Electronic Supplementary Information

Ligand / PTC-free intramolecular Heck reaction: Synthesis of pyrroloquinoxalines and their evaluation against PDE4 / luciferase / oral cancer cell growth in vitro and zebrafish in vivo

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Experimental Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane. $^1$H NMR and $^{13}$C NMR spectra were determined in CDCl$_3$ solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts ($\delta$) are relative to tetramethylsilane (TMS, $\delta$ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constants ($J$) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Microanalyses were performed using Perkin–Elmer 2400 C H N S/O analyzer.

1. **General Procedure for the preparation of 3-Chloro-N-aryl quinoxalin-2-amine (4/5)$^1$**

![Chemical structure](image)

A mixture of 2,3-dichloroquinoxaline 1/2 (1.0 mmol), an appropriate amine 3 (1.0 mmol) and AlCl$_3$ (1.25 mmol) in 1,2-dichloroethane (5 mL) was stirred at 80°C for 10-12 h under a nitrogen atmosphere. After completion of the reaction, the mixture was cooled to room temperature, poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with cold water (2 × 10 mL), brine (4 mL) and dried over anhydrous Na$_2$SO$_4$ and concentrated under vacuum. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using ethyl acetate/hexane to give the desired product 4/5.

2. **Preparation of N-benzyl-3-chloroquinoxalin-2-amine (4h/5g)**
A mixture of 2,3-dichloroquinoxaline 1/2 (0.01 mmol) and benzylamine 6 (0.015 mmol) in EtOH (5 mL) was heated under reflux for 5 h. After completion of the reaction, the reaction mass was cooled to room temperature and ethanol was removed under reduced pressure. The resulting solid was washed with water and dried to afford the desired products 4h and 5g.


Allyl bromide (3 mmol) was added to a solution of N-substituted quinoxaline-2-amine derivatives 4/5 (1 mmol) and sodium hydride in THF (10 mL) under nitrogen atmosphere. The reaction mixture was then stirred for 2 h at room temperature. After completion (confirmed by TLC), the mixture was diluted with ice water (3 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (5 mL), dried using anhydrous Na2SO4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane as eluent to afford the N-allylated quinoxaline-2-amine derivatives 7/8.

3.1. Preparation of N-allyl-3-chloro-N-phenylquinoxaline-2-amine (7a)

Yellow liquid; Yield: 85%; Rf (5% ethylacetate/n-Hexane): 0.60; IR (KBr) vmax: 2923, 1539, 1490, 1409, 1226 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.89 (d, J = 8.8 Hz, 1H), 7.86 (d, J =
9.2 Hz, 1H), 7.66 (td, \(J = 8.0, 1.2\) Hz, 1H), 7.55 (td, \(J = 8.0, 1.2\) Hz, 1H), 7.31 (t, \(J = 7.6\) Hz, 2H), 7.16 (t, \(J = 7.6\) Hz, 1H), 7.02 (d, \(J = 7.6\) Hz, 2H), 6.20-6.07 (m, 1H), 5.27 (dd, \(J = 17.2, 1.4\) Hz, 1H), 5.13 (dd, \(J = 10.0, 1.4\) Hz, 1H), 4.71 (d, \(J = 5.6\) Hz, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta 149.6, 146.5, 142.3, 140.2, 138.3, 134.2, 130.0, 129.2 (2C), 127.6, 127.3, 126.9, 124.9, 123.6 (2C), 117.3, 56.3\); m/z (CI): 296 (M\(^+\), 100%); HPLC: 94.6%, column: Symmetry C-18 75*4.6 mm 3.5\(\mu\)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210 nm, retention time 4.9 min.

### 3.2. Preparation of N-allyl-3-chloro-N-p-tolylquinoxaline-2-amine (7b)

![Chemical Structure](image)

Yellow liquid; Yield: 90%; \(R_f\) (5% ethylacetate/n-Hexane): 0.60; IR (KBr) \(\nu_{max}\): 2924, 1522, 1511, 1412, 1227 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.90-7.80\) (m, 2H), 7.64 (td, \(J = 7.6, 1.2\) Hz, 1H), 7.52 (td, \(J = 7.6, 1.2\) Hz, 1H), 7.11 (d, \(J = 8.2\) Hz, 2H), 6.91 (d, \(J = 8.2\) Hz, 2H), 6.19-6.06 (m, 1H), 5.24 (dd, \(J = 17.6, 1.4\) Hz, 1H), 5.12 (dd, \(J = 10.0, 1.4\) Hz, 1H), 4.66 (d, \(J = 5.6\) Hz, 2H), 2.34 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta 149.7, 143.9, 142.1, 140.2, 138.2, 134.8, 134.2, 130.0, 129.8 (2C), 127.6, 127.0, 126.8, 123.9 (2C), 117.3, 56.4, 20.9\); m/z (CI): 309 (M\(^+\), 100%); HPLC: 99.6%, column: Symmetry C-18 75*4.6 mm 3.5\(\mu\)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210 nm, retention time 5.2 min.

### 3.3. Preparation of N-allyl-3-chloro-N-(4-methoxyphenyl)quinoxaline-2-amine (7c)
3.4. Preparation of N-allyl-3-chloro-N-(4-fluorophenyl)quinoxaline-2-amine (7d)

Yellow liquid; Yield: 85%; Rf (5% ethylacetate/n-Hexane): 0.71; IR (KBr) ν\text{max}: 2921, 1506, 1410, 1219, 1072, 1410 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.92-7.82 (m, 2H), 7.66 (td, \(J = 8.0, 1.2\) Hz, 1H), 7.55 (td, \(J = 8.4, 1.2\) Hz, 1H), 7.10-6.98 (m, 4H), 5.22 (dd, \(J = 17.2, 1.6\) Hz, 1H), 5.14 (dd, \(J = 10.0, 1.2\) Hz, 1H), 4.64 (d, \(J = 6.0\) Hz, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): δ 160.2 (C-F \(J = 243.6\) Hz), 149.6, 142.5 (C-F \(J = 3.0\) Hz), 141.7, 140.0, 138.3, 133.8, 130.1 (2C), 127.6, 127.3, 126.9, 125.9 (C-F \(J = 8.3\) Hz), 117.7, 116.1 (C-F \(J = 22.8\) Hz, 2C), 56.6; m/z (CI): 314 (M+1, 100%); HPLC: 96.2%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (Gradient) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210 nm, retention time 6.0 min.
3.5. Preparation of N-allyl-3-chloro-N-(4-chlorophenyl)quinoxaline-2-amine (7e)

Yellow liquid; Yield: 80%; R_f (5% ethylacetate/n-Hexane): 0.54; IR (KBr) \( \nu_{\text{max}} \): 3061, 2925, 1511, 1424, 1238 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.92-7.83 (m, 2H), 7.67 (t, \( J = 7.6 \) Hz, 1H), 7.57 (t, \( J = 8.4 \) Hz, 1H), 7.27 (d, \( J = 8.8 \) Hz, 2H), 6.94 (d, \( J = 8.8 \) Hz, 2H), 6.18-6.03 (m, 1H), 5.25 (d, \( J = 17.2 \) Hz, 1H), 5.15 (d, \( J = 10.0 \) Hz, 1H), 4.68 (d, \( J = 6.0 \) Hz, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 149.3, 145.0, 142.1, 140.1, 138.5, 133.8, 130.2, 130.1, 129.3 (2C), 127.7, 127.6, 127.0, 124.7 (2C), 117.6, 56.2; m/z (CI): 330 (M\(^+\), 100%); HPLC: 99.6%, column: Symmetry C-18 75*4.6 mm 3.5\( \mu \)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (gradient) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.3 min.

3.6. Preparation of N-allyl-N-(4-bromophenyl)-3-chloroquinoxaline-2-amine (7f)

Yellow liquid; Yield: 95%; R_f (5% ethylacetate/n-Hexane): 0.72; IR (KBr) \( \nu_{\text{max}} \): 2920, 2853, 1539, 1484, 1416, 1226 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.92-7.85 (m, 2H), 7.68 (td, \( J = 7.6, 1.6 \) Hz, 1H), 7.58 (td, \( J = 8.4, 1.6 \) Hz, 1H), 7.42 (dd, \( J = 7.2, 2.0 \) Hz, 2H), 6.88 (dd, \( J = 7.2, 2.0 \) Hz, 2H), 6.11-6.04 (m, 1H), 5.26 (dd, \( J = 17.2, 1.6 \) Hz, 1H), 5.15 (dd, \( J = 10.4, 1.2 \) Hz, 1H), 4.69 (d, \( J = 5.6 \) Hz, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 149.3, 145.6, 142.1, 140.1, 138.6, 133.8, 132.3 (2C), 130.2, 127.7 (2C), 127.0, 124.9 (2C), 117.7, 117.6, 56.2; m/z (CI): 375 (M+1, 100%); HPLC: 96.2%, column: Symmetry C-18 75*4.6 mm 3.5\( \mu \)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (gradient) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210 nm, retention time 6.0 min.
3.7. Preparation of N-allyl-3-chloro-N-(3-fluorophenyl)quinoxalin-2-amine (7g)

Yellow liquid; Yield: 85%; \( R_f \) (5%ethylacetate/n-Hexane) 0.5; IR (KBr)\( v_{\text{max}} \): 3068, 2927, 1600, 1540, 1479, 1410 cm\(^{-1}\); 1H NMR (400 MHz, CDCl\(_3\)): 7.84 (d, \( J = 8.4 \) Hz, 1H), 7.82 (d, \( J = 8.8 \) Hz, 1H), 7.62 (td, \( J = 7.6, 1.4 \) Hz, 1H), 7.52 (t, \( J = 7.6 \) Hz, 1H), 7.21-7.13 (m, 1H), 6.76 (td, \( J = 8.0, 2.0 \) Hz, 1H), 6.70-6.62 (m, 2H), 6.07-5.96 (m, 1H), 5.22 (dd, \( J = 17.2, 1.6 \) Hz, 1H), 5.08 (dd, \( J = 10.4, 1.2 \) Hz, 1H), 4.65 (d, \( J = 5.2 \) Hz, 2H); 13C NMR (100 MHz, CDCl\(_3\)): 163.3 (C-F, \( J = 244.4 \) Hz), 149.2, 148.1 (C-F, \( J = 9.9 \) Hz), 142.5, 140.0, 138.7, 133.8, 130.2, 130.1, 127.9, 127.7, 127.0, 118.4 (C-F \( J = 2.9 \) Hz), 117.4, 111.4 (C-F, \( J = 21.3 \) Hz), 110.0 (C-F, \( J = 23.3 \) Hz), 56.1; m/z (CI): 314 (M+1, 100%); HPLC: 99.1%, column: Symmetry C-18 75*4.6 mm 3.5\( \mu \)m, mobile phase A: 0.1 % Formic Acid in water mobile phase, 0.5/50 B: ACN (gradient) T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210 nm, retention time 5.3 min.

3.8. Preparation of N-allyl-N-benzyl-3-chloroquinoxaline-2-amine (7h)

White liquid; Yield: 80%; \( R_f \) (5% ethylacetate/n-Hexane): 0.55; IR (KBr) \( v_{\text{max}} \): 3024, 2920, 1543, 1427 cm\(^{-1}\); 1H NMR (400 MHz, CDCl\(_3\)): 7.86 (d, \( J = 8.4 \) Hz, 1H), 7.79 (d, \( J = 7.2 \) Hz, 1H), 7.62 (td, \( J = 8.4, 1.6 \) Hz, 1H), 7.50 (td, \( J = 8.0, 1.2 \) Hz, 1H), 7.42 (d, \( J = 7.2 \) Hz, 2H), 7.32 (t, \( J = 6.8 \) Hz, 2H), 7.30-7.21 (m, 1H), 6.12-5.99 (m, 1H), 5.24 (d, \( J = 17.2 \) Hz, 2H), 4.78 (s, 2H), 4.13 (d, \( J = 6.0 \) Hz, 2H); 13C NMR (100 MHz, CDCl\(_3\)): 151.8, 140.8, 139.9, 137.8, 137.8, 133.8, 130.0, 128.3 (2C), 128.1 (2C), 127.5, 127.1, 126.8, 126.7, 118.6, 52.9, 52.7; m/z (CI): 310 (M+1, 100%); HPLC: 91.1%, column: Symmetry C-18 75*4.6 mm 3.5\( \mu \)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (gradient) T/%B:
Preparation of N-allyl-3-chloro-7-methyl-N-phenylquinoxalin-2-amine (8a)

Brown liquid; Yield: 90%; R_f (5% ethylacetate/n-Hexane): 0.63; IR (KBr) \( \nu_{\text{max}} \): 2925, 1534, 1404, 1223, 1073 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.81-7.74 (m, 1H), 7.65 (s, 1H), 7.38 (dd, \( J = 8.8, 1.6 \) Hz, 1H), 7.30 (td, \( J = 7.6, 2.0 \) Hz, 2H), 7.14 (t, \( J = 7.2 \) Hz, 1H), 7.00 (dd, \( J = 7.2, 1.2 \) Hz, 2H), 6.19-6.06 (m, 1H), 5.26 (dd, \( J = 17.2, 1.6 \) Hz, 1H), 5.13 (10.0 Hz, 1H), 4.70 (d, \( J = 5.6 \) Hz, 2H), 2.54 (s, 3H); m/z (Cl): 310 (M+1, 100%); HPLC: 98.1%, column: Symmetry C-18 75*4.6 mm 3.5\( \mu \)m, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.1 min.

Preparation of N-allyl-3-chloro-7-methyl-N-p-tolylquinoxalin-2-amine (8b)

Yellow liquid; Yield: 90%; R_f (5% ethylacetate/n-Hexane): 0.62; IR (KBr) \( \nu_{\text{max}} \): 2922, 1532, 1512, 1403, 1222, 1074 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.75 (d, \( J = 8.4 \) Hz, 1H), 7.63 (s, 1H), 7.36 (dd, \( J = 8.4, 1.6 \) Hz, 1H), 7.11 (d, \( J = 8.4 \) Hz, 2H), 6.90 (d, \( J = 8.0 \) Hz, 2H), 6.20-6.06 (m, 1H), 5.23 (dd, \( J = 17.2, 1.6 \) Hz, 1H), 5.12 (d, \( J = 10.0 \) Hz, 1H), 4.65 (d, \( J = 5.6 \) Hz, 2H), 2.54 (s, 3H), 2.33 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 149.8, 144.1, 140.5, 136.6, 134.6, 134.3, 129.8 (2C), 129.2, 127.1, 126.4, 126.0, 123.8 (2C), 123.5, 117.2, 56.3, 21.7, 20.9; m/z (Cl): 324 (M+1, 100%); HPLC: 99.7%, column: Symmetry C-18 75*4.6 mm
3.5μm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.5 min.

3.11. Preparation of N-allyl-3-chloro-N-(4-methoxyphenyl)-7-methylquinoxalin-2-amine (8c)

![Chemical structure of 8c](image_url)

Yellow liquid; Yield: 80%; Rf (5% ethylacetate/n-Hexane): 0.55; IR (KBr) \(\nu_{\text{max}}\): 2926, 1612, 1509, 1242 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.73 (d, \(J = 8.0\) Hz, 1H), 7.62 (s, 1H), 7.34 (dd, \(J = 8.4, 1.6\) Hz, 1H), 6.96 (d, \(J = 8.8\) Hz, 2H), 6.84 (d, \(J = 8.8\) Hz, 2H), 6.17- 6.07 (m, 1H), 5.21 (dd, \(J = 17.2, 1.6\) Hz, 1H), 5.11 (d, \(J = 10.4\) Hz, 1H), 4.60 (d, \(J = 6.0\) Hz, 2H), 3.80 (s, 3H), 2.53 (s, 3H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 157.2, 149.8, 140.5, 139.6, 137.2, 136.4, 134.1, 132.0, 128.9, 127.1, 126.0 (2C), 125.9, 117.4, 114.4 (2C), 56.6, 55.3, 21.7; m/z (CI): 340 (M+1, 100%); HPLC: 99.1%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.1 min.

3.12. Preparation of N-allyl-3-chloro-N-(4-fluorophenyl)-7-methylquinoxalin-2-amine (8d)

![Chemical structure of 8d](image_url)

Yellow liquid; Yield: 85%; Rf (5% ethylacetate/n-Hexane): 0.70; IR (KBr) \(\nu_{\text{max}}\): 2924, 1606, 1402, 1146, 1073 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.76 (d, \(J = 8.4\) Hz, 1H), 7.64 (s, 1H), 7.38 (dd, \(J = 8.4, 1.6\) Hz, 1H), 7.07-6.95 (m, 4H), 6.18-6.04 (m, 1H), 5.22 (d, \(J = 17.2\) Hz, 1H), 5.13 (d, \(J = 10.4\) Hz, 1H), 4.63 (d, \(J = 5.6\) Hz, 2H), 2.55 (s, 3H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 160.0 (C-F \(J = 243.3\) Hz), 149.6, 142.6 (C-F \(J = 3.0\) Hz), 140.7, 140.1, 137.8,
136.7, 133.9, 132.2, 129.4, 127.1, 126.6 (C-F $J = 25.4$ Hz), 126.0, 125.8 (C-F $J = 8.2$ Hz), 117.6, 116.0 (C-F $J = 22.5$ Hz), 56.5, 21.7; m/z (CI): 327 (M$^+$, 100%); HPLC: 99.7%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.5 min.

3.13. Preparation of N-allyl-N-(4-bromophenyl)-3-chloro-7-methylquinoxalin-2-amine (8e)

Yellow liquid; Yield: 85%; R$_f$ (5% ethylacetate/n-Hexane): 0.77; IR (KBr) $v_{max}$: 2922, 1534, 1488, 1222, 1073 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.78 (d, $J = 8.8$ Hz, 1H), 7.65 (s, 1H), 7.45-7.38 (m, 3H), 6.90-6.81 (m, 2H), 6.14-6.04 (m, 1H), 5.26 (dd, $J = 17.2$, 1.2 Hz, 1H), 5.13 (d, $J = 10.4$ Hz, 1H), 4.67 (d, $J = 5.4$ Hz, 2H), 2.55 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 149.3, 145.7, 140.8, 140.1, 138.3, 137.0, 133.9, 132.2 (2C), 129.9, 127.2, 126.6, 126.2, 124.7 (2C), 117.5, 56.1, 21.7; m/z (CI): 390 (M+2, 100%); HPLC: 93.2%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 220 nm, retention time 5.6 min.

3.14. Preparation of N-allyl-3-chloro-N-(3-fluorophenyl)-7-methylquinoxalin-2-amine (8f)

Yellow liquid; Yield: 85%; R$_f$ (5% ethylacetate/n-Hexane): 0.56; IR (KBr) $v_{max}$: 3066, 2924, 1606, 1402 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): 7.73 (dd, $J = 8.8$, 4.4 Hz, 1H), 7.62 (s, 1H),
7.36 (dd, $J = 8.4, 1.6$ Hz, 1H), 7.19-7.13 (m, 1H), 6.78-6.71 (m, 1H), 6.68-6.61 (m, 2H), 6.07-5.96 (m, 1H), 5.23 (dd, $J = 17.2, 1.2$ Hz, 1H), 5.08 (dd, $J = 10.4, 1.2$ Hz, 1H), 4.65 (d, $J = 5.6$ Hz, 2H) 2.48 (s, 3H); m/z (CI): 328 (M+1, 100%); HPLC: 99.1%, column:Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A:0.1 % Formic Acid in water mobile phase, B: ACN (gradient) T/%B: 0/50, 0.5/50, 3/98, 10/98, 10.5/50, 12/50;flow rate: 1.0 mL/min, UV 220 nm, retention time 5.6 min.

3.15. Preparation of N-allyl-N-benzyl-3-chloro-7-methylquinoxalin-2-amine (8g)

![Chemical Structure](image)

Colorless liquid; Yield: 80%; $R_f$ (5% ethylacetate/n-Hexane): 0.64; IR (KBr) $ν_{max}$: 3447, 3032, 2921, 1563, 1517, 1312, 1065 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $δ$ 7.76 (d, $J = 8.4$ Hz, 1H), 7.59 (s, 1H), 7.44 (d, $J = 7.4$ Hz, 2H), 7.36-7.28 (m, 3H), 7.27-7.25 (m, 1H), 6.10-5.99 (m, 1H), 5.24 (dd, $J = 17.2, 10.8$ Hz, 2H), 4.78 (s, 2H), 4.13 (d, $J = 6.4$ Hz, 2H), 2.53 (s, 3H).$^{13}$C NMR (100 MHz, CDCl$_3$): $δ$ 151.9, 140.5, 139.9, 137.9, 136.2, 133.9, 132.1, 129.0, 128.3 (2C), 128.1 (2C), 127.0, 126.6, 125.9, 118.4, 52.9, 52.7, 21.7; m/z (CI): 324 (M+1, 100%); HPLC: 97.3%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 215 nm, retention time 6.1 min.

4. General procedure for the preparation of 1,3-disubstituted pyrrolo[2,3-b]quinoxalines (9/10)

![Chemical Structure](image)

Pd(OAc)$_2$ (0.015 mmol) was added to a solution of N-allylated quinoxaline-2-amine derivatives 7/8 (1 mmol) and K$_2$CO$_3$ (3.0 mmol) in DMF (10 mL). The reaction mixture was stirred for 2h at 100 °C. It was then allowed to come to room temperature and diluted with
water. The organic part was extracted using ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (5 mL) and dried with anhydrous Na₂SO₄, filtered and concentrated under vacuum. The obtained residue was purified by flash chromatography on silica gel using hexane/ethyl acetate to obtain the desired pyrrolo[2,3-b]quinoxalines 9/10.

4.1. Preparation of 3-methyl-1-phenyl-1H-pyrrolo[2,3-b]quinoxaline (9a)

Light yellow solid; Yield: 82%; Mp: 130-132°C; Rₚ (10% ethylacetate/n-hexane): 0.32; IR (KBr) v_max: 2928, 2857, 1595, 1502, 1433 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.27-8.24 (m, 1H), 8.16-8.12 (m, 1H), 7.95-7.90 (m, 3H), 7.71-7.64 (m, 2H), 7.56 (t, J = 8.0 Hz, 2H), 7.34 (t, J = 7.5 Hz, 1H), 2.56 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 143.6, 141.7, 140.2, 139.2, 137.9, 134.4, 129.5 (2C), 129.1, 128.6, 127.8, 126.8, 125.9, 122.5 (2C), 111.6, 8.8 (CH₃); m/z (Cl): 260 (M+1, 100%); HPLC: 97.51%; column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN (Isocratic) T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.4 min; Elemental analysis found C, 78.49; H, 5.06; N, 16.42; C₁₇H₁₃N₃ requires C, 78.74; H, 5.05; N, 16.20.

4.2. Preparation of 3-methyl-1-p-tolyl-1H-pyrrolo[2,3-b]quinoxaline (9b)

Light yellow solid; Yield: 90%; Mp: 128-129 °C; Rₚ (10% ethylacetate/n-hexane): 0.43; IR (KBr) v_max: 2925, 2871, 1605, 1518, 1434 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 8.29-8.21 (m, 1H), 8.15-8.09 (m, 1H), 7.88 (s, 1H), 7.75 (d, J = 8.2Hz, 2H), 7.71-7.64 (m, 2H), 7.36 (d, J = 8.2 Hz, 2H), 2.56 (s, 3H), 2.43 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 143.5, 141.8, 140.2, 139.3, 135.8, 135.3, 134.7, 130.0 (2C), 129.1, 128.6, 127.6, 126.6, 122.7 (2C), 111.2, 21.0 (C₆H₄CH₃), 8.7 (CH₃); m/z (Cl): 274 (M+1, 100%); HPLC: 99.8%; column: Symmetry C-18
75*4.6 mm 3.5\(\mu\)m; mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.8 min; Elemental analysis found C, 79.35; H, 5.46; N, 15.19; \(\text{C}_{18}\text{H}_{15}\text{N}_{3}\) requires C, 79.10; H, 5.53; N, 15.37.

4.3. Preparation of 1-(4-methoxyphenyl)-3-methyl-1\(H\)-pyrrolo[2,3-\(b\)]quinoxaline (9c)

Light yellow solid; Yield: 85%; \(\text{Mp}: 222-223\degree\text{C}\); \(R_f\) (10% ethylacetate/\(n\)-hexane): 0.36; IR (KBr) \(v_{\text{max}}\): 2919, 2850, 1447 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.27 (dd, \(J = 8.0, 2.8\) Hz, 1H), 8.13 (dd, \(J = 8.0, 2.8\) Hz, 1H), 7.84 (s, 1H), 7.75 (d, \(J = 8.8\) Hz, 2H), 7.71-7.64 (m, 2H), 7.09 (d, \(J = 8.8\) Hz, 2H), 3.90 (s, 3H), 2.56 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 157.9, 143.3, 141.8, 140.2, 139.3, 135.0, 131.0, 129.1, 128.5, 127.6, 126.6, 124.4 (2C), 114.7 (2C), 110.9, 55.6 (OCH\(_3\)), 8.7 (CH\(_3\)\); m/z (CI): 289 (M\(^+\), 100%); HPLC: 99.0%, column: Symmetry C-18 75*4.6 mm 3.5\(\mu\)m, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (gradient) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 265 nm, retention time 4.6 min; Elemental analysis found C, 74.59; H, 5.26; N, 14.62; \(\text{C}_{18}\text{H}_{15}\text{N}_{3}\text{O}\) requires C, 74.72, H, 5.23; N, 14.52.

4.4. Preparation of 1-(4-fluorophenyl)-3-methyl-1\(H\)-pyrrolo[2,3-\(b\)]quinoxaline (9d)

Light yellow solid; Yield: 81%; \(\text{Mp}: 157-160\degree\text{C}\); \(R_f\) (10% ethylacetate/\(n\)-hexane): 0.33; IR (KBr) \(v_{\text{max}}\): 2924, 2857, 1511, 1431, 1222 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.30-8.23 (m, 1H), 8.14-8.08 (m, 1H), 7.91-7.81 (m, 3H), 7.72-7.65 (m, 2H), 7.31-7.20 (m, 2H), 2.56 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 160.6 (C-F \(J = 244.4\) Hz), 143.4, 141.7, 140.3, 139.2,
134.3, 133.9 (C-F J = 3.0 Hz), 129.1, 128.5 (2C), 127.9, 126.9, 124.4 (C-F J = 8.3 Hz), 116.3 (C-F J = 22.8 Hz, 2C), 111.7, 8.7 (CH3); m/z (Cl): 278 (M+1, 100%); HPLC: 98.9%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 260 nm, retention time 4.4 min; Elemental analysis found C, 73.33; H, 4.45; N, 15.31; C17H12FN3 requires C, 73.63; H, 4.36; N, 15.15.

4.5. Preparation of 1-(4-chlorophenyl)-3-methyl-1H-pyrrolo[2,3-b]quinoxaline (9e)

Light yellow solid; Yield: 85%; Mp: 141-142 °C; Rf (10% ethylacetate/n-hexane): 0.28; IR (KBr) vmax: 2921, 1643, 1493, 1426 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.30-8.25 (m, 1H), 8.15-8.10 (m, 1H), 7.91-7.87 (m, 3H), 7.75-7.68 (m, 2H), 7.53 (d, J = 8.8 Hz, 2H), 2.56 (s, 3H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): 143.6, 141.7, 140.3, 139.2, 136.4, 133.7, 131.2, 129.5 (2C), 129.1, 128.5, 128.0, 127.0, 123.5 (2C), 112.3, 8.7 (CH3); m/z (Cl): 292 (M+1, 100); HPLC: 99.6%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.5 min; Elemental analysis found C, 69.70; H, 4.17; N, 14.18; C\(_{17}\)H\(_{12}\)ClN\(_3\) requires C, 69.51; H, 4.12; N, 14.30.

4.6. Preparation of 1-(4-bromophenyl)-3-methyl-1H-pyrrolo[2,3-b]quinoxaline (9f)

Light yellow solid; Yield: 92%; Mp: 216-218 °C; Rf (10% ethylacetate/n-hexane): 0.41; IR (KBr) vmax: 2924, 2857, 1591, 1496, 1427 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.31-8.24 (m, 1H), 8.18-8.11 (m, 1H), 7.92-7.84 (m, 3H), 7.77-7.68 (m, 3H), 2.57 (s, 3H); \(^13\)C NMR (100
MHz, CDCl₃): δ 143.6, 141.7, 140.3, 139.2, 136.9, 133.6, 132.5 (2C), 129.2, 128.5, 128.0, 127.0, 123.8 (2C), 118.9, 112.4, 8.7 (CH₃); m/z (CI): 339 (M+1, 100%); HPLC: 88.8%, column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 272 nm, retention time 5.1 min; Elemental analysis found C, 60.13; H, 3.76; N, 12.62 C₁₇H₁₂BrN₃ requires C, 60.37; H, 3.58; N, 12.42.

4.7. Preparation of 1-(3-fluorophenyl)-3-methyl-1H-pyrrolo[2,3-b]quinoxaline (9g)

![Chemical structure](image)

Light yellow solid; Yield: 90%; Mp: 128-130 °C; Rf (10% ethylacetate/n-hexane): 0.35; IR (KBr) v_max: 3061, 2927, 1728, 1488, 1179 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): 8.42-8.37 (m, 1H), 8.23-8.18 (m, 1H), 7.97 (s, 1H), 7.85 (d, J = 10.0 Hz, 1H), 7.78-7.69 (m, 3H), 7.53 (q, J = 8.0, 6.8 Hz, 1H), 7.06 (td, J = 8.0, 1.6 Hz, 1H), 2.60 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): 163.1 (C-F J = 245.8 Hz), 142.0, 139.1, 139.0, 138.8, 134.8, 130.7 (C-F J = 9.2 Hz), 128.6 (2C), 128.3, 128.1, 127.8, 117.3 (C-F J = 3.1 Hz), 112.8 (C-F J = 21.1 Hz), 112.0, 109.8 (C-F J = 25.8 Hz), 8.9 (CH₃); m/z (CI): 278 (M+1, 100%); HPLC: 96.2%, column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (gradient) T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.8 min; Elemental analysis found C, 73.40; H, 4.46; N, 15.34; C₁₇H₁₂FN₃ requires C, 73.63; H, 4.36; N, 15.15.

4.8. Preparation of 1-benzyl-3-methyl-1H-pyrrolo[2,3-b]quinoxaline (9h)

![Chemical structure](image)

Light yellow solid; Yield: 80%; Mp: 133-134 °C; Rf (10% ethylacetate/n-hexane): 0.42; IR (KBr) v_max: 2915, 2860, 1581, 1453, 1352 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.25 (dd, J = 8.0, 2.4 Hz, 1H), 8.11 (dd, J = 8.4, 2.0 Hz, 1H), 7.71-7.61 (m, 2H), 7.45 (s, 1H), 7.37-7.22
(m, 5H), 5.49 (s, 2H), 2.45 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 142.8, 142.2, 140.2, 139.3, 137.1, 135.1, 129.1, 128.8 (2C), 128.1, 127.8, 127.7 (2C), 127.5, 126.2, 109.9, 47.6 (CH$_2$), 8.7 (CH$_3$); m/z (CI): 274 (M+1, 100%); HPLC: 98.4%, column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 254 nm, retention time 4.1 min; Elemental analysis found C, 79.31; H, 5.56; N, 15.22; C$_{18}$H$_{15}$N$_3$ requires C, 79.10; H, 5.53; N, 15.37.

4.9. Preparation of 3,7-dimethyl-1-phenyl-1H-pyrrolo[2,3-b]quinoxaline (10a)

![Chemical structure of 10a]

Light yellow solid; Yield: 89%; Mp: 138-140 °C; R$_f$ (10% ethylacetate/n-hexane): 0.34; IR (KBr) $\nu_{max}$: 2923, 2857, 1596, 1504, 1429 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.13 (d, $J = 8.4$ Hz, 1H), 7.92-7.87 (m, 3H), 7.85 (s, 1H), 7.59-7.48 (m, 3H), 7.32 (t, $J = 7.4$ Hz, 1H), 2.59 (s, 3H), 2.54 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 141.8, 139.2, 138.0, 137.9, 133.6, 130.2, 129.4 (2C), 128.4, 128.0, 127.3, 125.7, 122.4 (3C), 111.5, 22.6 (CH$_3$ attached to quinoxaline), 8.7 (CH$_3$ attached to pyrrole); m/z (CI): 274 (M+1, 100%); HPLC: 98.9%, column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.7 min; Elemental analysis found C, 79.29; H, 5.57; N, 15.42; C$_{18}$H$_{13}$N$_3$ requires C, 79.10; H, 5.53; N, 15.37.

4.10. Preparation of 3,7-dimethyl-1-p-tolyl-1H-pyrrolo[2,3-b]quinoxaline (10b)

![Chemical structure of 10b]

Light yellow solid; Yield: 90%; Mp: 141-142 °C; R$_f$ (10% ethylacetate/n-hexane): 0.25; IR (KBr) $\nu_{max}$: 2917, 2864, 1517, 1431, 1186 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.13 (d, $J = 8.4$, 1H), 7.89 (s, 1H), 7.84-7.81 (m, 1H), 7.75 (d, $J = 8.8$ Hz, 2H), 7.50 (dd, $J = 8.4$, 2.0 Hz, 1H), 7.35 (d, $J = 8.4$ Hz, 2H), 2.59 (s, 3H), 2.54 (s, 3H), 2.43 (s, 3H); $^{13}$C NMR (100 MHz,
4.11. Preparation of 1-(4-methoxyphenyl)-3,7-dimethyl-1H-pyrrolo[2,3-b]quinoxaline (10c)

Pale yellow solid; Yield: 80%; Mp: 219-221 °C; Rf (10% ethylacetate/n-Hexane) 0.30; IR (KBr) $\nu_{\text{max}}$: 2925, 1443, 1254 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.13 (d, $J = 8.4$ Hz, 1H), 7.88 (s, 1H), 7.80-7.71 (m, 3H), 7.50 (d, $J = 8.4$ Hz, 1H), 7.07 (d, $J = 8.8$ Hz, 2H), 3.88 (s, 3H), 2.59 (s, 3H), 2.54 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 158.0, 140.7, 139.4, 135.1, 130.8, 130.6, 130.6, 128.1, 127.3, 124.9, 124.7, 124.6, 114.7, 114.7 (2C), 110.3, 55.6 (OCH$_3$), 22.6 (CH$_3$ attached to quinoxaline), 14.1 (CH$_3$ attached to pyrrole); m/z (Cl): 304 (M+1, 100%); HPLC: 99.87%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.3 min; Elemental analysis found C, 75.47; H, 5.66; N, 13.65; C$_{19}$H$_{17}$N$_3$O requires C, 75.23; H, 5.65; N, 13.85.

4.12. Preparation of 1-(4-fluorophenyl)-3,7-dimethyl-1H-pyrrolo[2,3-b]quinoxaline (10d)
Light yellow solid; Yield: 85%; Mp: 142 °C; Rf (10% ethylacetate/n-hexane): 0.35; IR (KBr) v\text{max}: 2921, 2867, 1514, 1442, 1220 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.14 (d, J = 8.8, 1H), 7.88 (s, 1H), 7.86-7.78 (m, 2H), 7.79 (s, 1H), 7.52 (dd, J = 8.8, 1.6 Hz, 1H), 7.27-7.24 (m, 2H), 2.60 (s, 3H), 2.54 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 160.5 (C-F J = 244.4 Hz), 142.8, 141.8, 139.3, 138.8, 138.2, 133.5, 129.3, 128.6, 127.9 (C-F J = 6.0 Hz), 127.3, 124.2 (C-F J = 8.4 Hz, 2C), 116.2 (C-F J = 22.8 Hz, 2C), 111.7, 21.7 (CH₃ attached to quinoxaline), 8.7 (CH₃ attached to pyrrole); m/z (Cl): 292 (M⁺+ 1, 100); HPLC: 99.6%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic acid in water, mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 4.5 min; Elemental analysis found C, 74.40; H, 4.86; N, 14.21; C₁₈H₁₄FN₃ requires C, 74.21; H, 4.84; N, 14.42.

4.13. Preparation of 1-(4-bromophenyl)-3,7-methyl-1H-pyrrolo[2,3-b]quinoxaline (10e)

![Chemical Structure](image)

Light yellow solid; Yield: 84%; Mp: 140-142 °C; Rf (10% ethylacetate/n-hexane): 0.33; IR (KBr) v\text{max}: 2917, 1517, 1431, 1186, 816 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.13 (d, J = 8.4Hz, 1H), 7.89 (s, 1H), 7.83 (d, J = 9.2 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.58-7.50 (m, 2H), 2.60 (s, 3H), 2.54 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 142.9, 141.7, 140.4, 139.2, 138.4, 132.8, 132.4, 130.4, 129.4, 128.6, 127.9, 127.3, 123.6 (2C), 118.7, 112.3, 21.8 (CH₃ attached to quinoxaline), 8.7 (CH₃ attached to pyrrole); m/z (Cl): 352 (M⁺, 100), 354 (M⁺+2, 98); HPLC: 98.0%, column: Symmetry C-18 75*4.6 mm 3.5μm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (Isocratic) T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 280 nm, retention time 5.5 min; Elemental analysis found C, 61.19; H, 4.06; N, 11.77; C₁₈H₁₄BrN₃ requires C, 61.38; H, 4.01; N, 11.93.

4.14. Preparation of 1-(3-fluorophenyl)-3,7-dimethyl-1H-pyrrolo[2,3-b]quinoxaline (10f)
Yellow solid; Yield: 90%; Mp: 103-105°C; Rf (10% ethylacetate/n-hexane): 0.32; IR (KBr) $v_{\text{max}}$: 3046, 2925, 1603, 1432, 814 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.15 (d, $J = 8.4$ Hz, 1H), 8.03 (d, $J = 8.4$ Hz, 1H), 7.92 (s, 1H), 7.86 (t, $J = 8.4$ Hz, 1H), 7.69 (d, $J = 8.0$ Hz, 1H), 7.53 (m, 1H), 7.02 (td, $J = 8.4$, 1.6 Hz, 1H), 2.61 (s, 3H), 2.54 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.1 (C-F $J = 243.0$ Hz), 141.9, 139.2, 138.6, 137.5, 133.2, 130.7, 130.6, 130.5, 129.8, 128.1, 127.4 (2C), 117.0 (C-F $J = 3.0$ Hz), 112.4 (C-F $J = 22.6$ Hz), 109.5 (C-F $J = 25.5$ Hz), 21.8 (CH$_3$ attached to quinoxaline), 8.8 (CH$_3$ attached to pyrrole); m/z (CI): 292 (M+1, 100%); HPLC: 98.1%, column: Symmetry C-18 75*4.6 mm 3.5$\mu$m, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (gradient) T/%B: 0/50, 5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 270 nm, retention time 5.4 min; Elemental analysis found C, 74.40; H, 4.86; N, 14.32; C$_{18}$H$_{14}$FN$_3$ requires C, 74.21; H, 4.84; N, 14.42.

**4.15. Preparation of 1-benzyl-3,7-methyl-1H-pyrrolo[2,3-b]quinoxaline (10g)**

Light yellow solid; Yield: 91%; Mp: 125-127 °C; Rf (10% ethylacetate/n-hexane): 0.37; IR (KBr) $v_{\text{max}}$: 2920, 2867, 1601, 1450, 1354, 1183 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): 8.14 (d, $J = 8.4$ Hz, 1H), 7.89 (s, 1H), 7.48 (d, $J = 8.8$ Hz, 1H), 7.45-7.40 (m, 1H), 7.37-7.25 (m, 5H), 5.48 (s, 2H), 2.60 (s, 3H), 2.44 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): 142.1, 139.3, 138.7, 137.8, 137.2, 134.7, 134.4, 128.7, 128.7 (2C), 127.9, 127.8, 127.7 (2C), 126.9, 109.9, 47.6 (CH$_2$), 21.8 (CH$_3$ attached to quinoxaline), 8.8 (CH$_3$ attached to pyrrole); m/z (CI): 288 (M+1, 100%); HPLC: 98.7%, column: Symmetry C-18 75*4.6 mm 3.5$\mu$m, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN T/%B: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 255 nm, retention time 4.4 min; Elemental analysis found C, 74.40; H, 5.90; N, 14.78; C$_{19}$H$_{17}$N$_3$ requires C, 74.41, H, 5.96; N, 14.62.
Biology

PDE4B protein production and purification

PDE4B cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-Mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 % NP40). Recombinant His-tagged PDE4B protein was purified as previously described in a literature.1 Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4 °C and supernatant was collected. Supernatant was mixed with Ni-NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris-HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10 % glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 hour at 4 °C. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4 °C and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris-HCl pH 8.5, 1 M KCl, 10 mM 2-Mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS-PAGE. Eluates containing PDE4B protein were pooled and stored at -80 °C in 50% glycerol until further use.

PDE4B enzymatic assay

The inhibition of PDE4B enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer’s recommendations. Briefly, 10 ng of PDE4B enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 µM) for 1 h. The reaction was halted with stop solution followed by incubation with detection reagent for 10
minutes in dark. Luminescence values (RLUs) were measured by a Multilabel plate reader (Perkin Elmer 1420 Multilabel counter). The percentage of inhibition was calculated using the following formula and IC\textsubscript{50}s were computed using GraphPad Prism Version 5.04 software.

\[
\text{% inhibition} = \frac{(RLU \text{ of vehicle control} - RLU \text{ of inhibitor})}{RLU \text{ of vehicle control}} \times 100
\]

**Reference:**


**Molecular modeling studies (PDE4B binding):**

**Method:** The docking analysis of molecules was performed using Maestro, version 9.2 [1] implemented from Schrödinger molecular modeling suite. All molecules were sketched in 3D format using build module of maestro and LigPrep module was used to produce low-energy conformers of the molecules. The crystal structure coordinates of target protein (PDE4B, PDB ID: 1XMY) [2] were obtained from the protein data bank. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS-2005 force field. The grid for molecular docking was generated with bound co-crystallized ligand. Molecules were docked using Glide in extra-precision mode [3], with up to three poses saved per molecule. The ligands were kept flexible by producing the ring conformations and by penalizing non-polar amide bond conformations, whereas the receptors were kept rigid throughout the docking studies. The lowest energy conformations were selected and, the ligand interactions (Docking score, hydrogen bonding and hydrophobic interaction) with target protein were determined.

**References**


**Table 1**: Glide score and contributing XP parameters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>GScore</th>
<th>LipophilicEvdW</th>
<th>PhobEn</th>
<th>PhobEnHB</th>
<th>HBond</th>
<th>Electro</th>
<th>LowMW</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>-8.02</td>
<td>-4.53</td>
<td>-1.65</td>
<td>-0.75</td>
<td>-0.55</td>
<td>-0.15</td>
<td>-0.5</td>
</tr>
<tr>
<td>9d</td>
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<td>-4.50</td>
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<td>-0.75</td>
<td>-0.53</td>
<td>-0.17</td>
<td>-0.5</td>
</tr>
<tr>
<td>Rolipram</td>
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<td>-4.65</td>
<td>-1.98</td>
<td>-1.95</td>
<td>-1.49</td>
<td>-0.51</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein-ligand vdW energy
PhobEn: Hydrophobic enclosure reward
PhobEnHB: Reward for hydrophobically packed hydrogen bond
HBond: Rewards for hydrogen bonding interaction between ligand and protein
Electro: Electrostatic reward
LowMW: Reward for ligands with low molecular weight
**Figure S1:** Binding mode of 9a at the active site of PDE4B.
**Molecular modeling studies (Firefly Luciferase)**

**Method:** The docking analysis of molecules was performed using Maestro, version 9.2 [1] implemented from Schrödinger molecular modeling suite. The crystal structure coordinates of target protein (Firefly Luciferase, PDB ID: 4E5D) [2] were obtained from the protein data bank. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS-2005 force field. The grid for molecular docking was generated with bound co-crystallized ligand. Molecules were docked using Glide in extra-precision mode [3], with up to three poses saved per molecule. The ligands were kept flexible by producing the ring conformations and by penalizing non-polar amide bond conformations, whereas the receptors were kept rigid throughout the docking studies.

**References**


**Table 2:** Glide score and contributing XP parameters.

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<tr>
<th>Compound</th>
<th>GScore</th>
<th>LipophilicEvdW</th>
<th>Electro</th>
<th>LowMW</th>
<th>RotPenal</th>
<th>PhobicPenal</th>
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</thead>
<tbody>
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<tr>
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<td>-0.50</td>
<td>0.10</td>
<td>0.17</td>
</tr>
</tbody>
</table>

LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy
Electro: Electrostatic reward
LowMW: Reward for ligands with low molecular weight
RotPenal: Rotatable bond penalty
PhobicPenal: Penalty for exposed hydrophobic ligand group

![Diagram of molecular structure with labeled amino acids]
Fig. S3: Binding mode of 9a at the binding site of firefly luciferase.
Cell Proliferation assay

The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on normal and cancer cells was evaluated using the SRB (Sulphorhodamine B) cell proliferation assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as to vehicle-treated cells. Further, this assay is the standardized assay of choice for anticancer compound screening at the National Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number.

In brief, the assay was performed as follows: Cancer and non-cancer cells were seeded in 96-well plates and incubated overnight. We chose the non-cancerous 293T cell line (human epithelial cells) and the cancerous cell line CAL27 (human tongue adenosquamous carcinoma...
cells) for the evaluation. The optimum cell numbers to be seeded were determined by a growth curve analysis for each cell line. In the initial (single dose) screen, compounds (dissolved in 100% DMSO to a stock concentration of 100mM) were added to the adhered cells at a final concentration of 10 μM. After 72h of treatment, the cells were washed with phosphate-buffered saline and then ice-cold 10% trichloroacetic acid added to the cells to precipitate all proteins for 1h at 4 °C. The cells were then washed with water. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 30 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye solubilized with 10 mM Tris solution. Absorbance of solubilized dye was measured at a wavelength of 590 nm.

Percentage growth was determined by the formula

\[ \left[ \frac{A_t - A_0}{A_c - A_0} \right] \times 100 \]

where, \( A_t \) = absorbance after 72h of test compound treatment, 
\( A_0 \) = Absorbance at time 0,  
\( A_c \) = Absorbance after 72h without treatment.

The known cytotoxic agent Gemcitabine was used as a positive control in the assay.

References


Zebrafish embryo studies:

Materials and Methods:

**Husbandry:** Zebra fish obtained from a local vendor were maintained in in-house built recirculatory system under 14-10 h light dark cycle and 28 °C temperature. Breeding of zebrafish was allowed in breeding chamber and embryos obtained were maintained in petridishes (Westerfield, 2000).
**Teratogenicity assay:** Stock solution of each compound was prepared in 100% DMSO and serial dilutions were done to obtain required working stock solutions in 0.1% DMSO. The embryos at 24 h stage were removed and dechorinated manually using forceps. The embryos were distributed in 24 well plate (3/well) and respective concentration of compound was added to each well where n=6. The embryos were tested with concentrations starting from 1µM to 30µM. The plate was incubated at 28 °C until 5dpf. The embryos were washed with PBS and anesthetized using tricaine (0.008%). Morphological scoring was done based on the procedure previously described (Panzica-Kelly et al, 2010).

**Statistical analysis:** Compounds were tested for teratogenicity. Each embryo was scored based on their level of toxicity from 5 being non toxic and 0.5 being highly toxic. Statistical analysis for scoring was done using graphpad prism software using two ways ANOVA. The graph shown in Fig. 6 represents the teratogenic scoring given compared to the positive control Phenobarbital.

**Hepatotoxicity assay:** The working stocks of the compounds were prepared by serial dilutions as described above. The embryos of 4dpf were distributed in 24 well plates along with 250 µL of 0.1% DMSO with 6 embryos in each well. Each well is added with the respective working stock solutions to obtain the final concentration of 1, 3, 10 and 30µM concentration of the drug. The plate was incubated at 28 °C until 7dpf.

Embryos were washed with E3 medium on 7dpf and anaesthetized using tricaine. The qualitative scoring is given to the liver based on the toxicity level (Normal, Mild Hepatotoxicity, Severe Hepatotoxicity and Death).

**Apoptosis Assay:** 24hpf embryos were taken and dechorionated manually. The working stock solutions were prepared by serial dilution as described earlier. Three embryos were distributed in each well of 24 well plates along with 250µl of 0.1% DMSO. Each well was added with 250 µL of respective concentration to obtain final working concentration. Embryos were incubated at 28 °C for 24 h and 48 h.

The embryos at respective time periods (24 h and 48 h) were washed thrice with E3 medium and incubated with acridine orange (concentration: 2µg/ml) solution for 30 min. Then embryos were washed again with E3 medium to wash the acridine orange solution. The embryos were anaesthetized using tricaine and images were taken using zeiss florescence microscope using GFP filter under 5× magnification.
$^1$H NMR of 9b

$^{13}$C NMR of 9b
$^1$H NMR of 9d

$^{13}$C NMR of 9d
$^1$H NMR of 9e

$^{13}$C NMR of 9e
$\text{^1H NMR of 9g}$

$\text{^{13}C NMR of 9g}$
$^1$H NMR of 9h

$^{13}$C NMR of 9h
$^{1}H$ NMR of 10a

$^{13}C$ NMR of 10a
$^1$H NMR of 10b

$^{13}$C NMR of 10b
$^1$H NMR of 10c

$^{13}$C NMR of 10c
$^1$H NMR of 10d
$^{13}$C NMR of 10d

![13C NMR spectrum of 10d with chemical shifts and a molecular structure diagram]
$^1$H NMR of 10e

$^{13}$C NMR of 10e
$^1$H NMR of 10f

$^{13}$C NMR of 10f
$^1$H NMR of 10g
$^{13}$C NMR of 10g