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Design, Synthesis and Characterisation of New Trifluoroethyl Dihydroquinoxaline Carboxylate Amino Derivatives and Evaluation of their Biological Activities

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In this work, a series of novel 2,2,2-trifluoroethyl-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate amino derivatives (**10a-v**) was synthesised and their structures were confirmed by ^{1}H NMR, IR and mass spectral analysis. The synthesised quinoxaline derivatives were screened for antibacterial and anticancer activity. The antibacterial activity results showed that derivatives **10c**, **10e**, **10f**, **10h**, **10n**, **10o**, **10p**, **10t** and **10u** had excellent antibacterial activity against all the tested bacterial strains, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The anticancer activity of the derivatives was evaluated against two human cancer cell lines, MCF-7 and HepG-2. The results showed that derivative **10m** exhibits more potent activity compared to the standard drug erlotinib, while the remaining four compounds, **10i**, **10j**, **10k** and **10l**, show activity almost comparable to that of the standard drug. The molecular docking studies also support the *in vitro* anticancer activity results.

Keywords: Trifluoroethyldihydroquinoxaline-carboxylate amino derivatives, Biological activities, Molecular docking.

INTRODUCTION

Dihydroquinoxalines are a class of synthetic organic heterocyclic compounds consist of a six-membered ring [1]. Moreover, in recent years, quinoxaline ring have been grabbing the attention of synthetic chemists for their multifaceted pharmacological and medicinal applications [2]. Reports confirmed that quinoxalines, in particular display wide range of biological activities such as antimicrobial and antiviral [3] (Hepatitis-B), antibacterial [4-7], anticancer [8] activities and various precursers crystal studies [9,10].

The increasing prevalence of antimicrobial resistance has intensified the search for novel and more effective antimicrobial agents. Quinoxaline is an important heterocyclic scaffold with a wide range of pharmacological activities including antimicrobial and anticancer effects [11-13]. Several quinoxaline-based compounds have exhibited promising antitumor activity, and drug candidates such as CQS (4-amino-*N*-(5-chloroquinoxalin-2-yl)benzenesulphonamide) and XK-469 [2-[4-(7-chloroquinoxalin-2-yl)oxyphenoxy]propanoic acid] have advanced to clinical evaluation, which reflects the therapeutic potential of this scaffold [14,15]. In present study, new quinoxaline derivatives were synthesised and evaluated for their *in vitro* anticancer and antimicrobial activities. Incorporation of fluorinated substituents, particularly the 2,2,2-trifluoroethyl group, is known to improve hydrophobic interactions, lipophilicity, metabolic stability and electronic properties, which may enhance target binding, pharmacokinetic behaviour and EGFR inhibitory activity [14-18]. The biological performance of these derivatives depends on the molecular architecture and substitution pattern. Despite extensive studies on quinoxaline-based EGFR inhibitors, the incorporation of a 2,2,2-trifluoroethyl moiety into the quinoxaline framework has received little attention.

The limited exploration of 2,2,2-trifluoroethyl-substituted quinoxaline derivatives provided the basis for the present investigation. A series of trifluoroethyl-dihydroquinoxaline-6-carboxylate derivatives bearing structurally diverse amino and

hydrazinyl substituents at the C-3 position was synthesised and characterised. The synthesised compounds were subsequently evaluated for their *in vitro* antibacterial and anticancer activities.

EXPERIMENTAL

All chemicals were used as received without further purification. The purity of the synthesised compounds was monitored by thin-layer chromatography (TLC) using Merck 60F254 silica gel TLC plates. Melting points were obtained on a hot stage melting point apparatus Ernst Leitz Wetzlar, Germany and are uncorrected. The ^{1}H NMR recorded using Mercury plus spectrometer (operating at 400 MHz for ^{1}H NMR) and chemical shifts referenced to TMS. The ESI mass spectra at an ionising voltage of 70 eV obtained using Shimadzu QP5050A quadrupole-mass spectrometer.

Synthesis of 4-(methylamino)-3-nitrobenzoic acid (2): To a solution of 4-chloro-3-nitrobenzoic acid (**1**, 100 g, 0.510 mol) in methanol (500 mL), triethylamine (390 mL, 2.976 mol) was added to methylamine hydrochloride (97.5 g, 1.488 mol) and stirred at 60 ºC in an autoclave for 12 h. The progress of the reaction was monitored by TLC. The reaction mass was concentrated completely under vacuum to get the crude compound, water (200 mL) was added and it was extracted with dichloro-methane (2 × 500 mL). The organic layer was dried over sodium sulphate and the organic layer was concentrated under reduced pressure to afford the pure compound as a yellow solid. Yield: 85%; m.p.: 195.2-196.1 ºC, yellow solid, IR (KBr, ν_{max}, cm^{-1}): 3417 for (-OH/-NH), 3101 for aromatic (-CH), 1717 (C=O), 1357 for (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.513-8.518 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.175-8.201 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.925-7.946 (d, *J* = 8.4 Hz, 1H, Ar-H), 3.907 (s, 3H, -CH_3); Mass (*m/z*): 197.0 (M+1).

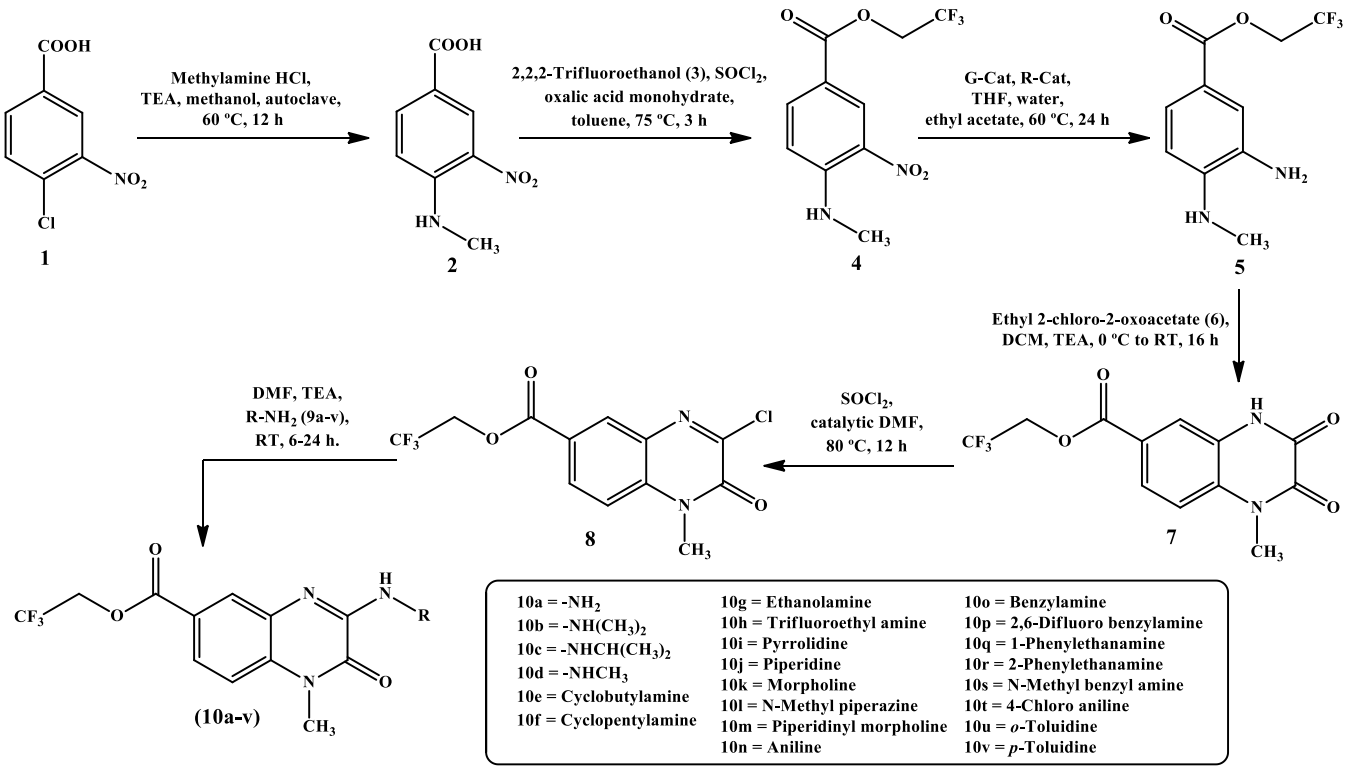
Synthesis of 2,2,2-trifluoroethyl 4-(methylamino)-3-nitrobenzoate (4): To a solution of compound **2** (100 g, 0.510 mol) in toluene (500 mL), thionyl chloride (1.275 mol) was added and stirred at 75 ºC for 3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was concentrated under vacuum pressure, then 2,2,2-trifluoroethanol (**3**) (350 mL) was added and stirred at room temperature for 3 h. Again, the progress of the reaction was monitored with TLC, the reaction mass was quenched with water (200 mL) and it was extracted with ethyl acetate (2 × 200 mL). The organic layer was washed with a 10% aq. $NaHCO_3$ solution and dried over sodium sulphate and the organic layer was concentrated under reduced pressure to afford the pure compound as a yellow solid. Yield: 98%; m.p.: 114.2-118.3 ºC, yellow solid. IR (KBr, ν_{max}, cm^{-1}): 3384 (-NH), 3107 (-CH, arom.), 1722 (C=O), 1331 (NO_2), 1167 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.670-8.705 (q, 1H, NH, D_2O exchangeable), 8.638-8.643 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.985-8.012 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.097-7.120 (d, *J* = 9.2 Hz, 1H, Ar-H), 4.935-5.003 (q, 2H, C$\underline{H_2}$-CF_3), 3.014-3.027 (d, 3H, NH-C$\underline{H_3}$); Mass (*m/z*): 276.8 (M-1)

Synthesis of 2,2,2-trifluoroethyl 3-amino-4-(methylamino) benzoate (5): To a solution of water (5.0 vol) and G-cat (3.0 times) at 75 ºC, compound **4** (100 g, 0.359 mol) in ethyl acetate (10.0 vol) was added and stirred at 60 ºC for 24 h. The progress of the reaction was monitored with TLC. The reaction mass was cooled and then R-cat (0.1 times) was added and stirred at 60 ºC for 30 min. Then the reaction mass was filtered and washed with excess water. The filtrate was extracted with ethyl acetate and the organic layer was concentrated under reduced pressure to afford the crude compound, which was isolated using MTBE at 50 ºC to afford the pure compound as a brown solid. Yield: 90%; m.p.: 98.1-101.4 ºC, IR (KBr, ν_{max}, cm^{-1}): 3424 (-NH), 2970 (-CH arom), 1697 (C=O), 1169 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.272-7.297 (dd, *J* =2.0 Hz, 1H, Ar-H), 7.177-7.182 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.418-6.439 (d, *J* = 8.4 Hz, 1H, Ar-H), 5.572-5.604 (q, 1H, NH, D_2O exchangeable), 4.816-4.885 (q, 2H, C$\underline{H_2}$-CF_3), 4.771 (br, s, 2H, -NH_2, D_2O exchangeable), 2.784-2.796 (d, 3H, NH-C$\underline{H_3}$); Mass (*m/z*): 248.4 (M+1).

Synthesis of 2,2,2-trifluoroethyl-1-methyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (7): To a solution of compound **5** (75 g, 0.302 mol) in dichloromethane (10.0 vol) and triethylamine (0.906 mol), ethyl-2-chloro-2-oxoacetate (**6**) (0.513 mol) was added at 0 ºC and the reaction mass was stirred for 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was quenched with ice-cold water (5.0 vol), stirred for 30 min and filtered under reduced pressure to afford the pure compound as an off-white solid. Yield: 95%; m.p.: >250 ºC, IR (KBr, ν_{max}, cm^{-1}): 3361 (-NH), 3137 (-CH arom), 1744, 1682 (C=O), 1168 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.148 (s, 1H, NH, D_2O exchangeable), 7.836-7.841 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.771-7.797 (dd, *J* = 1.6 Hz, 1H, Ar-H), 7.497-7.519 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.980-5.048 (q, 2H, C$\underline{H_2}$-CF_3), 3.537 (s, 3H, N-C$\underline{H_3}$); Mass (*m/z*): 302.4 (M+1).

Synthesis of 2,2,2-trifluoroethyl-3-chloro-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (8): In the reaction vessel, compound **7** (50 g, 0.165 mol) and thionyl chloride (5.0 vol) were added, followed by catalytic DMF (0.5 vol) and refluxed for 12 h. The reaction was monitored by TLC, the reaction mass was quenched with ice-cold water and extracted with dichloromethane (2 × 200 mL), the organic layer was washed with 10% aq. $NaHCO_3$ solution and dried over sodium sulphate and the organic layer was concentrated under reduced pressure to afford the pure compound as an off-white solid. Yield: 98%; m.p.: 146.4-149.6 ºC, IR (KBr, ν_{max}, cm^{-1}): 3066 (-CH aom), 1731, 1668 (C=O), 1162 (C-F), 760 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.255-8.260 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.184-8.211 (dd, *J* = 10.8 Hz, 1H, Ar-H), 7.760-7.782 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.016-5.084 (q, 2H, C$\underline{H_2}$-CF_3), 3.690 (s, 3H, N-C$\underline{H_3}$); Mass (*m/z*): 321.0 (M+1).

General procedure for the synthesis of trifluoroethyl-dihydroquinoxaline-carboxylate amino derivatives (10a-v): In the reaction vessel, compound **8** (1.0 g, 0.003 mol) was dissolved in DMF (10.0 vol) and triethylamine (0.009 mol) was added, followed by the corresponding amines (**9a-v**) (0.0045 mol) and stirred at room temperature for 12 h. The progress was monitored with TLC. After completion of the reaction, it was quenched with water (10.0 mL) and the solid was filtered under reduced pressure to obtain the pure compounds **(10a-v),** respectively (**Scheme-I**).



Scheme-I: Synthesis of 2,2,2-trifluoroethyl-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate amino derivatives (**10a-v**)

2,2,2-Trifluoroethyl 3-amino-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10a): Yield: 96%; m.p.: 213.0-220.8 ºC, white solid; anal. calcd. (found) % for $C_{12}H_{10}F_3N_3O_3$: C, 47.85 (47.84); H, 3.35 (3.34); N, 13.95 (13.92); IR (KBr, ν_{max}, cm^{-1}): 3463 (-NH), 3139 (-CH arom), 1723, 1660 (C=O), 1176 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.858-7.863 (d, *J* = 2.0Hz, 1H, Ar-H), 7.772-7.798 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.495-7.517 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.360 (br, 2H, -NH_2, D_2O exchangeable), 4.967-5.034 (q, 2H, CH_2), 3.645 (s, 3H, CH_3); Mass (*m/z*): 302.1 (M+1).

2,2,2-Trifluoroethyl 3-(dimethylamino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6carboxylate (10b): Yield: 85%; m.p.: 158.9-164.8 ºC, white solid; anal. calcd. (found) % for $C_{14}H_{14}F_3N_3O_3$: C, 51.07 (51.04); H, 4.29 (4.27); N, 12.76 (12.75); IR (KBr, ν_{max}, cm^{-1}): 3082 (-CH arom), 2976, 2939 (-CH aliph.), 1729, 1642 (C=O), 1287 (C-N), 1170 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.911-7.916 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.775-7.801 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.476-7.497 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.971-5.039 (q, 2H, CH_2), 3.318 (s, 3H, CH_3), 3.096-3.11 (d, 6H, 2 × CH_3); Mass (*m/z*): 330.1 (M+1).

2,2,2-Trifluoroethyl 3-(isopropylamino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10c): Yield: 80%; m.p.: 169.3-185.1 ºC, white solid; anal. calcd. (found) % for $C_{15}H_{16}F_3N_3O_3$: C, 52.48 (52.46); H, 4.70 (4.67); N, 12.24 (12.23); IR (KBr, ν_{max}, cm^{-1}): 3415 (-NH), 3119 (-CH), 2973 (-CH), 1664 and 1623 (C=O), 1209 (C-N), 1171 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.896-7.901 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.752-7.779 (dd, *J* = 8.8 Hz, 1H, Ar-H), 7.479-7.501 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.996-5.033 (q, 2H, CH_2), 4.232-4.318 (m, 1H, -CH), 3.631 (s, 3H, CH_3), 1.223-1.239 (d, 6H, 2 × CH_3); Mass (*m/z*): 344.2 (M+1).

2,2,2-Trifluoroethyl1-methyl-3-(methylamino)-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10d): Yield: 70%; m.p.: 149.3-156.1 ºC, off-white solid; anal. calcd. (found) % for $C_{13}H_{12}F_3N_3O_3$: C, 49.53 (49.52); H, 3.84 (3.83); N, 13.33 (13.31); IR (KBr, ν_{max}, cm^{-1}): 3463 (-NH), 3180, 3139 (-CH), 2974 (-CH), 1723, 1660 (C=O), 1284 (C-N), 1176 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.858-7.863 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.772-7.798 (dd, *J* = 8.4 Hz, 1H, Ar-H), 7.495-7.517 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.967-5.034 (q, 2H, CH_2), 3.645 (s, 3H, CH_3), 3.048 (s, 3H, CH_3); Mass (*m/z*): 316.2 (M+1).

2,2,2-Trifluoroethyl-3-(cyclobutylamino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10e): Yield: 55%, light brown semi-solid; anal. calcd. (found) % for $C_{16}H_{16}F_3N_3O_3$: C, 54.09 (54.06); H, 4.54 (4.52); N, 11.83 (11.82); IR (KBr, ν_{max}, cm^{-1}): 3424 (-NH), 2968 (-CH), 2865 (-CH), 1715, 1662 (C=O), 1329 (C-N), 1166 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.009-8.028 (d, 1H, -NH, D_2O exchangeable), 7.907-7.912 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.769-7.796 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.496-7.517 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.972-5.040 (q, 2H, CH_2), 3.637 (s, 3H, -CH_3), 2.116-2.306 (m, 4H, 2 × CH_2), 1.657-1.736 (m, 2H, CH_2); Mass (*m/z*): 356.1 $(M+1)^+$.

2,2,2-Trifluoroethyl 3-(cyclopentylamino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10f): Yield: 62%, light brown semi-solid; anal. calcd. (found) % for $C_{17}H_{18}F_3N_3O_3$: C, 55.28 (55.27); H, 4.91 (4.90); N, 11.38 (11.36); IR (KBr, ν_{max}, cm^{-1}): 3371 (-NH), 3011 (-CH), 2971, 2868 (-CH), 1731, 1654 (C=O), 1277 (C-N), 1174 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.912-7.917 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.764-7.790 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.582-

7.601 (d, 1H, -NH, D_2O exchangeable), 7.494-7.515 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.971-5.038 (q, 2H, CH_2), 4.320-4.408 (m, 1H, -CH), 3.637 (s, 3H, $-CH_3$), 1.921-2.002 (m, 2H, CH_2), 1.528-1.718 (m, 2H, 2 × CH_2); Mass (*m/z*): 370.1 $(M+1)^+$.

2,2,2-Trifluoroethyl-3-((2-hydroxyethyl)amino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10g): Yield: 40%; m.p.: 233.3-239.1 ºC, light brown solid; anal. calcd. (found) % for $C_{14}H_{14}F_3N_3O_4$: C, 48.70 (48.69); H, 4.09 (4.08); N, 12.17 (12.15); IR (KBr, ν_{max}, cm^{-1}): 3361 (-NH), 3051 (-CH), 2960 (-CH), 1731, 1677 (C=O), 1256 (C-N), 1154 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.928-7.948 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.520-7.801 (m, *J* = 6.8 Hz, 2H, Ar-H), 4.977-5.038 (q, 2H, CH_2), 4.822 (br, 1H, -NH, D_2O exchangeable), 3.501-3.651 (m, 4H, 2 × CH_2), 2.888 (s, 3H, $-CH_3$); Mass (*m/z*): 346.1 $(M+1)^+$.

2,2,2-Trifluoroethyl1-methyl-2-oxo-3-((2,2,2-trifluoroethyl)amino)-1,2-dihydroquinoxaline-6-carboxylate (10h): Yield: 68%; m.p.: 216.4-221.2 ºC, off-white solid; anal. calcd. (found) % for $C_{14}H_{11}F_6N_3O_3$: C, 43.88 (43.87); H, 2.89 (2.86); N, 10.96 (10.95).IR (KBr, ν_{max}, cm^{-1}): 3328 (-NH), 3070 (-CH), 2962 (-CH), 1739, 1657 (C=O), 1227 (C-N), 1160 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.311-8.342 (t, 1H, -NH, D_2O exchangeable), 7.979 (s, 1H, Ar-H), 7.862-7.884 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.575-7.597 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.984-5.051 (q, 2H, CH_2), 4.200-4.284 (m, 4H, 2 × CH_2), 3.672 (s, 3H, CH_3); Mass (*m/z*): 381.9 (M-1).

2,2,2-Trifluoroethyl 1-methyl-2-oxo-3-(pyrrolidin-1-yl)-1,2-dihydroquinoxaline-6-carboxylate (10i): Yield: 69%; m.p.: 186.5-1898 ºC, white solid; anal. calcd. (found) % for $C_{16}H_{16}F_3N_3O_3$: C, 54.09 (54.08); H, 4.54 (4.52); N, 11.83 (11.81); IR (KBr, ν_{max}, cm^{-1}): 2971 (-CH arom), 2872 (-CH aliph.), 1738 (C=O), 1258 (C-N), 1178 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.855-7.859 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.724-7.750 (dd, *J* = 1.6 Hz, 1H, Ar-H), 7.434-7.455 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.963-5.031 (q, 2H, CH_2), 4.036 (br, 4H, 2 × CH_2), 3.576 (s, 3H, CH_3), 1.882 (br, 4H, 2 × CH_2); Mass (*m/z*): 356.1 (M+1).

2,2,2-Trifluoroethyl1-methyl-2-oxo-3-(piperidin-1-yl)-1,2-dihydroquinoxaline-6-carboxylate (10j): Yield: 86%; m.p.: 127.9-133.0 ºC, white solid; anal. calcd. (found) % for $C_{17}H_{18}F_3N_3O_3$: C, 55.28 (55.26); H, 4.91 (4.90); N, 11.38 (11.36); IR (KBr, ν_{max}, cm^{-1}): 3053 (-CH), 2941, 2861 (-CH), 1729, 1650 (C=O), 1273 (C-N), 1167 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 7.923 (s, 1H, Ar-H), 7.802-7.823 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.492-7.513 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.972-5.040 (q, 2H, CH_2), 3.882 (br, 4H, 2 × CH_2), 3.601 (s, 3H, CH_3), 1.631 (br, 6H, 3 × CH_2); Mass (*m/z*): 370.2 (M+1).

2,2,2-Trifluoroethyl-1-methyl-3-morpholino-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10k): Yield: 96%; m.p.: 203.2-207.1 ºC, off-white solid; anal. calcd. (found) % for $C_{16}H_{16}F_3N_3O_4$: C, 51.76 (51.76); H, 4.34 (4.34); N, 11.32 (11.30); IR (KBr, ν_{max}, cm^{-1}): 3034 (-CH), 2992, 2858 (-CH), 1728, 1650 (C=O), 1230 (C-N), 1161 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.956-7.960 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.839-7.865 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.531-7.553 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.976-5.044 (q, 2H, CH_2), 3.900-3.922 (m, 4H, 2 × CH_2), 3.704-3.727 (m, 4H, 2 × CH_2), 3.611 (s, 3H, $-CH_3$); Mass (*m/z*): 372.1 (M+1).

2,2,2-Trifluoroethyl1-methyl-3-(4-methylpiperazin-1-yl)-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10l): Yield: 89%; m.p.: 196.3-200.4 ºC, off-white solid; anal. calcd. (found) % for $C_{17}H_{19}F_3N_4O_3$: C, 53.12 (53.12); H, 4.98 (4.97); N, 14.58 (14.56); IR (KBr, ν_{max}, cm^{-1}): 3045 (-CH), 2947, 2844 (-CH), 1723, 1669 (C=O), 1249 (C-N), 1159 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.956-7.961 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.834-7.860 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.529-7.551 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.977-5.045 (q, 2H, CH_2), 3.906 (m, 4H, 2 × CH_2), 3.614 (s, 3H, $-CH_3$), 2.423-2.444 (m, 4H, 2 × CH_2), 2.207 (s, 3H, $-CH_3$); Mass (*m/z*): 385.1 (M+1).

2,2,2-Trifluoroethyl1-methyl-3-(4-morpholinopiperidin-1-yl)-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10m): Yield: 92%; m.p.: 155.4-165.8 ºC, off-white solid; anal. calcd. (found) % for $C_{21}H_{25}F_3N_4O_4$: C, 55.50 (55.49); H, 5.55 (5.54); N, 12.33 (12.31); IR (KBr, ν_{max}, cm^{-1}): 3044 (-CH arom.), 2953 (-CH aliph.), 1731, 1648 (C=O), 1271 (C-N), 1119 (C-O-C), 1176 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.935-7.939 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.814-7.840 (dd, *J* = 1.6 Hz, 1H, Ar-H), 7.506-7.528 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.975-5.043 (q, 2H, CH_2), 4.847-4.878 (m, 2H, CH_2), 3.605 (s, 3H, $-CH_3$), 3.646-3.566 (m, 4H, 2 × CH_2), 2.944-3.003 (m, 2H, CH_2), 2.467-2.477 (m, 6H, 3 × CH_2), 1.420-1.892 (m, 4H, 2 × CH_2); Mass (*m/z*): 455.2 (M+1).

2,2,2-Trifluoroethyl-1-methyl-2-oxo-3-(phenylamino)-1,2-dihydroquinoxaline-6-carboxylate (10n): Yield: 94%; m.p.: >250 ºC, off-white solid; anal. calcd. (found) % for $C_{18}H_{14}F_3N_3O_3$: C, 57.30 (57.29); H, 3.74 (3.73); N, 11.14 (11.12); IR (KBr, ν_{max}, cm^{-1}): 3311 (-NH), 3063 (-CH arom.), 2948 (-CH aliph.), 1668 (C=O), 1283 (C-N), 1172 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.646 (s, 1H, -NH, D_2O exchangeable), 8.134-8.154 (d, *J* = 8.0 Hz, 2H, 2 × Ar-H), 8.069-8.074 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.895-7.922 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.607-7.629 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.373-7.413 (t, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.070-7.107 (t, *J* = 7.2 Hz, 1H, Ar-H), 5.007-5.075 (q, 2H, CH_2), 3.724 (s, 3H, CH_3); Mass (*m/z*): 378.1 (M+1).

2,2,2-Trifluoroethyl-3-(benzylamino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10o): Yield: 83%; m.p.: 190.4-216.2 ºC, off-white solid; anal. calcd. (found) % for $C_{19}H_{16}F_3N_3O_3$: C, 58.31 (58.30); H, 4.12 (4.10); N, 10.74 (10.73); IR (KBr, ν_{max}, cm^{-1}): 3322 (-NH), 3029 (-CH), 2947 (-CH), 1741, 1665 (C=O), 1298 (C-N), 1168 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.417-8.449 (t, 1H, -NH, D_2O exchangeable), 7.882-7.887 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.776-7.802 (dd, *J* = 2.0 Hz, 1H, Ar-H), 7.510-7.532 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.371-7.389 (d, *J* = 7.2 Hz, 2H, 2 × Ar-H), 7.284-7.322 (t, *J* = 8.0 Hz, 2H, 2 × Ar-H), 7.197-7.233 (t, *J* = 7.2 Hz, 1H, Ar-H), 4.964-5.032 (q, *J* = 6.4 Hz, 2H, CH_2), 4.625-4.641 (q, 2H, CH_2), 3.656 (s, 3H, CH_3); Mass (*m/z*): 392.1 (M+1).

2,2,2-Trifluoroethyl-3-((2,6-difluorobenzyl)amino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10p): Yield: 66%; m.p.: 184.2-198.5 ºC, white solid; anal. calcd. (found) % for $C_{19}H_{14}F_5N_3O_3$: C, 53.40 (53.38); H, 3.30 (3.29); N, 9.83 (9.82); IR (KBr, ν_{max}, cm^{-1}): 3314 (-NH), 3057 (-CH), 2952 (-CH), 1737, 1672 (C=O), 1257 (C-N), 1166 (C-F); ^{1}H

NMR (400 MHz, DMSO-d_6, δ ppm): 8.166-8.194 (t, J = 5.6 Hz, 1H, Ar-H), 7.908-7.913 (d, J = 3.2 Hz, 1H, Ar-H), 7.777-7.803 (dd, J = 1.6 Hz, 1H, Ar-H), 7.490-7.512 (d, J = 8.8 Hz, 1H, Ar-H), 7.324-7.399 (m, J = 14.8 Hz, 1H, Ar-H), 7.028-7.092 (m, J = 8.8 Hz, 2H, 2 × Ar-H), 4.980-5.048 (q, 2H, CH_2), 4.659-4.673 (d, J = 5.6 Hz, 2H, CH_2), 3.622 (s, 3H, CH_3); Mass (*m/z*): 428.2 (M+1).

2,2,2-Trifluoroethyl-1-methyl-2-oxo-3-((1-phenylethyl)-amino)-1,2-dihydroquinoxaline-6-carboxylate (10q): Yield: 59%; m.p.: 100.1-102.8 ºC, off-white solid; anal. calcd. (found) % for $C_{20}H_{18}F_3N_3O_3$: C, 59.26 (59.24); H, 4.48 (4.47); N, 10.37 (10.35); IR (KBr, ν_{max}, cm^{-1}): 3328 (-NH), 3027 (-CH), 2965, 2930 (-CH), 1731, 1662 (C=O), 1282 (C-N), 1174 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.129-8.150 (d, 1H, -NH, D_2O exchangeable), 7.862 (s, 1H, Ar-H), 7.757-7.778 (d, J = 8.4 Hz, 1H, Ar-H), 7.465-7.482 (d, J = 6.8 Hz, 3H, 3 × Ar-H), 7.290-7.326 (t, J = 7.2 Hz, 2H, 2 × Ar-H), 7.184-7.220 (t, J = 7.2 Hz, 1H, Ar-H), 5.304-5.370 (m, 1H, CH), 4.966-5.029 (q, 2H, CH_2), 3.656 (s, 3H, CH_3), 1.547-1.564 (d, J = 6.8 Hz, 3H, CH_3); Mass (*m/z*): 406.2 (M+1).

2,2,2-Trifluoroethyl-1-methyl-2-oxo-3-(phenethyl-amino)-1,2-dihydroquinoxaline-6-carboxylate (10r): Yield: 56%; m.p.: 116.0-120.1 ºC, off-white solid; anal. calcd. (found) % for $C_{20}H_{18}F_3N_3O_3$: C, 59.26 (59.24); H, 4.48 (4.45); N, 10.37 (10.35); IR (KBr, ν_{max}, cm^{-1}): 3344 (-NH), 3058, 3028 (-CH), 2944 (-CH), 1731, 1655 (C=O), 1275 (C-N), 1174 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.944-7.949 (d, J = 2.0 Hz, 1H, Ar-H), 7.857-7.886 (t, 1H, -NH, D_2O exchangeable), 7.777-7.804 (dd, J = 2.0 Hz, 1H, Ar-H), 7.503-7.525 (d, J = 8.8 Hz, 1H, Ar-H), 7.270-7.322 (m, 4H, 4 × Ar-H), 7.182-7.219 (m, J = 6.4 Hz, 1H, Ar-H), 4.978-5.046 (q, 2H, CH_2), 3.629-3.680 (m, 2H, CH_2), 2.928-2.964 (t, 2H, CH_2) and 3.641 (s, 3H, CH_3); Mass (*m/z*): 406.2 (M+1).

2,2,2-Trifluoroethyl-3-(benzyl(methyl)amino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10s): Yield: 80%; m.p.: 114.5-119.9 ºC, off-white solid; anal. calcd. (found) % for $C_{20}H_{18}F_3N_3O_3$: C, 59.26 (59.24); H, 4.48 (4.45); N, 10.37 (10.34); IR (KBr, ν_{max}, cm^{-1}): 3069, 3034 (-CH arom.), 2942 (-CH aliph.), 1722, 1662 (C=O), 1296 (C-N), 1153 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.920-7.924 (d, J = 1.6 Hz, 1H, Ar-H), 7.804-7.830 (dd, J = 1.6 Hz, 1H, Ar-H), 7.506-7.528 (d, J = 8.8 Hz, 1H, Ar-H), 7.302-7.353 (m, J = 20.4 Hz, 4H, 4 × Ar-H), 7.237-7.274 (m, J = 6.4 Hz, 1H, Ar-H), 5.128 (s, 2H, CH_2), 4.970-5.038 (q, 2H, CH_2), 3.617 (s, 3H, CH_3), 3.190 (s, 3H, CH_3); Mass (*m/z*): 406.2 (M+1).

2,2,2-Trifluoroethyl-3-((4-chlorophenyl)amino)-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (10t): Yield: 56%; m.p.: 202.2-206.8 ºC, white solid; anal. calcd. (found) % for $C_{18}H_{13}ClF_3N_3O_3$: C, 52.51 (52.49); H, 3.18 (3.17); N, 10.21 (10.20); IR (KBr, ν_{max}, cm^{-1}): 3316 for (-NH), 3072 (-CH arom.), 2973 (-CH aliph.), 1726, 1671 (C=O), 1279 (C-N), 1162 (C-F), 758 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.199-8.222 (d, J = 9.2 Hz, 1H, Ar-H), 8.081 (s, 1H, -NH, D_2O exchangeable), 7.877-7.951 (m, J = 13.2 Hz, 2H, 2 × Ar-H), 7.609-7.631 (d, J = 8.8 Hz, 1H, Ar-H), 7.429-7.482 (d, J = 21.2 Hz, 2H, 2 × Ar-H), 4.993-5.077 (q, 2H, CH_2), 3.717 (s, 3H, CH_3); Mass (*m/z*): 412.1 (M+1).

2,2,2-Trifluoroethyl-1-methyl-2-oxo-3-(*o*-tolylamino)-1,2-dihydroquinoxaline-6-carboxylate (10u): Yield: 53%; m.p.: 191.1-198.4 ºC, white solid; anal. calcd. (found) % for $C_{19}H_{16}F_3N_3O_3$: C, 58.31 (58.29); H, 4.12 (4.10); N, 10.74 (10.72); IR (KBr, ν_{max}, cm^{-1}): 3373 (-NH), 3059 (-CH), 2992 (-CH), 1734, 1670 (C=O), 1252 (C-N), 1165 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.040 (s, 1H, -NH, D_2O exchangeable), 7.991-8.010 (d, J = 7.6 Hz, 1H, Ar-H), 7.920-7.924 (d, J = 1.6 Hz, 1H, Ar-H), 7.865-7.892 (dd, J = 2.0 Hz, 1H, Ar-H), 7.599-7.629 (d, J = 12.0 Hz, 1H, Ar-H), 7.283-7.312 (m, J = 5.2 Hz, 2H, 2 × Ar-H), 7.108-7.144 (t, J = 7.2 Hz, 1H, Ar-H), 4.973-5.041 (q, 2H, CH_2), 3.729 (s, 3H, CH_3), 2.289 (s, 3H, CH_3); Mass (*m/z*): 392.1 (M+1).

2,2,2-Trifluoroethyl-1-methyl-2-oxo-3-(*p*-tolylamino)-1,2-dihydroquinoxaline-6-carboxylate (10v): Yield: 59%; m.p.: 214.6218.1 ºC, white solid, Anal. calcd. (found) % for $C_{19}H_{16}F_3N_3O_3$: C, 58.31 (58.29); H, 4.12 (4.10); N, 10.74 (10.72); IR (KBr, ν_{max}, cm^{-1}): 3322 (-NH), 3050 (-CH), 2925 (-CH), 1729 and 1716 (C=O), 1278 (C-N), 1170 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.030-8.035 (d, J = 2.0 Hz, 1H, Ar-H), 7.989-8.010 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 7.868-7.895 (dd, J = 2.0 Hz, 1H, Ar-H), 7.580-7.602 (d, J = 8.8 Hz, 1H, Ar-H), 7.181-7.202 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 4.998-5.066 (q, 2H, CH_2), 3.707 (s, 3H, CH_3), 3.294 (s, 3H, CH_3); Mass (*m/z*): 392.2 (M+1).

MTT assay: Each 96-well tissue culture microtiter plate well received 100 µL of full medium containing 1×10^4 cells. The plates were incubated for 18 h at 37 ºC in a humidified environment with 5% CO_2 before to the experiment. After removing the media, each well was incubated for 24 h at 37 ºC with 0.5, 1 and 2 µM of the test samples and erlotinib. After removing the medium, 10 µL of MTT dye was added. Plates were incubated at 37 ºC for 2 h. The formazan crystals that were produced were dissolved in 100 µL of extraction buffer. The optical density (O.D.) was determined at 570 nm using a Thermo Scientific microplate reader (Multimode Varioskan Instrument). The final concentration of DMSO in the culture medium did not exceed 0.25%.

Docking studies: Auto dock 4.2 was used for molecular docking studies. The protein was downloaded from protein data bank which is having pdb id: 4HJO. Ligand and water are removed from the protein and calculated Gasteiger charges after addition of polar hydrogens. The ligands are drawn using chem draw 12 and saved as .mol file after energy minimisation. Then they are converted in to pdb file using discovery studios. Grid box is generated by taking 60 points on three coordinate axes. Lamarckian GA (4.2) algorithm was employed to generate dpf file. Cygwin interface was used to obtain the dlg file from where the results were extracted. The 2D and 3D images are being rendered using Schrödinger's maestro v9.5 visualizer interface.

Antibacterial activity: The broth dilution method was used to determine the minimum inhibitory concentration (MIC), defined as the lowest concentration of an antibacterial agent that inhibits visible bacterial growth under standardised test conditions. The assay was performed in 96-well microtiter plates by inoculating bacterial suspensions into a liquid growth medium containing serial dilutions of the test comp-

ounds. After incubation for 16-20 h, bacterial growth was examined and the MIC was recorded as the lowest concentration that completely inhibited visible growth.

RESULTS AND DISCUSSION

The synthesis of the novel 2,2,2-trifluoroethyl-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate amino derivatives (**10a-v**) is outlined in **Scheme-I**. 4-Chloro-3-nitrobenzoic acid (**1**) was aminated with methylamine hydrochloride in an autoclave at 60 ºC to afford 4-(methylamino)-3-nitrobenzoic acid (**2**). Esterification of compound 2 with thionyl chloride and 2,2,2-trifluoroethanol (**3**) at 75 ºC yielded 2,2,2-trifluoroethyl 4-(methylamino)-3-nitrobenzoate (**4**). Reduction of the nitro group using G-Cat furnished 2,2,2-trifluoroethyl 3-amino-4-(methylamino)benzoate (**5**). Cyclisation of compound **5** with ethyl 2-chloro-2-oxoacetate (**6**) produced 2,2,2-trifluoroethyl-1-methyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**7**). Chlorination of compound **7** with SOCl2 in the presence of a catalytic amount of DMF afforded 2,2,2-trifluoroethyl-3-chloro-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate (**8**). Nucleophilic substitution of compound **8** with the appropriate amines (**9a-v**) in DMF using triethylamine as the base furnished the target 2,2,2-trifluoroethyl-1-methyl-2-oxo-1,2-dihydroquinoxaline-6-carboxylate amino derivatives (**10a-v**).

The structures of the synthesised compounds (**10a-v**) were confirmed by ^{1}H NMR, IR and mass spectrometry. The spectral data were consistent with the proposed structures, and the mass spectra displayed the expected $[M + H]^+$ molecular ion peaks.

Antibacterial activity: The synthesised compounds (**10a-v**) were evaluated for their antibacterial activity using the agar well diffusion method against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus* and *B. subtilis*). Ciprofloxacin (7 µg/mL) was used as the reference drug, while 1% DMSO served as the negative control. The antibacterial activity results (Table-1) revealed that the synthesised derivatives (**10a-v**) exhibited varying degrees of inhibition against the tested bacterial strains. Compounds **10c**, **10e**, **10f**, **10h**, **10n**, **10o**, **10p**, **10t** and **10u** displayed the highest antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*.

Anticancer activity: The newly synthesised derivatives (**10a-v**) antitumor activity was assessedon two human cancer cell lines, such as MCF-7 and HepG-2, using the MTT assay. The activity results (Table-2) indicate that the derivative **10m** shows more potent activity as compared to the standard drug, erlotinib and the remaining four compounds, **10i**, **10j**, **10k** and **10l**, show almost nearer activity as compared to the standard drug. The remaining compounds showed less activity as compared to **10i**, **10j**, **10k**, **10l** and **10m**.

The structure–activity relationship (SAR) analysis revealed that the nature of the substituent attached to the quinoxaline scaffold markedly influenced the anticancer activity. Among the synthesised compounds, the morpholinopiperidine derivative **10m** exhibited the highest activity, exceeding that of the reference drug erlotinib. The *N*-methylpiperazine derivative

TABLE-1
ANTIBACTERIAL ACTIVITY DATA OF SYNTHESISED COMPOUNDS (**10a-v**) (CONCENTRATION USED 25 µg/mL OF DMSO)

Compound	R	Zone of inhibition (mm) and the values in parenthesis are MIC (µg/mL) of the correspond molecule towards bacteria tested			
		Gram-negative		Gram-positive	
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
10a	Amine	5.4 (56.6)	7.2 (62.6)	6.1 (60.4)	8.5 (59.2)
10b	Dimethylamine	7.2 (49.2)	5.6 (52.4)	8.2 (53.2)	4.7 (59.0)
10c	Isopropylamine	4.3 (55.8)	5.1 (54.6)	4.7 (57.6)	3.5 (56.7)
10d	Methylamine	7.5 (49.2)	5.8 (52.4)	8.4 (53.2)	4.1 (59.0)
10e	Cyclobutylamine	4.3 (62.4)	6.4 (59.4)	5.9 (57.6)	4.7 (59.7)
10f	Cyclopentylamine	6.1 (64.7)	6.9 (66.8)	7.2 (62.3)	6.9 (64.6)
10g	Ethanolamine	7.6 (53.4)	7.9 (56.4)	8.2 (58.6)	8.9 (65.4)
10h	Trifluoroethyl amine	5.6 (62.0)	6.3 (67.2)	5.6 (69.5)	7.6 (59.6)
10i	Pyrrolidine	28.6 (7.3)	27.3 (8.1)	29.2 (7.5)	28.4 (10.5)
10j	Piperidine	27.1 (11.2)	28.7 (8.9)	26.3 (9.3)	28.0 (11.4)
10k	Morpholine	29.3 (7.7)	28.1 (8.2)	27.2 (9.1)	29.3 (9.9)
10l	N-Methyl piperazine	26.6 (10.1)	27.2 (9.4)	29.1 (11.2)	28.7 (8.7)
10m	Piperidinyl morpholine	7.3 (69.2)	8.3 (65.4)	7.5 (67.4)	6.5 (64.2)
10n	Aniline	5.2 (67.5)	7.1 (69.4)	6.3 (70.2)	6.1 (62.4)
10o	Benzylamine	7.6 (56.7)	4.6 (64.7)	6.3 (57.3)	7.6 (64.4)
10p	2,6-Difluoro benzylamine	6.3 (59.4)	7.3 (62.7)	6.9 (69.2)	7.6 (59.3)
10q	1-Phenylethanamine	7.3 (67.3)	6.3 (62.5)	7.6 (70.2)	5.6 (62.3)
10r	2-Phenylethanamine	7.8 (65.4)	7.9 (63.5)	6.7 (67.4)	5.7 (69.4)
10s	N-Methyl benzyl amine	5.5 (66.4)	6.4 (56.7)	6.9 (56.4)	7.2 (67.5)
10t	4-Chloro aniline	7.4 (62.7)	5.6 (66.7)	6.4 (69.5)	7.8 (64.5)
10u	*o*-Toluidine	6.5 (>163)	6.1 (>178)	6.4 (>156)	7.2 (>163)
10v	*p*-Toluidine	2.6 (>250) (NA)	3.6 (>189)	6.3 (>192)	1.2 (>150)
A	Ciprofloxacin (7 µg/mL of DMSO)	32.8 (6.5)	30.9 (4.3)	33.6 (6.9)	31.5 (7.0)

NA = Not active

TABLE-2
In vitro ANTICANCER ACTIVITY DATA OF 2,2,2-TRIFLUOROETHYL-1-METHYL-2-OXO-1,2-DIHYDRO QUINOXALINE-6-CARBOXYLATE AMINO DERIVATIVES (**10a-v**) WITH IC_{50} IN μM[a]

Entry	-R	MCF-7[b]	HepG-2[c]	MCF-10A[d]
10a	Amine	48.02 ± 1.03	50.20 ± 0.15	Not determined
10b	Dimethylamine	52.10 ± 0.04	55.12 ± 0.19	Not determined
10c	Isopropylamine	56.06 ± 0.02	57.03 ± 0.12	Not determined
10d	Methylamine	48.20 ± 0.04	53.75 ± 0.06	Not determined
10e	Cyclobutylamine	60.05 ± 0.05	68.12 ± 0.08	Not determined
10f	Cyclopentylamine	65.02 ± 0.04	67.02 ± 0.10	Not determined
10g	Ethanolamine	56.01 ± 0.42	69.30 ± 0.09	Not determined
10h	Trifluoro ethylamine	52.06 ± 1.04	60.10 ± 1.02	Not determined
10i	Pyrrolidine	17.02 ± 0.12	18.40 ± 0.03	96.20 ± 0.02
10j	Piperidine	16.56 ± 0.04	17.05 ± 0.06	97.02 ± 0.10
10k	Morpholine	16.12 ± 0.06	16.12 ± 0.06	94.06 ± 0.04
10l	*N*-Methyl piperazine	15.23 ± 0.10	16.01 ± 0.10	98.12 ± 0.02
10m	Piperidinyl morpholine	10.20 ± 0.02	12.05 ± 0.13	98.04 ± 0.12
10n	Aniline	38.23 ± 0.02	42.03 ± 0.05	Not determined
10o	Benzylamine	41.05 ± 0.12	43.12 ± 0.06	Not determined
10p	2,6-Difluorobenzylamine	38.10 ± 0.15	40.16 ± 0.02	Not determined
10q	1-Phenylethanamine	43.12 ± 0.11	45.18 ± 0.13	Not determined
10r	2-Phenylethanamine	45.02 ± 0.03	46.10 ± 0.06	Not determined
10s	N-Methyl benzylamine	44.05 ± 0.14	48.02 ± 0.07	Not determined
10t	4-Chloro aniline	39.10 ± 1.23	43.12 ± 0.02	Not determined
10u	*o*-Toluidine	45.02 ± 0.16	47.10 ± 0.04	Not determined
10v	*p*-Toluidine	46.13 ± 0.14	48.04 ± 0.08	Not determined
Erlotinib		15.06 ± 0.08	10.75 ± 0.30	94.10 ± 0.16

[a]IC_{50} after 26 h of incubation, each data represents as mean ± SD values from three different experiments performed in triplicates. [b]MCF-7: breast cancer cell line. [c]HepG-2: liver cancer cell line. [d]MCF-10A: breast healthy cell line.

10l showed the next highest activity. Compounds **10i**, **10j**, and **10k**, bearing pyrrolidine, piperidine and morpholine moieties, respectively, displayed activity comparable to erlotinib. Derivatives **10a-d**, **10g** and **10h**, containing aliphatic substituents, were less active than the nitrogen-containing cyclic analogues. Compounds **10e** and **10f**, containing cyclic amines, also exhibited relatively lower activity. Aromatic amine derivatives **10n-v** displayed moderate activity, superior to the aliphatic derivatives but generally lower than the most active nitrogen-containing cyclic analogues.

The cytotoxicity of the most active compounds (**10i-m**) was evaluated against the normal breast epithelial cell line (MCF-10A). All selected compounds exhibited low toxicity, with IC_{50} values greater than 98.12 μM, reflecting good selectivity for cancer cells. The higher anticancer activity of the pyrrolidine-, piperidine-, morpholine- and N-methyl-piperazine-substituted derivatives may arise from favourable ionic and hydrogen-bonding interactions with biological targets. The basic nitrogen atoms present in these substituents can improve water solubility, cellular uptake and pharmacokinetic properties, which may account for their enhanced biological activity.

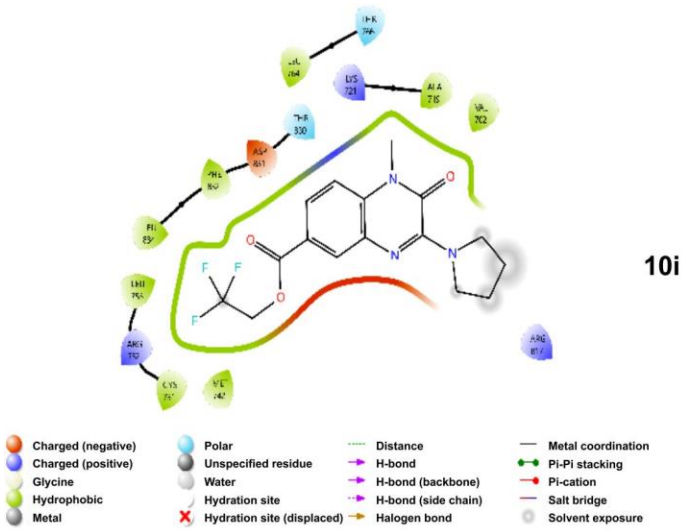
Molecular docking studies: The molecular docking study of compounds **10i-m** against the epidermal growth factor receptor (EGFR; PDB ID: 4HJO) [19-23] revealed stronger predicted binding than the reference drug erlotinib (Table-3). The binding energies of the synthesised derivatives ranged from -9.34 to -10.82 kcal/mol. Compound **10k** exhibited the most favourable binding energy (-10.82 kcal/mol) and the lowest inhibition constant (11.76 nM). It formed a hydrogen bond with MET769 at a bond distance of 1.92 Å, which supports stable ligand-protein binding. Compounds **10i** (-10.02 kcal/mol, 45.17 nM), **10m** (-9.87 kcal/mol, 58.64 nM) and **10j** (-9.77 kcal/mol, 68.63 nM) likewise exhibited strong binding affinities. Compound **10l** displayed the lowest affinity within the series (-9.34 kcal/mol, 142.59 nM), although its docking score remained better than that of erlotinib. Most derivatives did not form hydrogen bonds with the receptor, implied that

TABLE-3
MOLECULAR DOCKING RESULTS OF COMPOUNDS (**10i**, **10j**, **10k**, **10l** AND **10m**) WITH EGFR (PDB ID-4HJO)

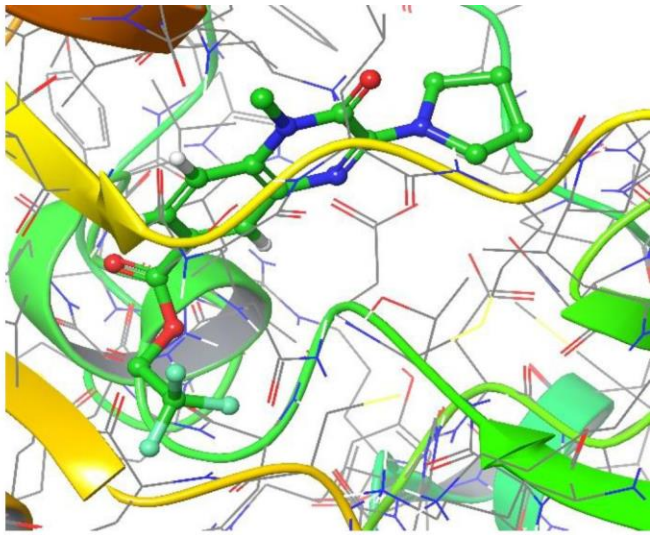
Entry	Binding energy (kcal/mol)	Inhibition constant (nM)	Number of hydrogen bonds	Residues involved in hydrogen bonding (bond length, Å)
10i	-10.02	45.17	–	–
10j	-9.77	68.63	–	–
10k	-10.82	11.76	1	MET769 (1.92)
10l	-9.34	142.59	–	–
10m	-9.87	58.64	–	–
Erlotinib	-7.68	2.30 μM	1	THR830 (2.25)

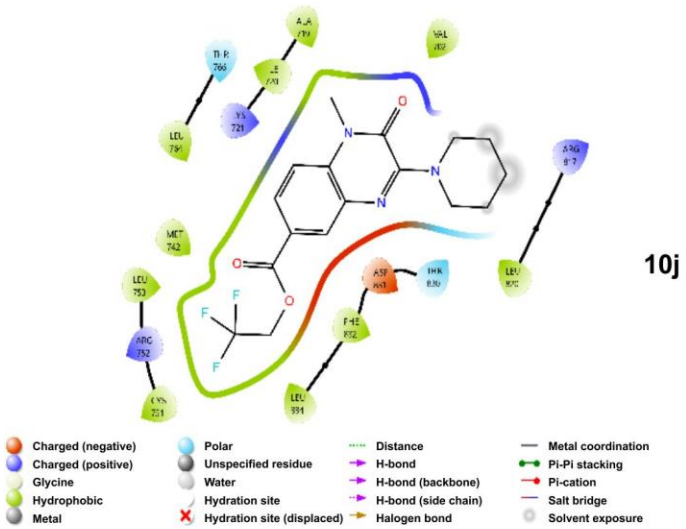
hydrophobic and van der Waals interactions contributed substantially to ligand binding within the EGFR active site. Although compound **10k** achieved the best docking score, compound **10m** displayed the highest *in vitro* anticancer activity. This difference reflects a recognised limitation of molecular docking, which estimates ligand binding in a rigid protein model and does not fully account for biological complexity (Fig. 1).

Erlotinib exhibited a binding energy of -7.68 kcal/mol with an inhibition constant of 2.30 μM and formed a hydrogen

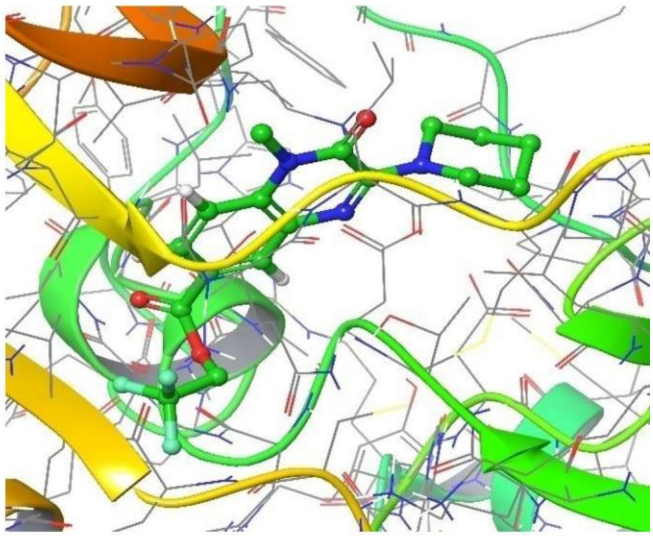


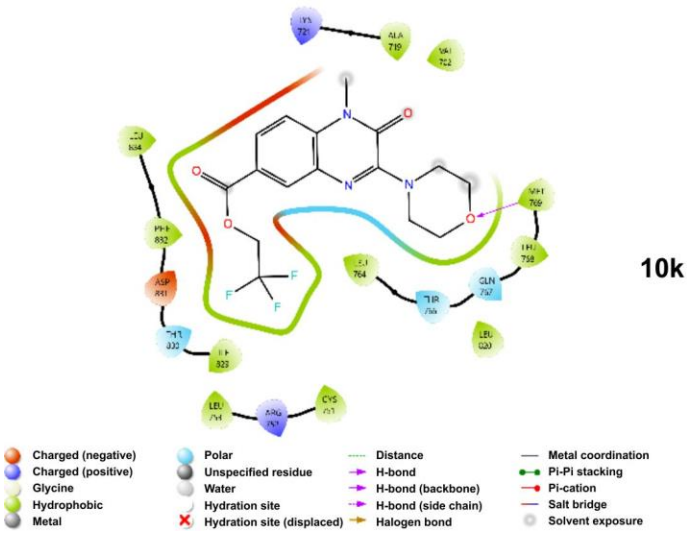
10i



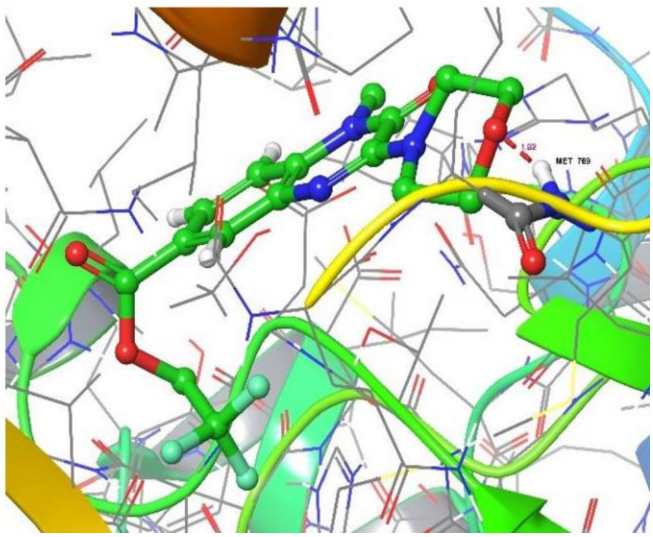


10j





10k



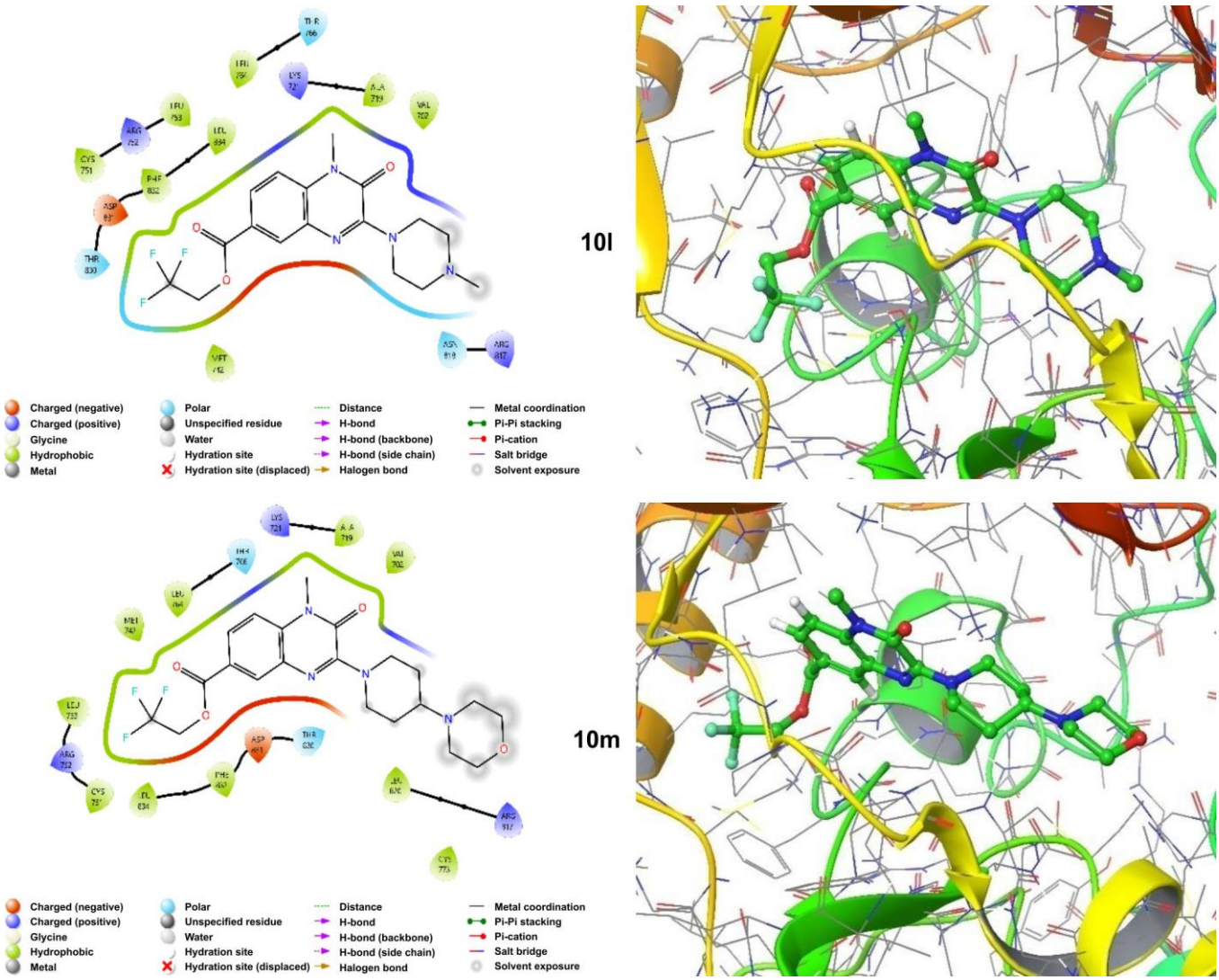


Fig. 1. 2D and 3D interactions of compounds **10i**, **10j**, **10k**, **10l** and **10m** with EGFR

bond with THR830 at a bond distance of 2.25 Å. The synthesised derivatives displayed more favourable docking scores than erlotinib, with compound **10k** emerging as the most promising candidate for further optimization as a potential EGFR inhibitor.

Conclusion

In summary, 22 new trifluoroethyl dihydroquinoxaline-carboxylate amino derivatives (**10a-v**) were synthesised from 4-chloro-3-nitrobenzoic acid. The *in vitro* antibacterial activity of these derivatives was evaluated against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*. Compounds **10c**, **10e**, **10f**, **10h**, **10n**, **10o**, **10p**, **10t** and **10u** exhibited excellent antibacterial activity against all the tested bacterial strains. The anticancer activity of the synthesised derivatives was evaluated against MCF-7 and HepG-2 cancer cell lines. Compound **10m** exhibited higher anticancer activity than the reference drug erlotinib, while compounds **10i**, **10j**, **10k** and **10l** displayed activity comparable to erlotinib. Molecular docking studies of compounds **10i-m** with EGFR (PDB ID: 4HJO) showed more favourable binding energies than erlotinib. Among these derivatives, compound **10k** exhibited the strongest binding affinity and formed a hydrogen bond with the MET769 residue, whereas erlotinib formed a hydrogen bond with the THR830 residue.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI.

All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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