–N(2)	−1.52	3.17
	Thr104(O)–N(2)	−2.50	2.97
	Asn102(N)–N(6)	−0.34	3.47
	Thr104(N)–N(6)	−1.73	3.06
	Thr104(O)–N(6)	−0.16	3.50
	Asn102(N)–O(8)	−2.49	3.10
3d	Asn102(N)–O(8)	−2.46	2.95
	Asn102(N)–N(6)	−0.03	3.58
	Thr104(O)–N(6)	−0.06	3.58
	Thr104(N)–N(6)	−1.74	3.11
	Thr104(O)–N(2)	−2.50	3.05
	Gly98(N)–N(2)	−1.52	3.02
	Gly99(N)–N(2)	−1.41	3.23

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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