

Synthesis and Biological Activities of New 3-Amino Derivatives of Methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate

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3-Amino derivatives of methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**7a-l**) were prepared using methyl 4-chloro-3-nitrobenzoate (**1**) as the starting material. The synthesized derivatives were characterized by ¹H NMR, mass and IR spectroscopy. The derivatives were tested for their antibacterial activity evaluated against four different bacterial strains, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The derivatives further screened for their anticancer activity against two breast cancer cell lines, MCF-7 and MDA-MB-231. The activity results indicate that the derivatives **7e** and **7f** show good activity as compared to the standard drug erlotinib. Further, the docking results also supports the *in vitro* anticancer activity results.

Keywords: 1,2-Dihydroquinoxaline-6-carboxylates, Docking studies, Antibacterial activity, Anticancer activity.

INTRODUCTION

Dihydroquinoxalines are a class of synthetic organic heterocyclic compounds containing a six-membered ring system [1]. Quinoxaline derivatives have received considerable attention from synthetic chemists due to their diverse pharmacological and therapeutic properties [2]. Literature reports show that quinoxaline derivatives possess a broad range of biological activities including antiviral activity, particularly against Hepatitis-B virus [3], antibacterial and antimicrobial activities [4-6] and anticancer properties [7-13]. Crystal structure of several quinoxaline derivatives have also been investigated [14,15]. Based on these reports and continued our interest in the heterocyclic chemistry systems, we undertook the synthesis of new heterocyclic compounds incorporating bioactive moieties and quinoxaline units. The synthesized compounds were evaluated for their antimicrobial activity against selected pathogenic microorganisms and for their anticancer activity against selected cancer cell lines [16-24].

In the present study, a series of 12 new 3-amino derivatives of methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**7a-l**) was synthesized. The target

compounds were synthesized by reacting methyl 3-chloro-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**5**) with a range of amines (**6a-l**), as outlined in **Scheme-I**. The structures of the synthesized compounds were established using ¹H NMR, mass and IR spectroscopy. Their biological potential was assessed through antimicrobial screening against selected pathogenic microorganisms and anticancer studies using selected cancer cell lines. The study was undertaken to examine the effect of structural variation in the synthesized 3-aminoquinoxaline derivatives on their biological activity and to identify compounds of potential pharmacological interest.

EXPERIMENTAL

All reagents and solvents compounds were utilized without any additional purification. Merck 60F₂₅₄ silica gel TLC plates were used to track each compound's purity. The melting points were measured using an uncorrected hot stage melting point instrument from Ernst LeitzWetzlar, Germany. The Mercury Plus spectrometer, which operates at 400 MHz for ¹H NMR, was used for recording the ¹H NMR and chemical

shifts were referred to TMS. The Shimadzu QP5050A quadrupole-mass spectrometer generated the ESI mass spectra at an ionising voltage of 70 eV.

LR grade 4-chloro-3-nitro methylbenzoate (**1**) was used as a starting material.

Synthesis of methyl 3-nitro-4-((2,2,2-trifluoroethyl)-amino)benzoate (2): To a solution of compound (**1**, 100.0 g, 0.4638 mol) in methanol (500.0 mL), triethylamine (195.3 mL, 1.3915 mol) and 2,2,2-trifluoroethylamine hydrochloride (TFA, 157.1 g, 1.1595 mol) were added and stirred at 60 °C in autoclave for 12 h. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated under vacuum. Water (200 mL) was added to the residue, and the aqueous phase was extracted with dichloromethane (2 × 500 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford compound **2** (109.0 g) as a yellow solid. Yield: 68%; m.p.: 124.2-128.3 °C, IR (KBr, $\nu_{\max}$, cm^{-1}): 3345 (-NH), 3095 (-CH arom.), 1716 (C=O), 1305 (NO_2), 1146 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 8.665-8.699 (t, 1H, NH, D_2O exchangeable), 8.632-8.637 (d, $J = 2.0$ Hz, 1H, Ar-H), 8.024-8.051 (dd, $J = 8.8$ Hz, 1H, Ar-H), 7.402-7.424 (d, $J = 8.8$ Hz, 1H, Ar-H), 4.386-4.474 (m, 2H, $\text{CH}_2\text{-CF}_3$), 3.844 (s, 3H, $-\text{CH}_3$); Mass (m/z): 277.0 (M^+).

Synthesis of 3-amino-4-((2,2,2-trifluoroethyl) amino)-methyl benzoate (3): A mixture of water (5 vol.) and G-cat (3.0 times) was heated to 75 °C. A solution of compound **2** (100 g, 0.3594 mol) in ethyl acetate (10 vol) was added and the reaction mixture was stirred at 75 °C for 24 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to 60 °C, followed by the addition of R-cat (0.1 times) and stirring was continued at 60 °C for 30 min. The reaction mixture was then filtered and washed with excess water. The filtrate was extracted with ethyl acetate and the combined organic layer was concentrated under reduced pressure to obtain the crude product. The crude product was purified using MTBE at 50 °C to afford compound **3** (80.0 g) as a brown solid. Yield: 90%; m.p.: 101.2-106.4 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3387 (-NH), 3008 (-CH arom.), 1683 (C=O), 1169 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 7.228-7.233 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.178-7.204 (dd, $J = 8.4$ Hz, 1H, Ar-H), 6.685-6.706 (d, $J = 8.4$ Hz, 1H, Ar-H), 5.741-5.774 (t, 1H, NH, D_2O exchangeable), 4.840 (br, s, 2H, $-\text{NH}_2$, D_2O exchangeable), 3.993-4.082 (m, 2H, $\text{CH}_2\text{-CF}_3$), 3.734 (s, 3H, $-\text{CH}_3$); Mass (m/z): 249.1 (M^+).

Synthesis of methyl 2,3-dioxo-1-(2,2,2-trifluoroethyl)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (4): To a solution of compound **3** (75.0 g, 0.3021 mol) in dichloromethane (10 vol), triethylamine (0.9065 mol) was added. The reaction mixture was cooled to 0 °C and ethyl 2-chloro-2-oxoacetate (0.4531 mol) was added slowly. The reaction mixture was stirred at 0 °C for 12 h and the progress of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with chilled water (5 vol) and stirred for 30 min. The resulting solid was collected by filtration under reduced pressure to afford compound **4** (69.0 g) as an off-white solid. Yield: 90%; m.p.: >250 °C, IR (KBr, $\nu_{\max}$, cm^{-1}): 3424 (-NH), 2991 (-CH arom), 1731 & 1715 (C=O), 1166 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 12.288 (s, 1H, NH, D_2O

exchangeable), 7.812-7.817 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.727-7.753 (dd, $J = 8.8$ Hz, 1H, Ar-H), 7.649-7.671 (d, $J = 8.8$ Hz, 1H, Ar-H), 5.092-5.160 (m, 2H, $\text{CH}_2\text{-CF}_3$), 3.856 (s, 3H, $-\text{CH}_3$); Mass (m/z): 303.1 (M^+).

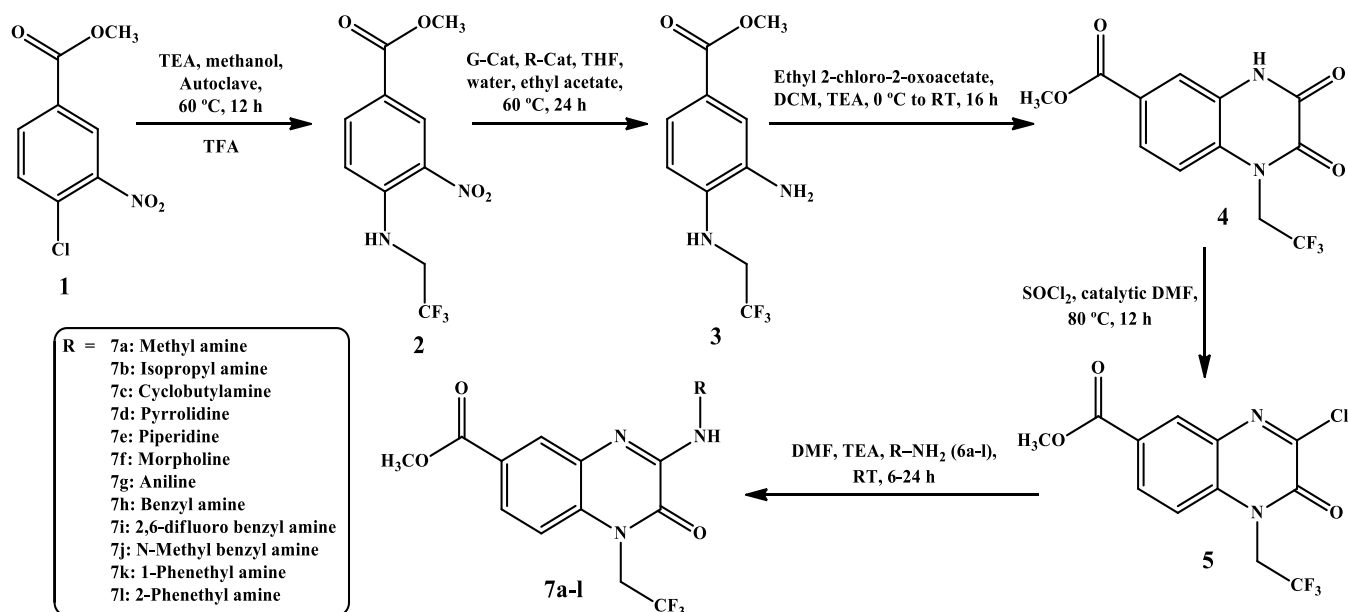
Synthesis of methyl 3-chloro-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (5): To compound **4** (60.0 g, 0.1985 mol), thionyl chloride (5 vol) was added, followed by catalytic dimethylformamide (0.5 vol). The reaction mixture was refluxed for 12 h and monitored by TLC. After completion, the reaction mixture was quenched with chilled water and extracted with dichloromethane. The organic layer was washed with 10% aqueous NaHCO_3 solution, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford compound **5** (54.0 g) as an off-white solid. Yield: 88%; m.p.: 146.9-152.6 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3076 (-CH arom.), 1714 & 1695 (C=O), 1166 for (C-F) and 764 for (C-Cl); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 8.224-8.229 (d, $J = 2.0$ Hz, 1H, Ar-H), 8.158-8.185 (dd, $J = 8.8$ Hz, 1H, Ar-H), 7.883-7.905 (d, $J = 8.8$ Hz, 1H, Ar-H), 5.242-5.309 (q, 2H, $\text{CH}_2\text{-CF}_3$), 3.899 (s, 3H, $-\text{CH}_3$); Mass (m/z): 321.0 (M^+).

General procedure for the synthesis of compounds (7): To a solution of compound **5** (1.0 g, 0.0031 mol) in DMF (10.0 vol), triethylamine (0.0093 mol) was added, followed by the corresponding amine (**6a-l**) (0.0046 mol). The reaction mixture was stirred at room temperature for 12 h and the progress of the reaction was monitored by TLC. After completion, water (10 mL) was added to quench the reaction and the resulting solid was collected by filtration under reduced pressure to obtain the respective pure compounds **7a-l**, as shown in Scheme-1.

Methyl 3-(methylamino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7a): Yield: 60%; m.p.: 159.8-163.1 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3407 (-NH), 3026 (-CH arom.), 2963 (-CH for aliph.), 1709 & 1670 (C=O), 1277 (C-N), 1164 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 8.001-8.039 (q, 1H, NH, D_2O exchangeable), 7.940-7.943 (d, $J = 1.2$ Hz, 1H, Ar-H), 7.729-7.755 (dd, $J = 8.8$ Hz, 1H, Ar-H), 7.653-7.674 (d, $J = 8.4$ Hz, 1H, Ar-H), 5.212-5.279 (q, 2H, CH_2), 3.862 (s, 3H, $-\text{CH}_3$), 2.921-2.933 (d, 3H, $-\text{CH}_3$); Mass (m/z): 316.2 (M^+);

Methyl 3-(isopropylamino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7b): Yield: 68%; m.p.: 142.2-148.4 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3349 (-NH), 2985 (-CH arom.), 2968 (-CH aliph.), 1666 & 1617 (C=O), 1226 (C-N), 1176 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 7.922-7.927 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.730-7.757 (dd, $J = 8.8$ Hz, 1H, Ar-H), 7.651-7.673 (m, 2H, Ar-H), 5.206-5.273 (q, 2H, CH_2), 4.231-4.317 (m, 1H, -CH), 3.864 (s, 3H, $-\text{CH}_3$), 1.231-1.247 (d, 6H, 2 × $-\text{CH}_3$); Mass (m/z): 344.1 (M^+).

Methyl 3-(cyclobutylamino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7c): Yield: 59%; m.p.: 142.2-147.9 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3337 (-NH), 3069 (-CH arom.), 2955 (-CH aliph.), 1713 & 1672 (C=O), 1326 (C-N), 1160 (C-F); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 8.160-8.178 (d, 1H, -NH, D_2O exchangeable), 7.919 (s, 1H, Ar-H), 7.734-7.756 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.645-7.666 (d, $J = 8.4$ Hz, 1H, Ar-H), 5.179-5.268 (m, 2H, CH_2), 4.488-4.587 (m, 1H, CH), 3.862 (s, 3H, $-\text{CH}_3$), 2.135-2.274 (m,



Scheme-I: Synthesis of 3-amino derivatives of methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**7a-l**)

4H, 2 × CH₂), 1.697-1.742 (m, 2H, CH₂); Mass (*m/z*): 356.1 (*M*⁺).

Methyl 2-oxo-3-(pyrrolidin-1-yl)-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7d): Yield: 62%; m.p.: 188.4-193.1 °C; IR (KBr, *v*_{max}, cm⁻¹): 2991 (-CH arom.), 2951 (-CH aliph.), 1716 (C=O), 1256 (C-N), 1162 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): 7.865-7.869 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.687-7.713 (dd, *J* = 8.8 Hz, 1H, Ar-H), 7.572-7.594 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.154-5.222 (q, 2H, CH₂), 4.056 (br, 4H, 2 × CH₂), 3.853 (s, 3H, CH₃), 3.633 (br, 2H, CH₂), 1.891 (br, 4H, 2 × CH₂); Mass (*m/z*): 356.1 (*M*⁺).

Methyl 2-oxo-3-(piperidin-1-yl)-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7e): Yield: 80%; m.p.: 136.2-140.0 °C; IR (KBr, *v*_{max}, cm⁻¹): 3028 (-CH arom.), 2941 (-CH aliph.), 1725 & 1679 (C=O), 1277 (C-N), 1160 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): 7.927 (s, 1H, Ar-H), 7.768-7.787 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.625-7.647 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.176-5.243 (q, 2H, CH₂), 3.860 (br, 7H, 2 × CH₂ & 1 CH₃) and 1.634 (m, 6H, 3 × CH₂); Mass (*m/z*): 370.2 (*M*⁺).

Methyl 3-morpholino-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7f): Yield: 91%; m.p.: 166.8-173.0 °C; IR (KBr, *v*_{max}, cm⁻¹): 3064 (-CH arom.), 2971 & 2954 (-CH aliph.), 1719 & 1664 (C=O), 1243 (C-N), 1151 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): *δ* 7.960 (s, 1H, Ar-H), 7.808-7.829 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.663-7.684 (d, *J* = 8.4 Hz, 1H, Ar-H), 5.187-5.253 (q, 2H, CH₂), 3.894 (br, 4H, 2 × CH₂), 3.864 (s, 3H, -CH₃), 3.725 (br, 4H, 2 × CH₂); Mass (*m/z*): 372.1 (*M*⁺);

Methyl 2-oxo-3-(phenylamino)-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7g): Yield: 94%; m.p.: 183.2-185.1 °C; IR (KBr, *v*_{max}, cm⁻¹): 3311 (-NH), 3063 (-CH arom.), 2948 (-CH aliph.), 1668 (C=O), 1283 (C-N), 1172 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): *δ* 9.770 (s, 1H, -NH, D₂O exchangeable), 8.146-8.163 (d, *J* = 6.8 Hz, 2H, 2 × Ar-H), 8.078 (s, 1H, Ar-H), 7.748-7.877 (dd, 2H, Ar-

H), 7.377-7.412 (t, *J* = 7.6 Hz, 2H, 2 × Ar-H), 7.079-7.119 (t, *J* = 8.0 Hz, 1H, Ar-H), 5.282-5.346 (q, 2H, CH₂), 3.889 (s, 3H, CH₃); Mass (*m/z*): 378.1 (*M*⁺);

Methyl 3-(benzylamino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7h): Yield: 78%; m.p.: 160.2-167.3 °C; IR (KBr, *v*_{max}, cm⁻¹): 3362 (-NH), 3010 (-CH arom.), 2964 (-CH aliph.), 1710 (C=O), 1299 (C-N), 1142 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): *δ* 8.562-8.592 (t, 1H, -NH, D₂O exchangeable), 7.895 (s, 1H, Ar-H), 7.655-7.761 (dd, *J* = 8.8 Hz, 2H, 2 × Ar-H), 7.221-7.413 (m, 6H, Ar-H), 5.217-5.277 (q, 2H, CH₂), 4.619-4.633 (d, 2H, CH₂), 3.852 (s, 3H, CH₃); Mass (*m/z*): 392.1 (*M*⁺).

Methyl 3-((2,6-difluorobenzyl)amino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7i): Yield: 66%; m.p.: 167.0-173.8 °C; IR (KBr, *v*_{max}, cm⁻¹): 3314 (-NH), 3057 (-CH arom.), 2952 (-CH aliph.), 1737 & 1672 (C=O), 1257 (C-N), 1166 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): *δ* 8.357-8.384 (t, *J* = 5.6 Hz, 1H, Ar-H), 7.906-7.911 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.752-7.778 (dd, *J* = 8.4 Hz, 1H, Ar-H), 7.655-7.677 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.330-7.404 (m, 1H, Ar-H), 7.042-7.105 (m, 2H, 2 × Ar-H), 5.191-5.258 (q, 2H, CH₂), 4.655-4.669 (d, 2H, CH₂) and 3.872 (s, 3H, CH₃); Mass (*m/z*): 428.2 (*M*⁺).

Methyl 3-(benzyl(methyl)amino)-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7j): Yield: 80%; m.p.: 170.0-178.5 °C; IR (KBr, *v*_{max}, cm⁻¹): 3089 & 3001 (-CH arom.), 2954 (-CH aliph.), 1718 & 1680 (C=O), 1279 (C-N), 1152 (C-F); ¹H NMR (400 MHz, DMSO-*d*₆, *δ* ppm): *δ* 7.932-7.937 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.774-7.801 (dd, *J* = 8.8 Hz, 1H, Ar-H), 7.649-7.671 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.243-7.358 (m, 5H, 5 × Ar-H), 5.202-5.269 (m, 2H, CH₂), 5.097 (s, 2H, CH₂), 3.860 (s, 3H, CH₃), 3.216 (s, 3H, CH₃); Mass (*m/z*): 406.2 (*M*⁺).

Methyl 2-oxo-3-((1-phenylethyl)amino)-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7k): Yield: 69%; m.p.: 154.2-159.4 °C; IR (KBr, *v*_{max}, cm⁻¹): 3069

(-CH arom.), 2971 & 2942 (-CH aliph.), 1722 & 1662 (C=O), 1269 (C-N), 1153 (C-F); ^1H NMR (400 MHz, DMSO- d_6 , δ ppm): δ 8.317-8.338 (d, 1H, -NH, D $_2$ O exchangeable), 7.869-7.874 (d, J = 2.0 Hz, 1H, Ar-H), 7.727-7.753 (dd, J = 8.4 Hz, 1H, Ar-H), 7.641-7.663 (d, J = 8.8 Hz, 1H, Ar-H), 7.477-7.495 (d, J = 7.2 Hz, 2H, 2 $\times$ Ar-H), 7.296-7.334 (t, J = 7.6 Hz, 2H, 2 $\times$ Ar-H), 7.188-7.224 (t, J = 7.2 Hz, 1H, Ar-H), 5.201-5.332 (m, 3H, CH & CH $_2$), 3.854 (s, 3H, CH $_3$), 1.552-1.570 (d, 3H, CH $_3$); Mass (m/z): 406.2 (M^{+1}).

Methyl 2-oxo-3-(phenethylamino)-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (7l): Yield: 56%; m.p.: 164.3-169.5 $^{\circ}\text{C}$, IR (KBr, ν_{max} , cm^{-1}): 3382 (-NH), 3028 (-CH arom.), 2969 (-CH aliph.), 1712 & 1695, 1230 (C-N), 1171 (C-F); ^1H NMR (400 MHz, DMSO- d_6 , δ ppm): 8.023-8.052 (t, 1H, -NH, D $_2$ O exchangeable), 7.959-7.964 (d, J = 2.0 Hz, 1H, Ar-H), 7.746-7.773 (dd, J = 8.8 Hz, 1H, Ar-H), 7.663-7.685 (d, J = 8.8 Hz, 1H, Ar-H), 7.276-7.328 (m, 4H, 4 $\times$ Ar-H), 7.181-7.224 (m, 1H, Ar-H), 5.213-5.281 (q, 2H, CH $_2$), 3.871 (s, 3H, CH $_3$), 3.624-3.676 (q, 2H, CH $_2$), 2.933-2.971 (t, 2H, CH $_2$); Mass (m/z): 406.2 (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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ with 0.5, 1 and 2 μM of the compounds and erlotinib. After removing the medium, 10 μL of MTT dye was added. Plates were incubated at 37 $^{\circ}\text{C}$ 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).

Docking studies: Molecular docking studies were carried out using AutoDock 4.2. The crystal structure of the target protein was obtained from the Protein Data Bank (PDB ID: 1M17). Water molecules and the co-crystallized ligand were removed from the protein structure, followed by the addition of polar hydrogens and calculation of Gasteiger charges. The ligands were drawn using ChemDraw 12.0 and saved in MOL format after energy minimization. The minimized structures were converted into PDB format using Discovery Studio. A grid box was generated with 60 grid points along each of the three coordinate axes. The Lamarckian Genetic Algorithm implemented in AutoDock 4.2 was used to generate the docking parameter file. Docking calculations were performed through the Cygwin interface, and the resulting docking log (DLG) files were analyzed to obtain the docking results. The docked ligand-protein interactions were visualized in 2D- and 3D formats using the Maestro v9.5 visualizer.

RESULTS AND DISCUSSION

The synthesis of the newly prepared 3-amino derivatives of methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**7a-l**) is outlined in **Scheme-I**. The synthesis started with the amination of methyl 4-chloro-3-nitrobenzoate (**1**) using trifluoroethylamine hydrochloride (TFA) in an autoclave at 60 $^{\circ}\text{C}$ to give methyl 3-nitro-4-((2,2,2-trifluoroethyl)amino)benzoate (**2**). Reduction of the nitro group

using G-cat afforded methyl 3-amino-4-((2,2,2-trifluoroethyl)-amino) benzoate (**3**). Treatment of compound **3** with ethyl 2-chloro-2-oxoacetate resulted in the cyclization to form methyl 2,3-dioxo-1-(2,2,2-trifluoroethyl)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**4**). Chlorination of compound **4** with SOCl $_2$ in the presence of a catalytic amount of DMF gave methyl 3-chloro-2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate (**5**). The final compounds **7a-l** were obtained by reacting compound **5** with the corresponding amines (**6a-l**) in DMF in presence of triethylamine.

The synthesized compounds were characterized by IR, ^1H NMR and mass spectrometry. The proposed structures of compounds **7a-l** were supported by their ^1H NMR spectra, which showed the expected signals arising from the aromatic and aliphatic proton environments. The mass spectra further supported the assigned structures, with molecular ion-related $[M+1]^+$ peaks corresponding to the expected molecular formulae of the respective compounds. The spectroscopic data were consistent with the proposed structures of the synthesized 3-aminoquinoxaline derivatives.

Bioassay

Antibacterial activity: Using the agar well diffusion method, the synthesized derivatives were screened for antimicrobial activity. Both Gram-positive and Gram-negative bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*, as well as *Escherichia coli* and *Pseudomonas aeruginosa*, were used to test their antibacterial activity. For comparison, ciprofloxacin at a concentration of 7 $\mu\text{g/mL}$ was used as the standard antibacterial agent. The negative control was a 1% DMSO solution that did not contain the test substances. The newly synthesized compounds **7a-l** showed varying degrees of inhibitory activity toward the chosen bacterial strains, according to the initial antibacterial screening results shown in Table-1. Notably, derivatives **7b**, **7c**, **7g**, **7h** and **7i** showed strong antibacterial action against all investigated microbes including the Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis* as well as the Gram-negative strains *Escherichia coli* and *Pseudomonas aeruginosa*.

Anticancer activity: The anticancer activity of the newly synthesized compounds **7a-l** was evaluated against two human breast cancer cell lines, MCF-7 and MDA-MB-231, using the MTT assay. Erlotinib was used as the reference drug, and the results are expressed as IC $_{50}$ values (μM) in Table-2. Among the synthesized derivatives, compounds **7e** and **7f** showed good anticancer activity against the tested cell lines when compared with erlotinib. The remaining derivatives showed lower activity under the same experimental conditions. The observed differences in activity may be related to the structural features of the substituents present in the synthesized derivatives.

The structure-activity relationship (SAR) study was used to examine the effect of different substituents attached to the quinoxaline nucleus on anticancer activity. Among the synthesized derivatives, compound **7e**, bearing a piperidine moiety, showed activity close to that of the reference drug erlotinib. Compound **7f**, containing a morpholine moiety, showed good activity, followed by **7e**. Derivatives **7a-d**, containing aliphatic nitrogen-based groups such as methylamine, isopro-

TABLE-1
RESULTS OF ANTIBACTERIAL BIOASSAY OF COMPOUNDS (**7a-l**) (CONCENTRATION USED 25 µg/mL OF DMSO)

Compound	R	Zones of inhibition (mm)			
		Gram-negative		Gram-positive	
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
7a	Methylamine	12.6	10.6	11.6	13.6
7b	Isopropylamine	32.6	30.6	33.6	30.6
7c	Cyclobutylamine	29.4	30.2	32.5	34.2
7d	Pyrrolidine	7.6	8.6	5.6	5.8
7e	Piperidine	4.0	5.0	4.0	5.0
7f	Morpholine	14.2	12.3	12.8	14.2
7g	Aniline	29.6	28.6	29.6	31.6
7h	Benzylamine	28.6	29.2	28.4	32.9
7i	2,6-Difluoro benzylamine	31.6	30.6	32.6	33.6
7j	1-Phenylethanamine	7.6	8.6	9.6	10.6
7k	2-Phenylethanamine	5.9	6.6	8.6	7.6
7l	N-Methyl benzyl amine	10.4	12.1	14.2	13.6
Std. drug	Ciprofloxacin (7 µg/mL of DMSO)	33.2	31.4	35.8	36.4

TABLE-2
In vitro ANTICANCER ACTIVITY OF 3-AMINO DERIVATIVES OF METHYL 2-OXO-1-(2,2,2-TRIFLUOROETHYL)-1,2-DIHYDROQUINOXALINE-6-CARBOXYLATE (**7a-l**) WITH IC₅₀ IN µM^[a]

Compound	^[b] MCF-7	^[c] MDA-MB-231	^[d] MCF-10A
7a	52.02 ± 0.15	56.02 ± 0.10	ND
7b	58.20 ± 0.16	62.10 ± 0.16	ND
7c	63.05 ± 0.12	65.02 ± 0.10	ND
7d	36.14 ± 0.05	39.05 ± 0.08	ND
7e	14.04 ± 0.07	15.10 ± 0.10	97.08 ± 0.09
7f	16.02 ± 0.04	17.05 ± 0.02	98.06 ± 0.06
7g	64.04 ± 0.12	69.30 ± 0.09	ND
7h	67.06 ± 0.12	69.10 ± 0.02	ND
7i	59.02 ± 0.10	62.40 ± 0.10	ND
7j	57.02 ± 0.07	62.05 ± 0.06	ND
7k	52.12 ± 0.07	57.10 ± 0.06	ND
7l	51.10 ± 0.10	53.10 ± 0.12	ND
Erlotinib	13.02 ± 0.05	15.06 ± 0.23	98.10 ± 0.14

Abbreviation: ND, not determined. ^[a]IC₅₀ 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]MDA-MB-231: breast cancer cell line. ^[d]MCF-10A: breast healthy cell line.

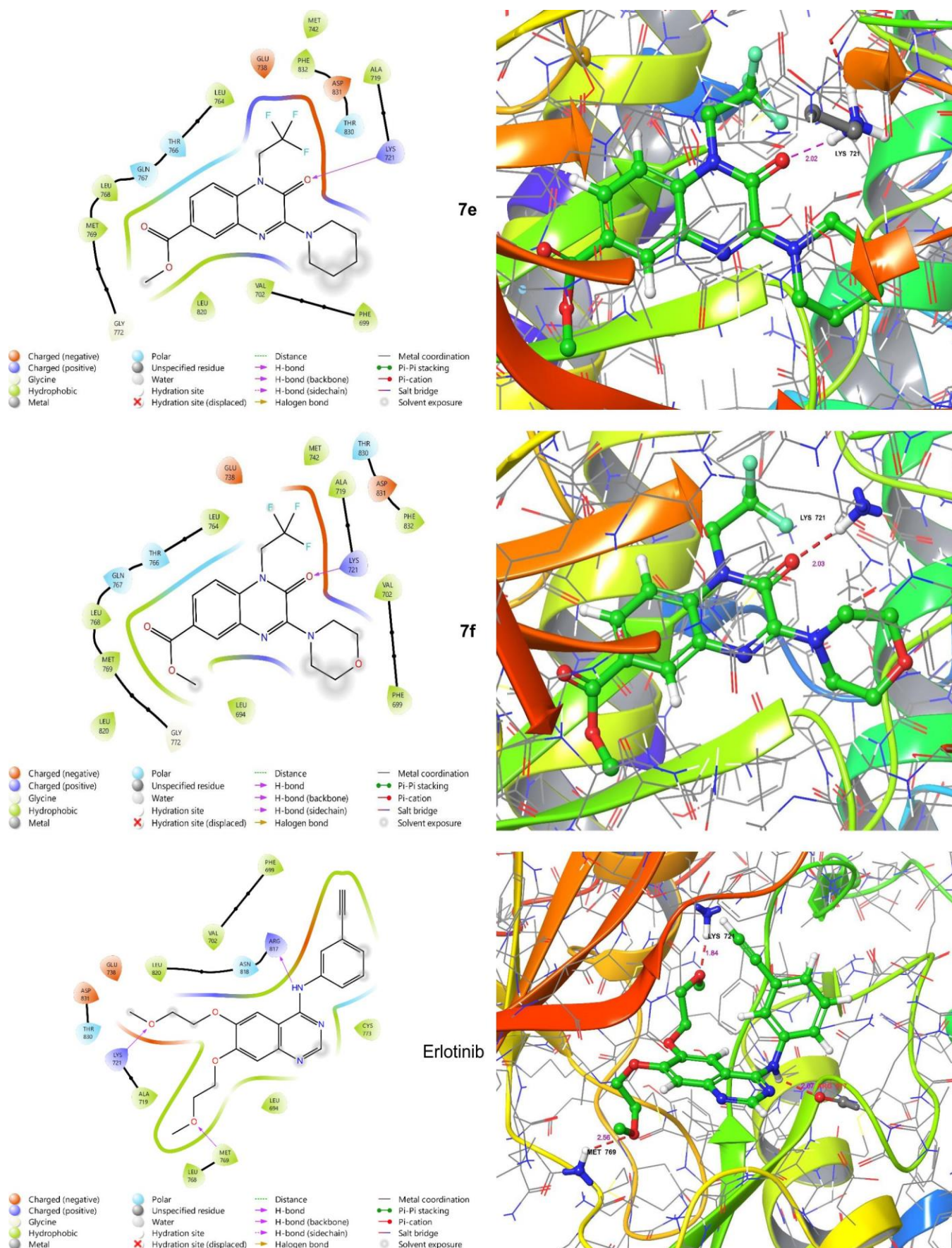
pylamine, cyclobutylamine and pyrrolidine, showed lower activity than erlotinib. Derivatives **7g-l**, containing aromatic amine substituents, showed comparatively lower activity against the tested cancer cell lines. These results suggest that the nature of the amine substituent attached to the quinoxaline nucleus has an important effect on the anticancer activity of the synthesized compounds.

The cytotoxicity of the two more active derivatives, **7e** and **7f**, was further evaluated using the normal breast cell line MCF-10A. Both compounds showed relatively low cytotoxicity toward the normal cell line, with IC₅₀ values close to 98.10 µM. This result suggests a difference in the response of the tested cancer and normal cell lines and supports further investigation of compounds **7e** and **7f**.

Molecular docking studies: Molecular docking studies were performed to investigate the binding interactions of compounds **7e** and **7f** with the epidermal growth factor receptor, PDB ID 1M17 [25]. The results revealed that both compounds exhibited favourable binding within the active site of EGFR and showed better docking scores than the reference inhibitor erlotinib. Compound **7e** demonstrated the highest binding affinity with a binding energy of -7.72 kcal/mol and an inhibition constant (K_i) of 2.18 µM, whereas compound **7f** showed a binding energy of -7.49 kcal/mol with a K_i value of 3.24 µM. In comparison, erlotinib exhibited a lower binding affinity with a docking score of -5.95 kcal/mol and a higher K_i value of 43.64 µM. Both compounds formed a key hydrogen bond interaction with the amino acid residue LYS721, with bond lengths of 2.02 Å for **7e** and 2.03 Å for **7f**, indicating stable ligand-receptor interactions (Table-3). Although erlotinib formed three hydrogen bonds involving LYS721, MET769 and ARG817, its overall binding energy was less favourable than those of the synthesized compounds (Fig. 1). These findings suggest that compounds **7e** and **7f** possess strong binding affinity toward EGFR and may serve as promising lead molecules for the development of novel EGFR-targeted anticancer agents.

TABLE-3
MOLECULAR DOCKING INTERACTION PARAMETERS OF COMPOUNDS **7e** AND **7f** WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR (PDB ID-1M17)

Entry	Binding energy (kcal/mol)	Inhibition constant (µM)	Number of hydrogen bonds	Residues involved in hydrogen bonding (bond length in Å)
7e	-7.72	2.18	1	LYS721 (2.02)
7f	-7.49	3.24	1	LYS721 (2.03)
Erlotinib	-5.95	43.64	3	LYS721 (1.84), MET769 (2.56), ARG817 (2.07)

Fig. 1. 2D and 3D interaction of compounds **7e**, **7f** and erlotinib

Conclusion

Twelve new 3-amino derivatives of methyl 2-oxo-1-(2,2,2-trifluoroethyl)-1,2-dihydroquinoxaline-6-carboxylate were synthesized from commercially available methyl 4-chloro-3-nitrobenzoate (**1**) in five steps. The synthesized compounds were evaluated for antibacterial activity against four bacterial strains, namely *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. Compounds **7b**, **7c**, **7g**, **7h**, and **7i** showed good antibacterial activity against all the tested bacterial strains. The compounds were further evaluated for anticancer activity against the breast cancer cell lines **MCF-7** and **MDA-MB-231**. Among the tested derivatives, compounds **7e** and **7f** showed good anticancer activity compared with the reference drug erlotinib. Molecular docking studies provided additional support for the observed anticancer activity and the proposed interaction of these compounds with the selected target. The present findings provide useful preliminary information on the biological activity of these newly synthesized quinoxaline derivatives.

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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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