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Synthesis and molecular docking study of pyrazole clubbed oxazole as antibacterial agents

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Abstract

We have developed a simple synthetic protocol for the preparation of novel 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-arylisoxazoles. The structure of synthesized compounds was elucidated by spectral techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass. The novel bioactive compounds **3a-t** were evaluated for in vitro antibacterial activity on several bacterial species. Compounds **3c** (–4–NO_2), **3o** (–4–F), and **3r** (–3,4–Cl_2) exhibited good in vitro antibacterial activity. Furthermore, molecular docking on *DNA gyrase subunit b* could shed some light on the mechanism of action which can serve as a guide for lead optimization.

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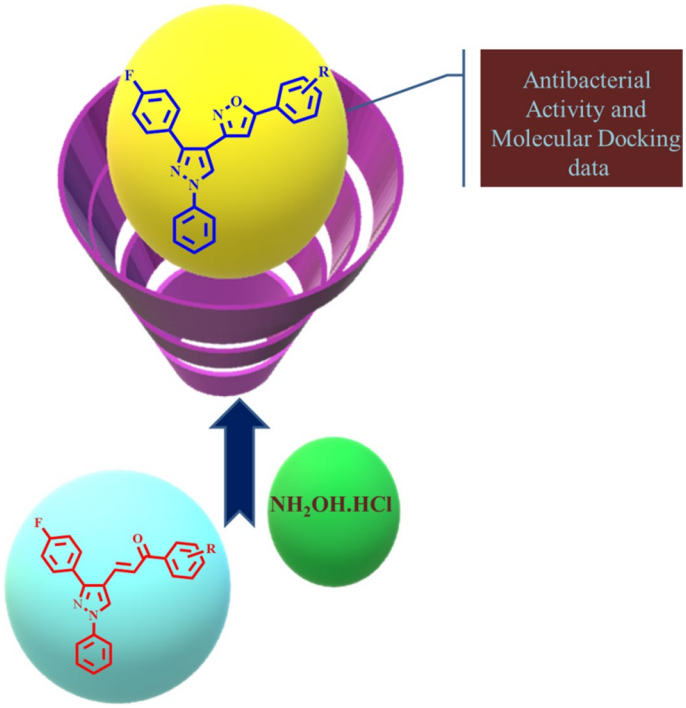
Graphic abstract



Keywords Pyrazole · Isoxazole · Antibacterial activity · Molecular modelling · Docking

Introduction

The inactivation of antibiotics because of the resistance towards the existing antimicrobial agents is one of the major challenges posed to the scientific community. The mortality and morbidity data, published by WHO, support this fact. Antibiotic resistance is now a growing threat to mankind [1]. Chemists are trying to find the solution to this problem [2, 3]. To find a solution to this pressing problem, our research group has extensively synthesized antimicrobial scaffolds with a novel mechanism of action against these pathogens [4–7]. Azoles are known for their antibacterial property. Selma SARAÇ and several other workers have synthesized and developed azole-based antimicrobial compounds that showed good-to-excellent activity [8–12]. In continuation, we have incorporated pyrazole and isoxazole as a building block of our targeted compounds and studied their antibacterial efficiency. Synthesis of isoxazoles goes back to 1883 when it was explained by Claisen in 1888 [13] utilising β-diketones and hydroxylamine. It ultimately gave 3,5-disubstituted isoxazoles. However, the formation of unsymmetrical 1,3-diketones can result in

mixtures of isomeric isoxazoles. Numerous research groups have developed isoxazoles through 1,3-dipolar cycloaddition or 3 + 2 cycloaddition. The major disadvantages of this method are low yield, side reactions, and poor regioselectivity [14]. With this objective, we synthesized isoxazoles in good yield from easily available reactants with simple workup. Furthermore, considering the significant place occupied by the heterocyclic structural unit among pharmaceutically important synthetic and natural materials, detailed antimicrobial profiling was carried out for these isoxazoles. Molecular docking study against crucial target *DNA gyrase subunit b* could provide valuable insights into the binding mode and affinity of these isoxazole analogs. The drug design concept on the basis of commercially available drugs containing pyrazole and isoxazole scaffolds is given in Fig. 1.

Experimental

Synthesis of 3-(4-fluorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (1) was achieved by reported method [15].

3-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-ones (2a-t) was prepared by Shaharyar et al. (2010) method [16].

Preparation of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-arylisoxazoles (3a-t)

A mixture of 3-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-arylprop-2-en-1-ones (2a-t) and hydroxylamine hydrochloride (0.03 mol) was refluxed in the ethanol (30 mL) at 120 °C for 15 h in the presence of NaOH which is used as a catalyst. Once the reaction mixture is cooled, it was poured into crushed ice. The obtained crude product was crystallized in CH_3CH_2OH (95%) to produce the final compounds (3a-t).

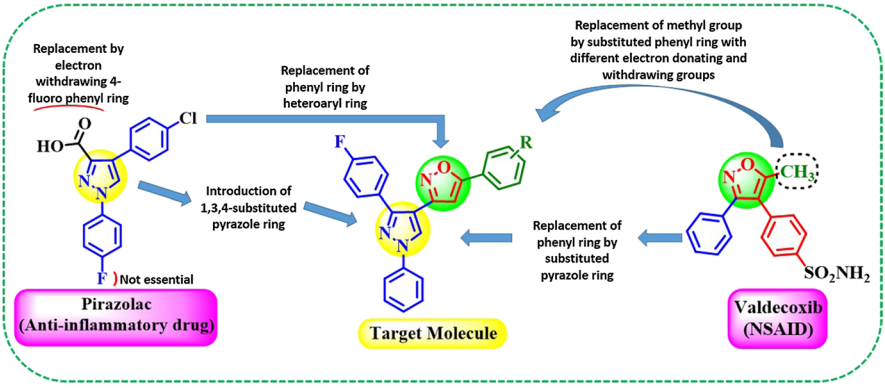


Fig. 1 Drug design concept on the basis of commercially available drugs containing pyrazole and isoxazole scaffold

Physical constants and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-phenylisoxazole (3a)

Yield 65%; m.p. 158–160 ºC; IR: 1095, 1450, 1598, 1544, 1649, 2848, 3055; ^{1}H NMR (DMSO-*d6*, 400 MHz): δ = 6.70 (s, 1H,=CH–isoxazole), 7.20–8.30 (m, 14H, Ar–H), 8.60 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-*d6*): δ = 98.2, 103.5, 116.2 (2), 119.8 (2), 125.4 (2), 126.2, 126.3, 128.3, 128.4, 129.3 (2), 129.4 (2), 130.5 (2), 131.4, 143.7, 145.2, 162.2, 162.7, 139.4; LCMS: m/z 381.2 (60.1%). Anal. Calcd. For $C_{24}H_{16}FN_3O$: C, 75.58; H, 4.23; N, 11.02. Found: C, 75.78; H, 4.29; N, 11.15.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(3-nitrophenyl)isoxazole (3b)

Yield 62%; mp 145–147 ºC; IR: 1060, 1435, 1523, 1570, 1565, 1690, 2865, 3030; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 6.80 (s, 1H,=CH–isoxazole), 7.00–8.30 (m, 13H, Ar–H–), 8.70 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 98.0, 103.5, 116.5 (2), 119.4 (2), 122.7, 123.7, 126.7, 128.4, 129.0 (2), 130.2 (2), 130.8, 131.5, 131.7, 131.9, 143.0, 145.3, 148.9, 162.7, 162.7, 169.0; LCMS: *m/z* 426.1 (100.0%). Anal. Cacld. For $C_{24}H_{15}FN_4O_3$: C, 67.60; H, 3.55; N, 13.14. Found: C, 67.67; H, 3.65; N, 13.19.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(4-nitrophenyl)isoxazole (3c)

Yield 69%; mp 148–150 ºC; IR: 1025, 1400, 1550, 1545, 1585, 1660, 2828, 3045; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 6.75 (s, 1H, = CH– isoxazole), 7.29–8.15 (m, 13H, Ar–H-), 8.63 (s, 1H, = CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 98.1, 103.3, 116.8 (2), 119.4 (2), 124.4 (2), 124.9 (2), 126.1, 129.1, 129.8, 130.7 (2), 131.9, 132.2, 143.8, 145.2, 147.4, 162.1, 162.8, 169.1; LCMS: *m/z* 426.1 (100.0%). Anal. Cacld. For $C_{24}H_{15}FN_4O_3$: C, 67.60; H, 3.55; N, 13.14. Found: C, 67.70; H, 3.75; N, 13.23.

Physical constant and characterization of 5-(3-bromophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3d)

Yield 62%; mp 151–153 ºC; IR: 540, 1010, 1460, 1545, 1600, 1670, 2820, 3010; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 6.72 (s, 1H, = CH–isoxazole), 7.19–8.25 (m, 13H, Ar–H–), 8.45 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 98.4, 103.0, 116.8 (2), 119.1 (2), 122.4, 124.9, 126.9, 128.0, 128.8, 129.3 (2), 130.4 (2), 131.2, 131.9, 132.0, 133.0, 132.2, 133.8, 143.9, 145.1, 162.0, 162.8, 169.6; LCMS: *m/z* 462.0 (26.6%). Anal. Cacld. For $C_{24}H_{15}BrFN_3O$: C, 62.62; H, 3.28; N, 9.13; Found: C, 62.70; H, 3.36; N, 9.23.

Physical constant and characterization of 5-(4-bromophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3e)

Yield 70%; mp 171–173 ºC; IR: 520, 1008, 1422, 1523, 1510, 1645, 2800, 3036; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.65$ (s, 1H, = CH- isoxazole), 7.35–8.30 (m, 13H, Ar–H-), 8.69 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 99.0$, 103.1, 116.9 (2), 119.5 (2), 123.7, 125.1, 126.7, 127.6 (2), 128.8, 129.8 (2), 130.1 (2), 131.3, 132.9 (2), 143.9, 145.8, 162.0, 162.9, 139.6; LCMS: *m/z* 459.0 (99.0%); Anal. Cacld. For $C_{24}H_{15}BrFN_3O$: C, 62.62; H, 3.28; N, 9.13; Found: C, 62.69; H, 3.35; N, 9.28.

Physical constant and characterization of 2-(3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazol-5-yl)phenol (3f)

Yield 69%; mp 175–177 ºC; IR: 1060, 1430, 1536, 1555, 1652, 2835, 3040, 3250; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 5.40$ (s, 1H,–OH), 6.77 (s, 1H,=CH–isoxazole), 7.02–8.20 (m, 13H, Ar–H–), 8.50 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.1$, 103.4, 116.2, 116.8 (2), 117.8, 119.3 (2), 121.1, 126.5, 126.9, 128.4, 129.6 (2), 130.3, 130.8, 131.8, 143.1, 145.9, 155.1, 162.8, 162.9, 169.0; LCMS: *m/z* 397.1 (90.0%). Anal. Cacld. For $C_{24}H_{16}FN_3O_2$: C, 72.54; H, 4.06; N, 10.57. Found: C, 72.60; H, 3.40; N, 9.35.

Physical constant and characterization of 3-(3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazol-5-yl)phenol (3 g)

Yield 68%; mp 185–187 ºC; IR: 1020, 1406, 1535, 1558, 1660, 2810, 3010, 3300; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 5.46$ (s, 1H, –OH), 6.64 (s, 1H, = CH–isoxazole), 6.86–8.20 (m, 13H, Ar–H–), 8.45 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.6$, 103.9, 114.9, 115.1, 116.8 (2), 117.4, 119.4 (2), 126.7, 128.9, 129.1 (2), 130.2 (3), 131.7, 131.5, 143.7, 145.3, 157.1, 162.0, 162.7, 169.5; LCMS: *m/z* 398.1 (27.3%). Anal. Cacld. For $C_{24}H_{16}FN_3O_2$: C, 72.54; H, 4.06; N, 10.57. Found: C, 72.59; H, 4.10, N, 10.63.

Physical constant and characterization of 4-(3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazol-5-yl)phenol (3 h)

Yield 65%; mp 188–190 ºC; IR: 1030, 1412, 1545, 1569, 1695, 2860, 3020, 3350; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 5.30$ (s, 1H, –OH), 6.52 (s, 1H,=CH–isoxazole), 6.60–8.00 (m, 13H, Ar–H–), 8.25 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.4$, 103.9, 116.1 (2), 116.8 (2), 119.1, 119.6 (2), 126.7, 127.3 (2), 128.4, 129.3 (2), 130.9 (2), 131.7, 143.8, 145.9, 158.3, 162.3, 162.8, 169.6; LCMS: *m/z* 398.1 (30.5%). Anal. Cacld. For $C_{24}H_{16}FN_3O_2$: C, 72.54; H, 4.06; N, 10.57. Found: C, 72.69; H, 4.09; N, 10.60.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(2-methoxyphenyl)isoxazole (3i)

Yield 67%; mp 196–198 ºC; IR: 1000, 1396, 1501, 1510, 1640, 2820, 2835, 3029; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.30$ (s, 3H, –OCH_3), 6.40 (s, 1H, = CH- isoxazole), 7.05–8.10 (m, 13H, Ar–H–), 8.21 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 56.3, 98.3, 103.7, 114.2, 116.2, 116.9 (2), 119.9, 121.4, 126.1, 126.3, 128.2, 129.3, 129.6 (2), 130.9 (2), 131.9, 143.9, 145.0, 155.9, 162.6, 162.8, 169.6; LCMS: *m/z* 411.1 (100.0%). Anal. Calcd. For $C_{25}H_{18}FN_3O_2$: C, 72.98; H, 4.41; N, 10.21. Found: C, 73.02; H, 4.49; N, 10.26.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(3-methoxyphenyl)isoxazole (3j)

Yield 70%; mp 205–207 ºC; IR: 1015, 1396, 1532, 1508, 1630, 2815, 2825, 3036; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.26$ (s, 3H, –OCH_3), 6.47 (s, 1H,=CH–isoxazole), 7.87–8.12 (m, 13H, Ar–H–), 8.26 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 55.9, 98.7, 103.1, 111.8, 114.8, 116.9 (2), 117.9, 119.3 (2), 126.9, 129.1 (2), 130.8, 130.7 (2), 131.1, 131.9, 143.9, 145.7, 161.9, 162.7, 162.8, 169.3; LCMS: *m/z* 411.1 (45.0%). Anal. Calcd. For $C_{25}H_{18}FN_3O_2$: C, 72.98; H, 4.41; N, 10.21. Found: C, 73.15; H, 4.55; N, 10.36.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(4-methoxyphenyl)isoxazole (3 k)

Yield 72%; mp 200–202 ºC; IR: 1040, 1339, 1549, 1515, 1638, 2839, 2828, 3059; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.30$ (s, 3H, –OCH_3), 6.53 (s, 1H,=CH–isoxazole), 7.87–8.12 (m, 13H, Ar–H–), 8.24 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 55.1, 98.4, 103.1, 114.9 (2), 116.6 (2), 118.1, 119.7 (2), 126.8, 127.7 (2), 128.4, 129.1 (2), 130.8 (2), 131.1, 143.3, 145.1, 160.6, 162.9, 162.6, 169.7; LCMS: *m/z* 412.1 (28.4%). Anal. Cacld. For $C_{25}H_{18}FN_3O_2$: C, 72.98; H, 4.41; N, 10.21. Found: C, 73.09; H, 4.57; N, 10.28.

Physical constant and characterization of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-(*p*-tolyl)isoxazole (3 l)

Yield 70%; mp 204–206 ºC; IR: 1032, 1345, 1310, 1540, 1512, 1625, 2830, 3040; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.30$ (s, 3H, –CH_3), 6.49 (s, 1H, = CH–isoxazole), 7.36–7.69 (m, 13H, Ar–H–), 8.14 (s, 1H, = CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 21.8$, 98.6, 103.9, 116.8 (2), 119.5 (2), 123.7, 124.3 (2), 126.0, 128.6, 129.6 (2), 129.7 (2), 130.1 (2), 131.8, 131.2, 143.1, 145.3, 162.7, 162.9, 169.0; LCMS: *m/z* 396.1 (28.8%). Anal. Cacld. For $C_{25}H_{18}FN_3O$: C, 75.93; H, 4.59; N, 10.63. Found: C, 75.99; H, 4.63; N, 10.66.

Physical constant and characterization of 5-(2-chlorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3 m)

Yield 71%; mp 209–211 ºC; IR: 835, 1045, 1306, 1568, 1522, 1636, 2839, 3060; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.49$ (s, 1H,=CH–isoxazole), 7.30–8.00 (m, 13H, Ar–H–), 8.05 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.1$, 103.2, 116.8 (2), 119.6 (2), 126.3, 127.1, 128.6, 129.9, 129.7 (2), 130.7, 130.9 (2), 131.8, 132.9, 136.7, 143.2, 145.9, 162.0, 162.8, 169.6; LCMS: *m/z* 404.1 (35.5%). Anal. Cacld. For $C_{24}H_{15}ClFN_3O$: C, 68.58; H, 3.50; N, 10.43. Found: C, 68.63; H, 3.56; N, 10.49.

Physical constant and characterization of 5-(4-chlorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3n)

Yield 68%; mp 156–158 ºC; IR: 850, 1059, 1296, 1563, 1536, 1670, 2852, 3075; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.41$ (s, 1H, = CH–isoxazole), 7.24–8.10 (m, 13H, Ar–H–), 8.12 (s, 1H, = CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.7$, 103.9, 116.7 (2), 119.6 (2), 124.1, 124.9 (2), 126.0, 128.3, 129.1 (4), 130.1 (2), 131.9, 134.0, 143.0, 145.0, 162.0, 162.9, 169.0; LCMS: *m/z* 417.1 (35.7%); Anal. Cacld. For $C_{24}H_{15}ClFN_3O$: C, 69.32; H, 3.64; N, 10.10. Found: C, 69.36; H, 3.74; N, 10.16.

Physical constant and characterization of 5-(4-fluorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3o)

Yield 75%; mp 135–137 ºC; IR: 1025, 1260, 1540, 1505, 1605, 2830, 3070; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.31$ (s, 1H, = CH–isoxazole), 7.03–8.06 (m, 13H, Ar–H–), 8.30 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 97.8$, 103.4, 116.5 (4), 119.1 (2), 122.9, 126.0, 127.3 (2), 128.3, 129.6 (2), 130.0 (2), 131.1, 143.2, 145.9, 162.5, 162.9 (2), 169.0; LCMS: *m/z* 400.1 (27.3%). Anal. Cacld. For $C_{24}H_{15}F_2N_3O$: C, 72.17; H, 3.79; N, 10.52. Found: C, 72.29; H, 3.82; N, 10.59.

Physical constant and characterization of 5-(2,4-difluorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3p)

Yield 73%; mp 151–153 ºC; IR: 1035, 1269, 1549, 1509, 1608, 2830, 3045; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.21$ (s, 1H, = CH–isoxazole), 6.80–8.06 (m, 12H, Ar–H–), 8.50 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.0$, 102.1, 103.2, 116.0, 116.1 (2), 119.3, 119.6 (2), 126.0, 128.6, 129.6 (2), 130.1, 130.3 (2), 131.0, 143.9, 145.3, 159.3, 161.1, 162.7, 162.9, 169.2; LCMS: *m/z* 418.1 (25.3%). Anal. Cacld. For $C_{24}H_{14}F_3N_3O$: C, 69.06; H, 3.38; N, 10.07. Found: C, 69.10; H, 3.42; N, 10.09.

Physical constant and characterization of 5-(2,4-dichlorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3q)

Yield 64%; mp 161–163 ºC; IR: 812, 1053, 1212, 1598, 1523, 1623, 2839, 3053; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.21$ (s, 1H, = CH- isoxazole), 7.80–8.30 (m, 12H, Ar–H–), 8.69 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 98.0, 103.0, 116.3 (2), 119.3 (2), 126.4, 127.6, 128.1, 129.1 (2), 130.1, 130.8 (2), 130.8, 131.2, 133.8, 135.0, 135.1, 143.2, 145.3, 162.3, 162.9, 169.4; LCMS: *m/z* 450.1 (26.2%). Anal. Cacld. For $C_{24}H_{14}Cl_2FN_3O$: C, 64.02; H, 3.13; N, 9.33. Found: C, 64.12; H, 3.18; N, 9.38.

Physical constant and characterization of 5-(3,4-dichlorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3r)

Yield 68%; mp 207–209 ºC; IR: 855, 1049, 1219, 1590, 1512, 1623, 2850, 3053; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.28$ (s, 1H, = CH–isoxazole), 7.75–8.15 (m, 12H, Ar–H–), 8.42 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.3$, 103.1, 116.0, 119.3 (2), 123.1, 126.3, 128.1, 128.8, 129.1 (2), 130.7 (2), 130.9, 131.0, 132.6, 133.8, 143.1, 145.3, 162.9, 162.8, 169.0; LCMS: *m/z* 451.0 (63.9%). Anal. Cacld. For $C_{24}H_{14}Cl_2FN_3O$: C, 64.02; H, 3.13; N, 9.33. Found: C, 64.10; H, 3.19; N, 9.39.

Physical constant and characterization of 5-(2-bromo-4-chlorophenyl)-3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazole (3 s)

Yield 76%; mp 213–215 ºC; IR: 510, 836, 1070, 1249, 1585, 1550, 1678, 2878, 3085; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.36$ (s, 1H,=CH–isoxazole), 7.52–8.29 (m, 12H, Ar–H–), 8.50 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 98.1$, 103.1, 116.1 (2), 119.3 (2), 121.0, 126.3, 128.6, 128.7, 129.7 (2), 130.1 (2), 130.3, 131.8, 131.9, 135.5, 137.1, 143.3, 145.3, 162.8, 162.7, 169.5; LCMS: *m/z* 493.0 (75.2%). Anal. Cacld. For $C_{24}H_{14}BrClFN_3O$: C, 58.26; H, 2.85; N, 8.49. Found: C, 58.32; H, 2.96; N, 8.53.

Physical constant and characterization of 4-(3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)isoxazol-5-yl)-3-methoxyphenol (3t)

Yield 70%; mp 217–219 ºC; IR: 1064, 1248, 1522, 1536, 1645, 2835, 2010, 3074, 3350; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.86$ (s, 3H,–OCH_3), 5.39 (s, 1H,–OH), 6.78 (s, 1H,=CH–isoxazole), 6.35–8.25 (m, 12H, Ar–H–), 8.60 (s, 1H,=CH–N < pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 56.3, 98.5, 103.1, 105.3, 107.3, 108.6, 116.5 (2), 119.1 (2), 126.0, 127.0, 128.6, 129.6 (2), 130.1 (2), 131.1, 143.2, 145.9, 154.6, 158.3, 162.0, 162.8, 169.7; LCMS: *m/z* 428.1 (28.5%). Anal. Cacld. For $C_{25}H_{18}FN_3O_3$: C, 70.25; H, 4.24; N, 9.83. Found: C, 70.29; H, 4.30; N, 9.93.

Results and discussion

Chemistry

The need of the hour is to develop simple yet effective methodologies in organic reactions. Organic chemists are constantly working to find newer routes for the synthesis of bioactive molecules. In continuation, a mixture of 3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(1)** and derivatives of acetophenone, in the same ratio, was stirred in methanolic potassium hydroxide solution for 4 h at room temperature. With the help of the Claisen–Schmidt condensation reaction, chalcones were produced **(2a-t)**. Then, chalcones and hydroxylamine hydrochloride (0.03 mol) were refluxed for 15 h at 120°C. This simple condensation reaction produced final compounds **(3a-t)**.

Characterization of newly synthesized compounds of the series was carried out by IR, NMR, and mass spectra, and the data with a detailed discussion are given in the experimental section.

IR spectrum of compound **3c** (molecular formula $C_{24}H_{15}FN_4O_3$, M.W. 426.41 gm/mol) shows an absorption band at 1025 cm^{-1} due to halogen group -C-F stretching. Compound **3c** has a stretching vibration at 1400 cm^{-1} indicating the presence of > C–N stretching corresponding to cyano group. 1550 cm^{-1} confirms the presence of the nitro group at the para position. Stretching vibration at 1545 and 1585 cm^{-1} is an indication of the presence of –C=N, –C=C stretching of the aromatic ring. The absorption band at 1660 cm^{-1} indicates the presence of –C=O stretching due to isoxazole ring. > C–H stretching vibrations at 2828 and 3045 cm^{-1} show the presence of aromatic ring.

The ^{1}H-NMR spectra of the final compound **3c** showed that the presence of singlet peak at δ = 8.63 ppm was due to one proton of pyrazole ring (C-12). One proton attached at isoxazole ring (C-2) gave a singlet peak at $\delta = 6.75$ ppm. In 7.29–8.15 ppm range, multiplet peaks appeared due to thirteen protons of the aromatic ring.

The chemical shifts of the compound **3c** have carbons that ranged from $\delta = 169.1$–98.1 ppm. Carbon (C-9) attached to the nitro (Ar–NO_2) group has a chemical shift at $\delta = 147.4$ ppm. The carbon-21 attached with fluoro group has a chemical shift value at $\delta = 162.1$ ppm. The carbons of the pyrazole ring of carbon C-12, C-13, and C-14 showed chemical shifts at $\delta = 131.9$, 103.3, and 145.2 ppm, respectively. The carbons of isoxazole ring, C-1, C-2, and C-3, confirmed with the value of the chemical shift at d = 162.8, 98.1, and 169.1 ppm, respectively. Aromatic carbons showed a chemical shift value between 116.8 and 143.8.

Discussion on antibacterial activity

With the intention of developing potential antibacterial agents, we synthesized twenty hybrid bioactive molecules. These compounds were screened for their antibacterial activity against gram-positive and gram-negative bacteria by Broth dilution method. For comparison of activity, we used ampicillin and chloramphenicol as

standard drugs. Details of bacterial strains, MTCC numbers, and antibacterial activity data are depicted in Table 1. Some of the compounds showed minimum inhibitory concentration (MIC) value less than the standard drug, while some compounds showed similar values with the standard drug. Compound **3c** displayed excellent activity towards *S. pyogenes* at MIC 12.5 µg/mL. Compound **3r** found very good towards *S. aureus*, while compound **3o** showed good results towards *E. coli* and *S. aureus.*

Molecular docking

With the increasing number and accuracy of X-ray crystal structures of target proteins, molecular docking has emerged as an important tool for the synthetic elaboration of novel therapeutics based on chemical scaffolds [17]. Therefore, to elucidate the plausible mechanism by which these novel isoxazole analogs could exhibit the antibacterial activity and to understand the SAR based on experimental findings, molecular docking against *DNA gyrase subunit b* (PDB ID:1KZN) was performed using the standard protocol implemented in GLIDE (Grid-Based Ligand Docking with Energetics) module of the Small Drug Discovery Suite (Schrödinger, LLC, New York, NY) [18–20]. *DNA gyrase*, encoded by the *gyrB gene*, catalyses the ATP-dependent negative supercoiling of double-stranded closed-circular DNA. Inhibition of this type II topoisomerase enzyme blocks the relaxation of supercoiled DNA, required for transcription and replication, resulting in disruption of DNA synthesis and, consequently, cell cycle arrest. Being exclusive to prokaryotes and essential across bacterial species for the survival of the organism qualifies this enzyme as an attractive target for antibacterial drugs.

Molecular docking showed that the docked conformations of all the heterocyclic analogues **(3a-3t)** could bind within the active site of *DNA gyrase* with varying affinities (Table 1) at coordinates proximity to the native structure (Chlorobiocin, docking score: −9.902) through the formation of various bonded and non-bonded interactions. An investigation into the per-residue interactions was also performed to identify the most significant thermodynamic interactions. This analysis is elaborated for the most active compound **3c** and summarized in Table 2 for other active compounds (**3o** and **3r**). The lowest energy docked conformation of **3c** (Fig. 2a) was observed to be snugly fitting into the active site of DNA gyrase with a significantly higher binding affinity (Glide score: −8.698, Glide energy: −50.489 kcal/mol). The compound is seen to be stabilized within the active site through an extensive network of favourable van der Waals (steric) interactions with Arg136, Pro79, Gly77, Arg76, and Glu50 through the 4-nitro phenylisoxazoles scaffold, while the other half of the molecule, i.e. 3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl scaffold, engaged in a similar type of favourable interactions with Val167, Thr165, Val120, Ile90, Ile78, Val71, Ala47, Asn46, and Val43 residues. Furthermore, the higher binding affinity of **3c** is also attributed to significant electrostatic (Columbic) interactions with Arg136, Gly77, Asp73, Glu50, Asp49, and Asn46 residues of the active site. This balanced network of steric and electrostatic interactions with the active site residues was observed for other active molecules **(3o—**Fig. 2b and **3r—**Fig. 2c) as

Table 1 Results of biological activities of compounds (3a-t)

Sr. No	–R	Minimum inhibitory concentrations(MIC) in µg/mL				Glide docking score
		E. c	*P. a*	*S. a*	*S. p*	
3a	–H	250	250	250	250	−6.177
3b	–3–NO_2	250	100	100	500	−7.627
3c	–4–NO_2	500	125	62.5	12.5	−8.698
3d	–3–Br	500	200	100	200	−7.499
3e	–4–Br	250	250	200	250	−6.353
3f	–2–OH	200	200	200	500	−6.548
3 g	–3–OH	125	125	250	250	−7.024
3 h	–4–OH	200	200	250	100	−7.553
3i	–2–OCH_3	250	250	100	250	−7.442
3j	–3–OCH_3	100	100	200	200	−7.655
3 k	–4–OCH_3	100	250	250	250	−7.483
3 l	–4–CH_3	200	250	200	250	−6.478
3 m	–2–Cl	125	250	250	200	−6.983
3n	–4–Cl	200	100	250	250	−7.558
3o	–4–F	50	100	50	500	−8.584
3p	–2,4-F_2	125	200	200	250	−6.957
3q	–2,4–Cl_2	200	250	250	100	−7.493
3r	–3,4–Cl_2	500	500	25	200	−8.565
3 s	–2–Br–4–Cl	500	250	125	200	−6.864
3t	–4–OH–3–OCH_3	200	250	200	250	−6.464
Ampicillin	100	100	250	100	–	–
Chloramphenicol	50	50	50	50	–	–

E. c.—Escherichia coli (MTCC-443), *P. a.—Pseudomonas aeruginosa* (MTCC-1688), *S. a.—Staphylococcus aureus* (MTCC-96), *S. p.—Streptococcus pyogenes* (MTCC-442)

well. Furthermore, all three actives, i.e. **3c, 3o,** and **3r**, exhibited a prominent pi–pi (π–π) stacking interactions with Arg76 residue (3c: 2.492 Å, 3o: 2.496 Å, 3r: 2.66 Å) through the aromatic ring attached to the isoxazole heterocycle which serves as "anchor" for binding of the ligand to the active site of an enzyme and facilitates the steric and electrostatic interactions. Overall this in silico binding study suggests that these novel heterocycles possess an excellent affinity for the *DNA gyrase* qualifying them for structure-based optimization to improve the affinity and selectivity. Glide docking scores of ampicillin and chloramphenicol are −4.426 and −5.769, respectively (Scheme 1).

Structure–activity relationship

An overview of the SAR showed that the antimicrobial activity is highly impacted by the variation of substituents on the aromatic ring of compounds **(3a-t)**. The

Table 2 The per-residue interaction analysis based on docking study for novel 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-arylisoxazole analogues with *DNA gyrase subunit b*

Code	Docking score	Glide interaction energy (kcal/mole)	Per-residues interactions		
			Van der Waals (kcal/mol)	Coulombic (kcal/mol)	Pi–Pi stacking (Å)
3c	−8.698	−50.489	Val167(−2.519), Thr165(−4.066), Arg136(−2.749), Val120(−1.892), Ile90(−1.863), Pro79(−3.112), Ile78(−6.177), Gly77(−1.956), Arg76(−4.75), Val71(−1.827), Glu50(−4.662), Ala47(−2.135), Asn46(−5.436), Val43(−1.467)	Arg136(−2.168), Gly77(−1.872), Asp73(−4.095), Glu50(−1.887), Asp49(-−1.921), Asn46(−1.558)	Arg76(2.492)
3o	−8.584	−48.481	Val167(−1.964), Thr165(−3.901), Arg136(−1.888), Val120(−1.654), Ile90(−1.472), Pro79(−2.927), Ile78(−6.087), Gly77(−1.934), Arg76(−4.455), Val71(−1.809), Glu50(−4.313), Ala47(−2.289), Asn46(−5.248), Val43(−1.445)	Arg136(−1.745), Gly77(−1.729), Asp73(−3.826), Glu50(−1.566), Asp49(−1.211), Asn46(−1.463)	Arg76(2.496)
3r	−8.565	−48.362	Val167(−1.826), Thr165(−3.881), Arg136(−1.791), Val120(−1.553), Ile90(−1.559), Pro79(−2.637), Ile78(−6.011), Gly77(−1.905), Arg76(−4.340), Val71(−1.805), Glu50(−4.286), Ala47(−2.083), Asn46(−5.187), Val43(−1.439)	Arg136(−1.689), Gly77(−1.621), Asp73(−3.415), Glu50(−1.528), Asp49(−1.010), Asn46(−1.367)	Arg76(2.66)

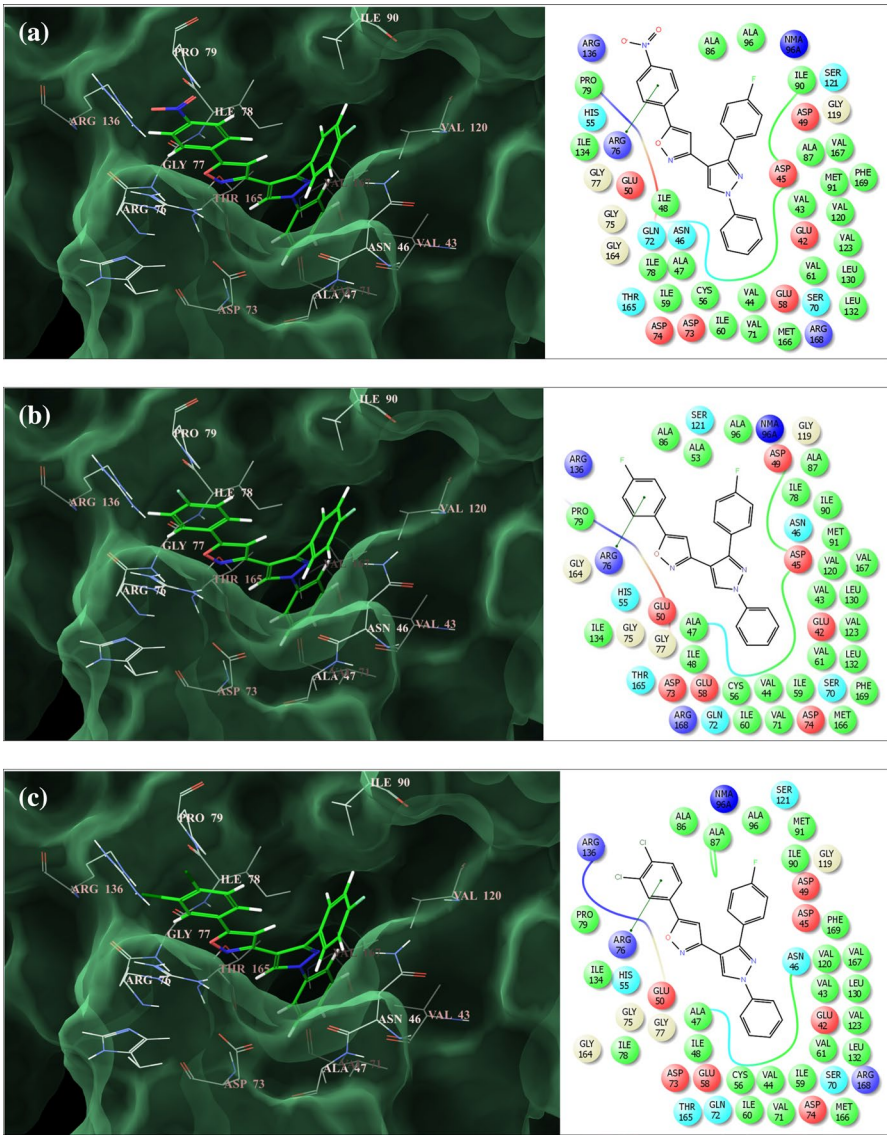


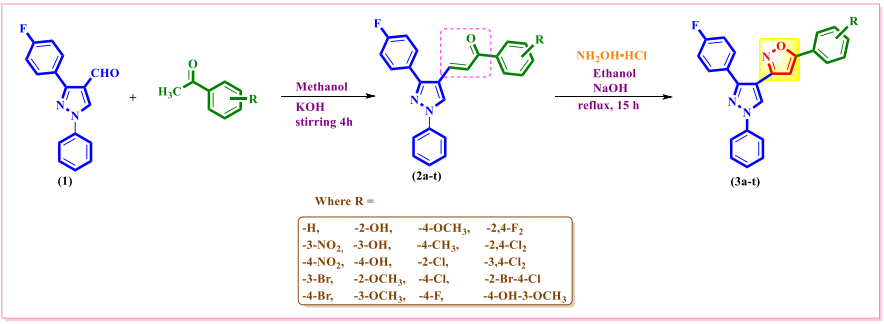
Fig. 2 **a** Binding mode of **3c** into the active site of *DNA gyrase subunit b*. (On right side, green lines signify pi–pi stacking interactions.) **b** Binding mode of **3o** into the active site of *DNA gyrase subunit b* (on right side: green lines signify pi–pi stacking interactions). **c** Binding mode of **3r** into the active site of *DNA gyrase subunit b* (on right side, green lines signify pi–pi stacking interactions). (Color figure online)

introduction of electron-withdrawing group at aromatic ring increased antibacterial potency of the compounds. Nitro group at *para* position, containing compound **3c** , showed maximum efficacy against *S. pyogenes,* i.e. inhibition at MIC 12.5 µg/mL which is also supported by the significantly higher binding affinity (Glide docking

score -8.698). Substitution of halogen (Fluoro) at 4th position (compound **3o**) of the substituted benzene ring also produced very good activity against *E. coli* and *S. aureus* (MIC 50 µg/mL and a docking score of −8.584). Furthermore, di-halogen substitution (**3r:–**3,4–Cl_2 at *meta* and *para* position) also incremented the activity moderately against *S. aureus* at MIC 25 µg/mL (docking score of −8.565).

Conclusion

Preparation of 3-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-arylisoxazoles was associated with the achievement of fine yield. Results of biological activity revealed that the electronic environment has a noteworthy impact on the antibacterial activity of the final compounds. Out of twenty reported bioactive compounds, **3c** (–4–NO_2), **3o** (–4–F), and **3r** (–3,4–Cl_2) displayed in vitro antibacterial activity. Antibacterial screening revealed that electron-withdrawing groups present in the product have potent activity towards bacterial strains. Furthermore, in silico binding affinity data against DNA gyrase could provide an insight into the binding mode and the associated thermodynamic interactions. The outcome will help in identifying more potent analogs in the coming times.



Scheme 1 Synthetic pathway of reported compounds (3a-t)

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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