

## Electronic Supporting Information

### Transition metal free hydrolysis / cyclization strategy in a single pot: Synthesis of fused furo *N*-heterocycles of pharmacological interest

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## Experimental

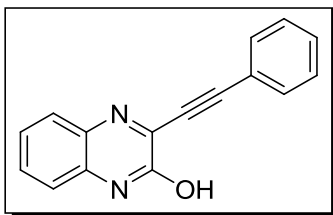
### Chemistry

**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 or iodine spray.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3/\text{DMSO-}d_6$  solution by using 400 and 100 MHz spectrometers (VARIAN 400 MR), 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), dd (doublet of doublet), td (triplet of doublet) and m (multiplet) as well as bs (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer (FT/IR-4200, JASCO). Melting points were determined by using melting point apparatus (Buchi melting point B-540) and are uncorrected. MS spectra were obtained on a mass spectrometer (AGILENT 6430 triple quadrupole LC-MS). Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

### General procedure for the preparation of **3**:

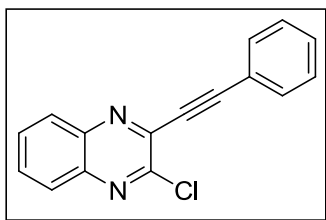
A mixture of 2,3-dichloro compound (**1**, 1.256 mmol), 10% Pd/C (0.0125 mmol), CuI (0.0125 mmol),  $\text{PPh}_3$  (0.05 mmol) and  $\text{Et}_3\text{N}$  (1.8844 mmol) in EtOH (4 mL) was stirred for 15 min. To this was added an appropriate terminal alkyne (**2**, 1.256 mmol), under a nitrogen atmosphere and the mixture was stirred at 60 °C for 2-4 h. After completion of the reaction over, the mixture was diluted with EtOAc (3 x 10 mL). The organic layers were collected, combined, washed with cold water (2 x 15 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under vacuum. The crude product was then purified by column chromatography on silica gel using EtOAc / *n*-hexane as a solvent system.

### **3-(Phenylethynyl)quinoxalin-2-ol (3aa)**



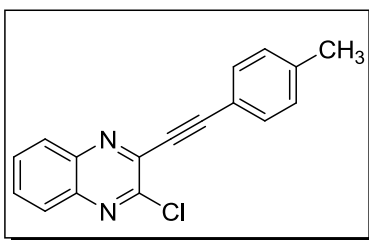
Off white semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ) ppm: 12.86-12.85 (m, 1H), 7.76 (d,  $J = 7.8$  Hz, 1H), 7.68-7.61 (m, 2H), 7.56 (dd,  $J = 9.2, 3.9$  Hz, 1H), 7.52-7.43 (m, 3H), 7.35-7.27 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ ) ppm: 154.4, 143.5, 132.7, 132.5 (2C), 132.2 (2C), 131.7, 130.6, 129.4 (2C), 124.1, 121.2, 115.9, 95.3, 86.8; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3226, 2138, 1461, 673. MS (ESI)  $m/z$ : 246.9 [M + H].

### 2-Chloro-3-(phenylethynyl)quinoxaline (3a)



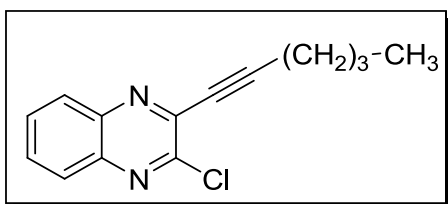
Light yellow solid; mp: 103-105 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.19-7.87 (m, 2H), 7.87-7.57 (m, 4H), 7.57-7.31 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 147.9, 140.7, 140.2, 138.5 (2C), 132.9, 131.9, 130.2 (2C), 129.4, 129.4, 128.9, 128.6, 121.1, 97.1, 85.4; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2213, 1497, 1193, 721; MS (ESI)  $m/z$ : 265.5 [M + H].

### 2-Chloro-3-(p-tolyethynyl)quinoxaline (3b)



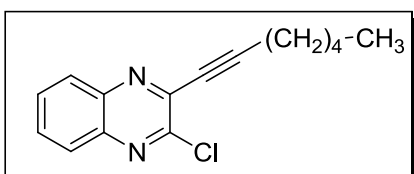
Light brown semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.14-8.08 (m, 1H), 8.05-8.00 (m, 1H), 7.83-7.75 (m, 2H), 7.63 (d,  $J = 8.0$  Hz, 2H), 7.27-7.21 (m, 2H), 2.43 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 144.3, 140.2, 140.0 (2C), 139.9 (2C), 137.7, 133.5, 129.9 (2C), 128.8, 128.7 (2C), 128.0, 90.3, 85.2, 21.2; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2891, 2218, 1522, 677; MS (ESI)  $m/z$ : 278.9 [M + H].

### 2-Chloro-3-(hex-1-ynyl)quinoxaline (3c)



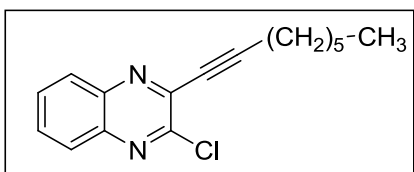
Brown liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.51 (d,  $J = 6.2$  Hz, 1H), 8.24 (d,  $J = 7.2$  Hz, 1H), 7.77-7.72 (m, 2H), 2.04-2.02 (m, 2H), 1.54-1.50 (m, 2H), 1.42-1.38 (m, 2H), 0.92 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 151.9, 148.7, 144.8, 143.9, 126.8, 126.5, 119.7, 117.3, 81.5, 79.7, 34.3, 23.5, 18.9, 13.3; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2938, 2853, 2198, 1546, 701; MS (ESI)  $m/z$ : 245.7 [M + H].

### 2-Chloro-3-(hept-1-ynyl)quinoxaline (3d)



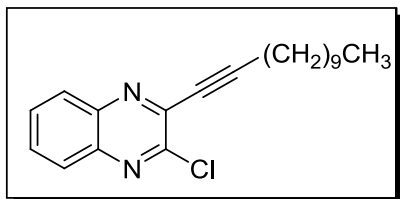
Light yellow semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.11 (d,  $J = 8.2$  Hz, 1H), 7.86 (d,  $J = 7.8$  Hz, 1H), 7.44-7.41 (m, 2H), 2.20-2.18 (m, 2H), 2.16-2.15 (m, 2H), 1.88-1.78 (m, 2H), 1.53-1.51 (m, 2H), 1.02 (t,  $J = 7.3$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 156.9, 149.0, 148.9, 129.0, 128.9 (2C), 123.8, 115.3, 112.8, 91.6, 79.3, 37.3, 30.0, 28.1, 14.2; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2957, 2830, 2213, 1504, 677; MS (ESI)  $m/z$ : 259.1 [M + H].

### 2-Chloro-3-(oct-1-ynyl)quinoxaline (3e)



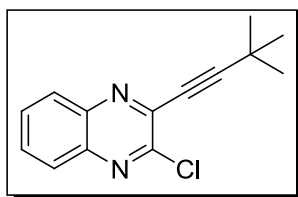
Light yellow semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 7.96 (d,  $J = 6.4$  Hz, 1H), 7.89 (d,  $J = 6.5$  Hz, 1H), 7.67-7.66 (m, 2H), 2.51 (t,  $J = 7.0$  Hz, 2H), 1.75-1.74 (m, 2H), 1.44-1.42 (m, 2H), 1.32-1.31 (m, 4H), 0.89 (t,  $J = 6.0$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 156.0, 144.4, 139.5, 129.4, 128.3 (2C), 126.9, 125.7, 106.6, 79.2, 72.0, 30.8, 29.5, 28.2, 27.3, 14.6; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2973, 2851, 2218, 1521, 768; MS (ESI)  $m/z$ : 273.2 [M + H].

### 2-Chloro-3-(dodec-1-ynyl)quinoxaline (3f)



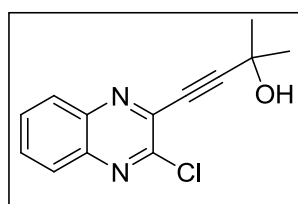
Light brown semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.07 (d,  $J = 7.4$  Hz, 1H), 8.00 (d,  $J = 7.4$  Hz, 1H), 7.80-7.70 (m, 2H), 2.59 (t,  $J = 7.0$  Hz, 2H), 1.76-1.67 (m, 2H), 1.58-1.46 (m, 2H), 1.30-1.24 (m, 12H), 0.87 (t,  $J = 6.7$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 148.0, 140.5, 140.1, 138.8, 131.0, 130.4, 128.6, 128.1, 100.2, 78.9, 31.8, 29.5, 29.4, 29.3, 29.0, 28.9, 27.9, 22.6, 19.7, 14.1; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2961, 2844, 2235, 1468, 647; MS (ESI)  $m/z$  : 329.1 [M + H].

### 2-Chloro-3-(3, 3-dimethylbut-1-ynyl) quinoxaline (3g)



Light yellow liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.14-7.89 (m, 2H), 7.81-7.56 (m, 2H), 1.44 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ) ppm: 151.9, 144.8, 140.9, 140.3, 129.5(2C), 128.1, 91.5, 79.7, 30.2, 29.7 (3C); IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) : 2893, 2264, 1531, 784; MS (ESI)  $m/z$ : 245.6 [M + H].

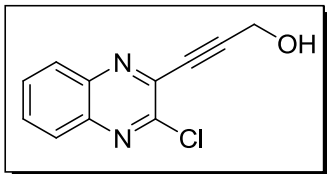
### 4-(3-Chloroquinoxalin-2-yl)-2-methylbut-3-yn-2-ol (3h)



Light green solid; mp: 77-79°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.07 (d,  $J = 6.2$  Hz, 1H), 8.03 (d,  $J = 6.8$  Hz, 1H), 7.82-7.74 (m, 2H), 2.61 (s, 1H), 1.73 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,

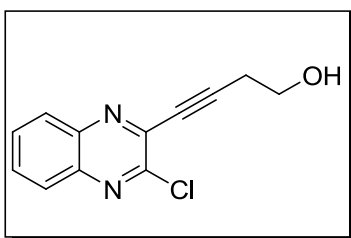
$\text{CDCl}_3$ ) ppm: 150.9, 147.1, 144.0, 141.7, 141.3, 129.3 (2C), 127.8 (2C), 96.2, 73.5, 50.2, 31.4;  
IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3358, 2858, 2217, 1527, 1102, 677; MS (ESI)  $m/z$ : 247.4 [M + H].

### 3-(3-Chloroquinoxalin-2-yl)prop-2-yn-1-ol (3i)



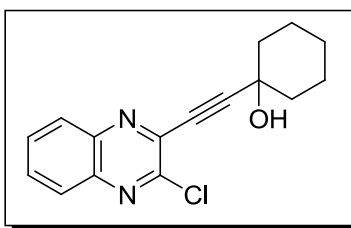
Brown semi solid; mp: 153-155°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.08-7.96 (m, 2H), 7.81-7.74 (m, 2H), 2.17 (s, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 151.1, 140.5, 140.2, 140.1, 130.6, 129.8 (2C), 129.5, 85.7, 84.9, 51.0; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3443, 2231, 1529, 697; MS (ESI)  $m/z$ : 219.3 [M + H].

### 4-(3-chloroquinoxalin-2-yl)but-3-yn-1-ol (3j)



Light green semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.13 (d,  $J = 6.4$  Hz, 1H), 8.19 (d,  $J = 6.3$  Hz, 1H), 7.73-7.71 (m, 2H), 4.15-4.13 (m, 2H), 3.20-3.19 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 153.1, 152.9, 145.5, 144.4, 143.7, 132.1, 131.3, 127.7, 99.4, 78.9, 63.0, 23.0; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3301, 2938, 2217, 1478, 718; MS (ESI)  $m/z$ : 232.9 [M + H].

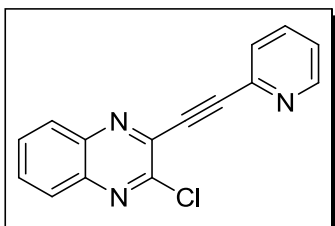
### 1-((3-Chloroquinoxalin-2-yl)ethynyl)cyclohexanol (3k)



Off white solid; mp: 134-136°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.07 (d,  $J = 6.4$  Hz, 1H), 7.99 (d,  $J = 6.4$  Hz, 1H), 7.82-7.80 (m, 2H), 2.42 (s, 1H), 2.13 (t,  $J = 9.4$  Hz, 2H), 1.83-1.66

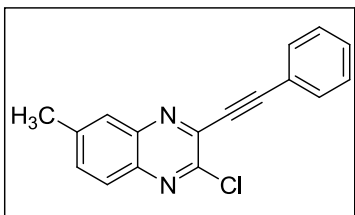
(m, 8H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 142.0 (2C), 141.8 (2C), 139 (2C), 135.0 (2C), 101.6, 69.1, 39.6 (2C), 39.6, 39.4, 25.0, 23.1; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3342, 2217, 1478, 718; MS (ESI)  $m/z$ : 287.1 [M + H].

### 2-Chloro-3-(pyridin-2-ylethynyl)quinoxaline (3l)



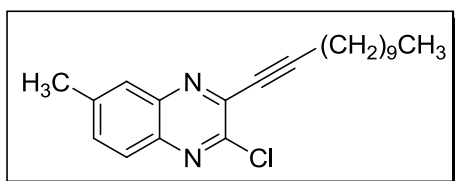
Light gray solid; mp: 134-136 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.72 (d,  $J = 4.7$  Hz, 1H), 8.17 (d,  $J = 4.4$  Hz, 1H), 8.07-7.99 (m, 1H), 7.85-7.80 (m, 2H), 7.75-7.73 (m, 2H), 7.39-7.33 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 151.6, 150.5, 146.6, 143.4, 141.1, 139.7, 132.8, 130.1, 129.9, 128.7, 128.2, 127.1, 109.9, 92.6, 84.2; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2203, 1518, 677; MS (ESI)  $m/z$ : 266.2 [M + H].

### 2-Chloro-6-methyl-3-(phenylethynyl)quinoxaline (3m)



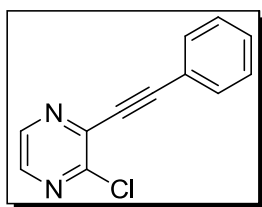
Light yellow semi solid;  $^1\text{H}$ NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.15-8.08 (m, 1H), 8.06-7.98 (m, 1H), 7.84-7.76 (m, 2H), 7.63 (d,  $J = 8.0$  Hz, 2H), 7.27-7.20 (m, 2H), 2.43 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 148.0, 140.7, 140.6, 140.2, 138.7, 132.4, 131.2, 130.6, 129.3, 128.8, 128.2, 118.1, 97.7, 85.1, 21.7; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2934, 2221, 1450, 791; MS (ESI)  $m/z$  : 278.9 [M + H].

### 2-Chloro-3-(dodec-1-ynyl)-6-methylquinoxaline (3n)



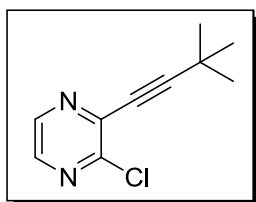
Light brown liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 7.91 (d,  $J = 8.5$  Hz, 1H), 7.85 (d,  $J = 8.5$  Hz, 1H), 7.79 (s, 1H), 7.73 (s, 1H), 7.57 (d,  $J = 8.5$  Hz, 2H), 2.56-2.55 (m, 4H), 1.75-1.66 (m, 2H), 1.56-1.46 (m, 4H), 1.32-1.30 (m, 8H), 0.87 (t,  $J = 6.7$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 141.9, 141.1, 133.2, 132.7, 128.1, 127.6, 127.5, 127.0, 99.8, 87.7, 31.8, 29.5, 29.4, 29.2, 29.0, 28.1, 28.0, 22.6, 21.8, 19.7, 14.0; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2934, 2852, 2213, 1530, 761; MS (ESI)  $m/z$  : 342.9 [M + H].

### 2-Chloro-3-(phenylethynyl)pyrazine (3o)



Light yellow liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.50 (d,  $J = 3.1$  Hz, 1H), 8.29 (d,  $J = 2.8$  Hz, 1H), 7.65 (d,  $J = 7.3$  Hz, 2H), 7.47-7.36 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 159.3, 146.8, 146.7, 139.9, 132.0, 128.4 (2C), 115.4, 113.9, 106.9, 97.0, 81.9; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2221, 1508, 674; MS (ESI)  $m/z$ : 214.9 [M + H].

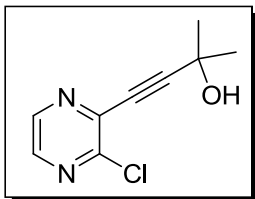
### 2-Chloro-3-(3,3-dimethylbut-1-ynyl)pyrazine (3p)



Light green liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.42 (d,  $J = 2.9$  Hz, 1H), 8.23 (t,  $J = 3.6$  Hz, 1H), 1.38 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 150.7, 141.9, 141.1, 139.9, 108.0, 75.5, 30.3 (3C), 28.4; IR(KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2949, 2237, 1494, 701; MS (ESI)  $m/z$ : 195.1 [M + H].

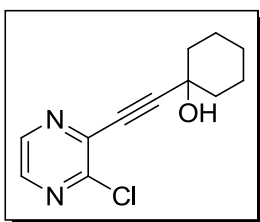


#### 4-(3-Chloropyrazin-2-yl)-2-methylbut-3-yn-2-ol (3q)



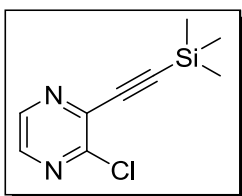
Light yellow liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.47 (d,  $J = 3.1$  Hz, 1H), 8.32 (d,  $J = 3.4$  Hz, 1H), 2.46 (s, 1H), 1.68 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 154.1, 144.8, 141.7, 138.3, 99.7, 78.8, 34.0, 28.0 (2C); IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3392, 2853, 2211, 1460, 655; MS (ESI)  $m/z$ : 196.1 [M + H].

#### 1-((3-Chloropyrazin-2-yl)ethynyl)cyclohexanol (3r)



Brown semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.46 (d,  $J = 2.4$  Hz, 1H), 8.29 (d,  $J = 2.4$  Hz, 1H), 2.44 (s, 1H), 2.09-2.07 (m, 2H), 1.77-1.74 (m, 4H), 1.61 (d,  $J = 9.1$  Hz, 2H), 1.33-1.21 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 150.7, 142.0, 141.8, 139.0, 80.2, 79.6, 39.4 (3C), 25.0 (2C), 23.1; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3432, 2853, 2243, 1529, 701; MS (ESI)  $m/z$ : 236.9 [M + H].

#### 2-Chloro-3-((trimethylsilyl)ethynyl)pyrazine (3s)



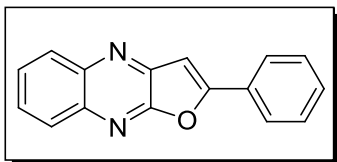
Light yellow liquid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 7.87 (d,  $J = 4.6$  Hz, 1H), 8.06-7.95 (d,  $J = 4.4$  Hz, 1H), 1.27 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 147.8, 140.3, 140.2, 137.8,

104.5, 99.4, -0.71 (3C); IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2938, 2210, 1546, 632; MS (ESI) m/z: 211.2 [M + H].

#### General procedure for the preparation of 4:

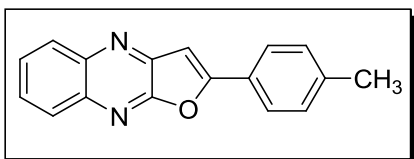
A mixture of compound **3** (0.75 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.38 mmol) in 1:4 DMSO-H<sub>2</sub>O (3 mL) was stirred at 80°C for 1-6 h. After completion of the reaction, the crude mass was diluted with ethyl acetate (20 mL) and water (10 mL) and then extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using EtOAc / *n*-hexane as a solvent system.

#### 2-Phenylfuro[2,3-*b*]quinoxaline (4a)<sup>1</sup>



White solid; mp: 258–260 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.21–8.20 (m, 2H), 8.12–8.10 (m, 2H), 7.85–7.83 (m, 2H), 7.66–7.61 (m, 3H), 7.23 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 161.1, 147.9, 140.7, 140.1, 138.8, 134.2 (2C), 131.1 (2C), 130.6, 128.7 (2C), 128.2 (2C), 114.3 (2C); HPLC: 97.1 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 7.2 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 1563, 1498, 771; MS (ESI) m/z: 246.9 [M + H].

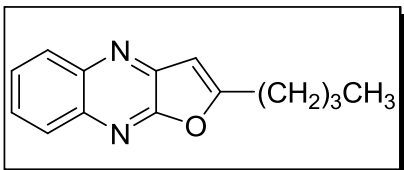
#### 2-*p*-Tolylfuro[2,3-*b*]quinoxaline (4b)



Light Yellow solid; mp: 205-207 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.18 (d, *J* = 5.6, 1H), 8.12 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.80-7.70 (m, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 2.47 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 158.9, 155.9,

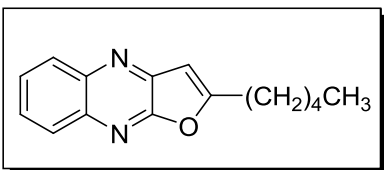
145.6, 143.0 (2C), 141.9, 130.2 (2C), 129.1, 128.8 (2C), 126.8, 124.3, 121.1, 120.6, 115.6, 20.9; HPLC: 98.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.43 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2837, 1481, 792; MS (ESI) m/z: 261.1 [M + H].

#### 2-Butylfuro[2,3-*b*]quinoxaline (4c)



Brown solid; mp: 103-105 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.16 (d, *J* = 6.7 Hz, 1H), 8.10 (d, *J* = 5.6 Hz, 1H) 7.78-7.77 (m, 2H), 6.02 (s, 1H), 2.88-2.67 (m, 2H), 2.55-2.40 (m, 2H), 2.16-1.89 (m, 2H), 0.88 (t, *J* = 6.3 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 153.7, 152.5, 145.4, 142.0 (2C), 136.1, 129.9, 129.6, 127.0, 124.5, 96.3, 31.6, 22.5, 19.6, 13.9; HPLC: 96.9%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.61 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2941, 2868, 1477, 735; MS (ESI) m/z : 226.8 [M + H].

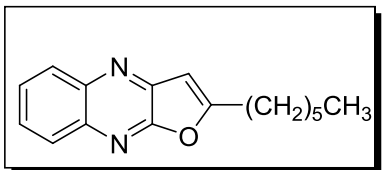
#### 2-Pentylfuro[2,3-*b*]quinoxaline (4d)



Light yellow semi solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.09 (d, *J* = 5.7 Hz, 1H), 8.06 (d, *J* = 5.6 Hz, 1H), 7.65 (d, *J* = 6.4 Hz, 2H), 6.63 (s, 1H), 2.86 (t, *J* = 7.5 Hz, 2H), 1.78-1.77 (m, 2H), 1.43-1.25 (m, 4H), 0.86 (t, *J* = 6.8 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 150.2, 145.4 (2C), 136.2, 136.1, 129.4 (2C), 127.1, 125.0, 108.4, 30.5, 30.3, 29.7, 22.5, 13.9; HPLC: 99.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20;

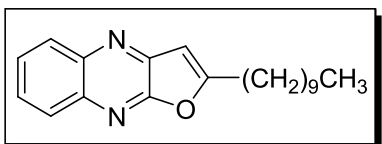
flow rate:1.0 mL/min; UV 229 nm, retention time 4.44 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2936, 2871, 1463, 784; MS (ESI) m/z: 240.8 [M + H].

#### 2-hexylfuro[2,3-b]quinoxaline (4e)



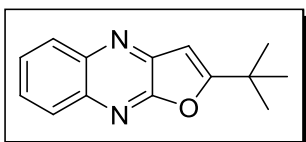
Dark brown semi solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.24 (d, *J* = 6.8 Hz, 1H), 8.14 (d, *J* = 6.7 Hz, 1H), 7.80-7.78 (m, 3H), 2.95 (t, *J* = 7.5 Hz, 2H), 1.93-1.91 (m, 2H), 1.47-1.46 (m, 2H), 1.40-1.37 (m, 4H), 0.93 (t, *J* = 6.9 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 156.0, 155.6, 141.7, 138.3, 128.6 (2C), 128.4 (2C), 128.0, 102.5, 31.4, 29.6, 28.8, 28.7, 22.4, 14.0. HPLC: 99.1%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.78 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2943, 2856, 1494, 692; MS (ESI) m/z: 254.9 [M + H].

#### 2-Decylfuro[2,3-b]quinoxaline (4f)



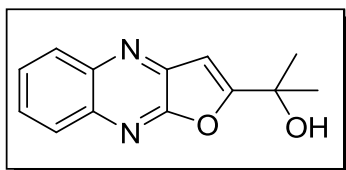
Brown solid; mp: 130-132°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.20 (d, *J* = 7.2 Hz, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.73-7.72 (m, 2H), 6.71 (s, 1H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.90-1.81 (m, 2H), 1.50-1.42 (m, 2H), 1.41-1.35 (m, 2H), 1.29-1.26 (m, 10H), 0.88 (t, *J* = 6.7 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 170.0, 154.1, 144.8, 141.7, 138.3, 128.6 (2C), 128.4, 128.0, 102.6 (2C), 31.8, 29.6, 29.5, 29.4, 29.2(C), 26.7, 22.6, 14.1; HPLC: 99.2%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.18 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2921, 2835, 1439, 721; MS (ESI) m/z : 310.9 [M + H].

#### 2-tert-Butylfuro[2,3-b]quinoxaline (4g)<sup>2</sup>



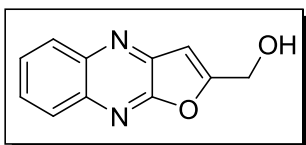
Light yellow solid; mp: 98-100 °C (found); 98°C (lit<sup>2</sup>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.17-8.12 (m, 1H), 8.10-8.05 (m, 1H), 7.73-7.67 (m, 2H), 6.65 (s, 1H), 1.46 (s, 9H); HPLC: 98.9%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 6.15 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2945, 2866, 1563, 1498, 771; MS (ESI) m/z: 227.1 [M + H].

#### 2-(Furo[2,3-*b*]quinoxalin-2-yl)propan-2-ol (4h)



Off white semi solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.14 (d, *J* = 6.2 Hz, 1H), 8.01 (d, *J* = 6.1 Hz, 1H), 7.78-7.77 (m, 2H), 7.43 (s, 1H), 2.04 (s, 1H), 1.66 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 163.3, 155.6, 141.6, 139.7, 129.7 (2C), 128.1 (2C), 123.7, 98.5, 60.7, 30.9 (2C). HPLC: 97.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 7.0 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 3328, 2837, 1566, 692; MS (ESI) m/z: 229.1 [M + H].

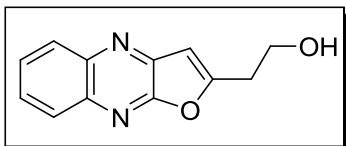
#### Furo[2,3-*b*]quinoxalin-2-ylmethanol (4i)



Light brown solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.16-7.91 (m, 1H), 7.78-7.77 (m, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.18 (s, 1H), 6.95 (s, 1H), 2.99 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 145.4, 136.2, 136.1, 129.4, 127.0, 125.0, 124.5, 115.7, 114.1, 108.4, 63.5; HPLC: 98.8%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in

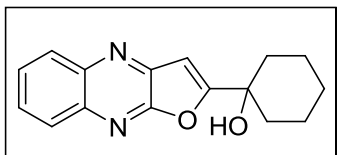
water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 4.9 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 3353, 2857, 1491, 724; MS (ESI) m/z: 200.9 [M + H].

#### 2-(furo[2,3-*b*]quinoxalin-2-yl)ethanol (4j).



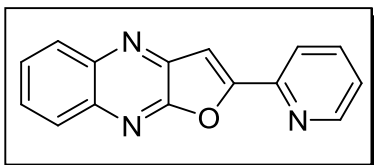
Light green semi solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.19-8.18 (m, 1H), 8.10-8.09 (m, 1H), 7.74-7.72 (m, 2H), 6.83 (s, 1H), 4.17 (t, *J* = 6.0 Hz, 2H), 3.21 (t, *J* = 6.0 Hz, 2H), 2.53 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 166.4, 153.9, 144.1, 141.5, 138.2, 128.7, 128.6, 128.1, 104.3, 59.5, 33.1, 29.6; HPLC: 95.7%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.22 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 3355, 2951, 2869, 1537, 1441, 818; MS (ESI) m/z: 214.9 [M + H].

#### 1-(Furo[2,3-*b*]quinoxalin-2-yl)cyclohexanol (4k).



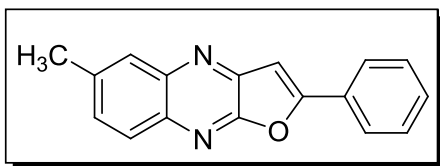
Light yellow solid; mp: 178-180°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 8.24-8.17 (m, 1H), 8.15-8.07 (m, 1H), 7.75-7.73 (m, 2H), 6.94 (s, 1H), 2.27 (s, 1H), 2.18 (m, 2H), 1.97 (d, *J* = 9.3 Hz, 2H), 1.88-1.87 (m, 2H), 1.70 (t, *J* = 9.5 Hz, 2H), 1.55-1.31 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 154.0, 144.2, 141.9, 138.5, 128.8 (2C), 128.7, 128.6, 128.2, 101.2, 71.2, 35.7 (2C), 25.1, 21.4 (2C); HPLC: 96.9%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.54 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 3367, 2923, 2852, 1546, 1432, 751; MS (ESI) m/z: 268.8 [M + H].

#### 2-(Pyridin-2-yl)furo[2,3-*b*]quinoxaline (4l)



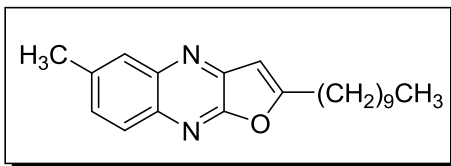
Light green solid; mp: 220-222°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.79 (d,  $J = 7.8$  Hz, 1H), 8.23 (s, 1H), 8.13 (d,  $J = 7.0$  Hz, 2H), 7.91 (d,  $J = 7.1$  Hz, 1H), 7.77-7.75 (m, 3H), 7.41 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 162.5, 154.4, 150.4, 147.4, 144.1, 142.5, 139.1, 137.1, 129.2 (2C), 129.0, 128.7, 128.5, 121.1, 104.1; HPLC: 97.18 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.05 % formic acid in water, mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 7.41 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3355, 2951, 2869, 1537, 1441, 818; MS (ESI)  $m/z$  : 247.9 [M + H].

#### 6-Methyl-2-phenylfuro[2,3-*b*]quinoxaline (4m)



Light yellow; mp: 182-184°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.04-8.07 (m, 2H), 7.94 (d,  $J = 7.2$  Hz, 1H), 7.46-7.58 (m, 5H), 7.27 (s, 1H), 2.52 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 140.7, 138.6, 131.1, 131.0, 130.6, 129.1(2C), 128.2, 128.1(2C), 127.5 (2C), 127.6, 126.0 (2C), 100.8, 21.8; HPLC: 98.9 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 6.42 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2843, 1533, 1428, 718; MS (ESI)  $m/z$  : 261.1 [M + H].

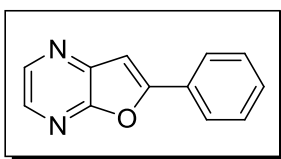
#### 2-Decyl-6-methylfuro[2,3-*b*]quinoxaline (4n)



Dark brown solid; mp: 74-76°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.01 (d,  $J = 8.5$  Hz, 1H), 7.89 (d,  $J = 7.2$  Hz, 1H), 7.55 (d,  $J = 8.6$  Hz, 1H), 6.68 (s, 1H), 2.91 (t,  $J = 7.5$  Hz, 2H), 2.59

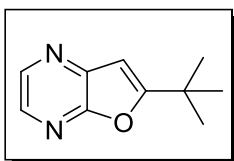
(s, 3H), 1.84 (d,  $J = 7.5$  Hz, 2H), 1.45 (d,  $J = 5.3$  Hz, 2H), 1.35-1.26 (m, 12H), 0.88 (t,  $J = 6.5$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 158.9, 155.9, 145.6 (2C), 139.4, 135.8, 129.1 (2C), 126.8, 120.6, 115.4, 94.6, 39.2, 33.5, 28.5, 28.4, 27.5, 24.3, 22.9, 18.7, 14.0; HPLC: 99.0 %; column: XBridge C-18 150\*4.6 mm  $5\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 5.42 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2918, 2857, 1471, 677; MS (ESI)  $m/z$ : 325.1 [M + H].

### 6-Phenylfuro[2,3-*b*]pyrazine (4o)<sup>3</sup>



Off white solid; mp: 107-109°C (found); 112–113°C (lit<sup>3</sup>);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.54 (d,  $J = 1.9$  Hz, 1H), 8.25 (d,  $J = 2.4$  Hz, 1H), 7.99 (d,  $J = 7.1$  Hz, 2H), 7.65-7.64 (m, 3H), 7.28 (s, 1H); HPLC: 99.1 %; column: XBridge C-18 150\*4.6 mm  $5\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 7.34 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2941, 1563, 1452, 788; MS (ESI)  $m/z$ : 196.8 [M + H].

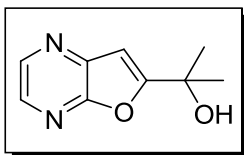
### 6-*tert*-Butylfuro[2,3-*b*]pyrazine (4p)<sup>4</sup>



Off white solid; mp: 75-77 °C (lit<sup>4</sup> 73-75 °C);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 7.96 (d,  $J = 2.1$  Hz, 1H), 7.85 (d,  $J = 2.0$  Hz, 1H), 7.62 (s, 1H), 1.35 (s, 9H); HPLC: 96.7 %; column: XBridge C-18 150\*4.6 mm  $5\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 229 nm, retention time 8.34 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2963, 1572, 1461, 659; MS (ESI)  $m/z$ : 177.1 [M + H].

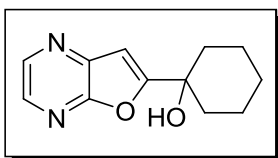
### 2-(Furo[2,3-*b*]pyrazin-6-yl)propan-2-ol (4q)





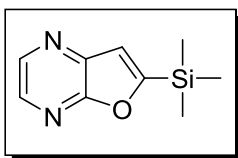
Light brown semi solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.48 (d,  $J = 2.7$  Hz, 1H), 8.19 (d,  $J = 2.7$  Hz, 1H), 6.93 (s, 1H), 3.08 (s, 1H), 1.72-1.67 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 162.3, 145.0 (2C), 144.0, 141.4, 96.5, 62.3, 33.8, 29.6; HPLC: 88.1 %; column: XBridge C-18 150\*4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.23 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3136, 2940, 1501, 1411, 723; MS (ESI) m/z: 179.1 [M + H].

#### 1-(Furo[2,3-b]pyrazin-6-yl)cyclohexanol (4r)



Off white solid; mp: 105-107°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.50 (d,  $J = 2.6$  Hz, 1H), 8.21 (d,  $J = 2.6$  Hz, 1H), 6.87 (s, 1H), 2.24-2.13 (m, 2H), 2.13-2.01 (m, 2H), 1.99-1.90 (m, 2H), 1.86-1.74 (m, 2H), 1.70-1.64 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 155.1, 141.8, 141.2, 137.2, 101.6, 84.0, 70.9, 36.0 (2C), 25.1, 21.5 (2C); HPLC: 96.8%; column: XBridge C-18 150\*4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.55 min; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3039, 2963, 2952, 2880, 1530, 1454; MS (ESI) m/z : 218.9 [M + H].

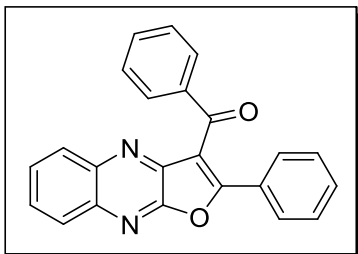
#### 6-(Trimethylsilyl)furo[2,3-b]pyrazine (4s)



Yellow semi solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) ppm: 8.74 (d,  $J = 3.1$  Hz, 1H), 8.58 (d,  $J = 2.9$  Hz, 1H), 7.61 (s, 1 H), -0.62 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm: 162.0, 144.8, 141.4, 140.4, 110.7, 108.9, -0.24 (3C); HPLC: 92.8%; column: XBridge C-18 150\*4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water mobile phase B:  $\text{CH}_3\text{CN}$  (gradient) T/B%:

0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.11 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 2941, 1530, 1252; MS (ESI) m/z : 192.9 [M + H].

### Phenyl(2-phenylfuro[2,3-*b*]quinoxalin-3-yl)methanone (6)<sup>5</sup>



Brown solid; mp: 152-154°C (lit<sup>5</sup> 149-151°C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) ppm: 7.96 (d, *J* = 7.2 Hz, 1H), 7.78-7.76 (m, 2H), 7.54 (d, *J* = 7.1 Hz, 1H), 7.29-7.21 (m, 5H), 7.19 (d, *J* = 6.4 Hz, 2H), 6.92-6.89 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) ppm: 185.9, 160.7, 159.9, 156.2, 152.3, 144.7, 143.4, 140.2, 132.3 (2C), 131.7, 129.9, 129.6 (2C), 129.5, 128.8, 128.6 (2C), 128.4, 128.2, 127.5, 125.5, 125.2; HPLC: 91.6 %; column: XBridge C-18 150\*4.6 mm 5μ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH<sub>3</sub>CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.59 min; IR (KBr)  $\nu_{max}$  (cm<sup>-1</sup>): 1621, 1522, 1431, 764; MS (ESI) m/z : 351.1 [M + H].

### Single crystal X-ray data for the compounds 4a:

Single crystals suitable for X-ray diffraction of compound **4a** were grown from 50% ethylacetate/n-hexane. Single crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at ambient temperature (298 K) on a Bruker SMART APEX CCD single crystal diffractometer using graphite monochromated Mo-K $\alpha$  radiation (0.71073 Å). Absorption corrections using multi  $\psi$ -scans were applied. Structure was solved using SHELXS-97, and refined by full-matrix least squares against  $F^2$  using SHELXL-97 software.<sup>6</sup> All non-hydrogen atoms were refined anisotropically. The hydrogen atoms on the carbon atoms were included in the structure factor calculation by using a riding model.

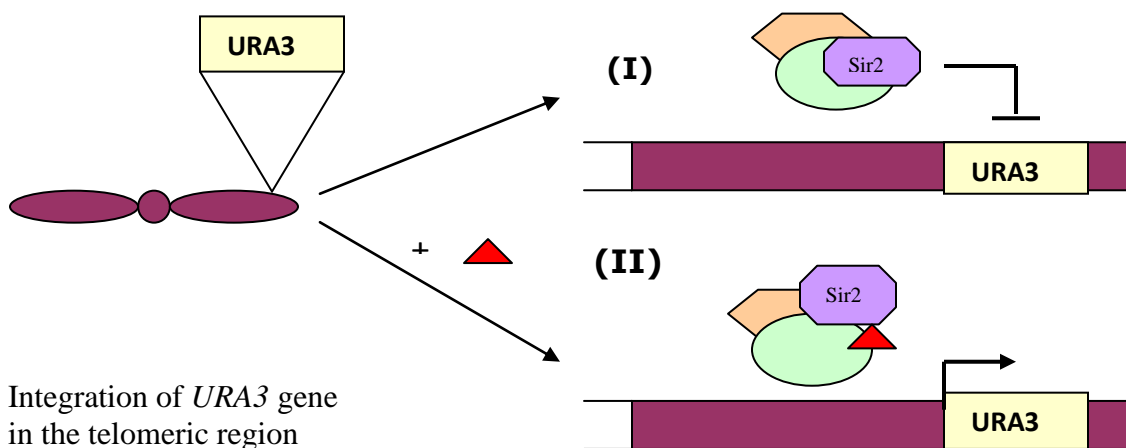
Crystal data of **4a** (CCDC No. 935821): Molecular formula = C<sub>16</sub>H<sub>9</sub>N<sub>2</sub>O, Formula weight = 245.25, Monoclinic, C2/c, *a* = 25.558 (3) Å, *b* = 4.5239 (5) Å, *c* = 23.971 (3) Å, *V* = 2384.4 (5) Å<sup>3</sup>, *T* = 298 K, *Z* = 8, *D<sub>c</sub>* = 1.366 Mg m<sup>-3</sup>,  $\mu$ (Mo-K $\alpha$ ) = 0.71073 mm<sup>-1</sup>, 9084 reflections were measured with 2122 unique reflections (*R*<sub>int</sub> = 0.0316), of which 2122 (*I* >

$2\sigma(I)$ ) were used for the structure solution. Final  $R_1$  (w  $R_2$ ) = 0.0545 (0.1424), 172 parameters. The final Fourier difference synthesis showed minimum and maximum peaks of -0.239 and +0.557 e.Å<sup>-3</sup> respectively. Goodness of fit = 1.056.

### Pharmacology:

#### A yeast cell based assay<sup>7</sup> for identification of potential inhibitors of HDAC Sir2 Reporter

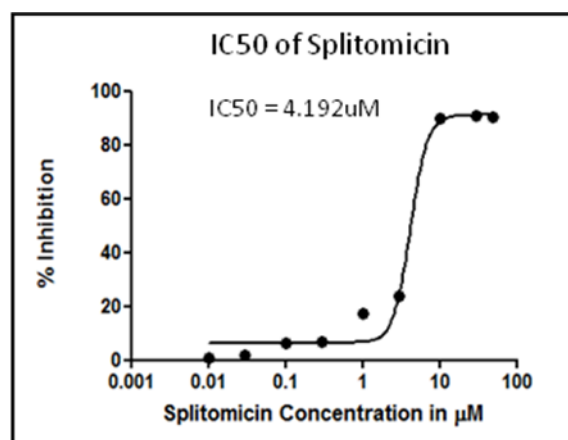
**silencing assay:** In this assay a yeast strain (TEL::URA3 strain (MAT $\alpha$  ura3-52 lys2-801 ade2-101 trp $\Delta$ 63 his3 $\Delta$ 200 leu3 $\Delta$ 200 leu2- $\Delta$ 1 TEL adh4::URA) was used in which, a reporter gene URA3 was inserted in the silenced telomeric region where it is silenced by yeast Sir2 protein. A compound having the Sir2 protein inhibitory effect will inhibit the Sir2 protein, and thus the URA3 gene will be expressed and this will result in the death of the yeast cell in presence of 5-fluoro orotic acid (5-FOA) through formation of toxic 5-fluorouracil. This assay can also test the toxicity of compounds. The cells when grown in absence of 5-FOA should grow if the compound is not toxic. However, in case of toxic compound yeast cells would die. The yeast strain was inoculated in 5.0 mL of YPDA media. The cells growing at the exponential phase were dispensed in the round bottom 96-well plate using cell dispenser. A Stock concentration of 10% 5-FOA was used to make a final concentration of 0.3% 5-FOA in the wells of 96-well plate. The compounds at a concentration of 50 uM were added to each well and the plates were incubated at 30 °C. Absorbance at 590 was measured using 96 well plate reader after 24 and 48h. The inhibitory effect of compounds was analyzed after plotting the OD vs concentration of the compound in Excel data sheet. Splitomicin was used as a control.



**Scheme S-1.** Cell based Sir2 mediated reporter silencing assay in yeast. **(I)** Growth in presence of 5-Fluoroorotic acid (5-FOA): Sir2 mediated silencing of URA3 gene permits growth in FOA. **(II)** No growth in presence of FOA: Inhibition of Sir2 results in expression of URA3 gene and cell death in presence of FOA.

### Dose Response study in yeast

Before performing the dose response with the test compound, the dose response with known inhibitor, which we used as a reference compound (Splitomicin), was performed. So, for this, yeast based reporter silencing assay, as described<sup>1</sup> was performed (Figure S-1) and the IC<sub>50</sub> was found to be 4.192 μM.



**Figure S-1:** IC<sub>50</sub> of the reference compound, Splitomicin

A dose response study was carried out with the compound **4e** using yeast cell based reporter silencing assay to determine its, IC<sub>50</sub> using Graph pad software. IC<sub>50</sub> for the compound **4e** in using yeast based reporter silencing assay was determined.

### Dose Response for Compound against Mammalian SIRT1:

A dose response study was carried out with the test compound, **4e** using In vitro cyclex SIRT1/Sir2 Deacetylase Fluorometric Assay Kit to determine its IC<sub>50</sub> using Graph pad software. IC<sub>50</sub> for the compound **4e** was determined.

### **Evaluation of Cytotoxicity effect of 4e on cancer cell lines: MTT assay**

We evaluated potential anti-tumor effects of **4e** on human cell lines. To this end, we incubated three different cell lines, HEPG2 (Hepatic Carcinoma cells) and Hela (Cervical Cancer cells) and in one non cancerous cell HEK293T in the presence of increasing logarithmic concentrations of **4e** (from 0.1 $\mu$ M to 100.0  $\mu$ M) and quantified for cell survival by the MTT assay. For MTT assay, specific cell numbers were seeded in 96-well plate for each cell line and were incubated with different concentrations of **4e** and DMSO (negative control) and the relative amount of viable cells were estimated by measuring the absorbance of the cell suspension after incubation with MTT (a tetrazole) for 2 hours at 37 °C and then dissolving the tetrazole by DMSO thereby forming purple colored product (formazon). We used Cambinol (already known sirtuin inhibitor) as an internal control. Increase in the concentration of **4e** strongly decreased the cell survival in the cell lines.

### **Docking studies:**

The docking analysis of molecules was performed using FRED, version 3.0<sup>8,9</sup> implemented from OpenEye Scientific Software. All molecules were sketched in 3D format with VIDA program of OpenEye. Omega<sup>10</sup> module was used to produce maximum conformers of the molecules and charges were added from mmff94s force field. The crystal structure coordinates of NAD-dependent protein deacetylase were obtained from the protein data bank (PDB ID: 1Q1A).<sup>11</sup> The protein is preprocessed by removal of water molecules and assigning bond orders. Hydrogens were added to the protein from the program Reduce version 3.1. The final protein was obtained by optimizing the added hydrogens using conjugate gradient algorithm from SZYBKI version 1.7. The grid for molecular docking was generated with bound co-crystallized ligand. Finally FRED was used to dock the conformers of the molecules in to the active site. Exhaustive conformation search was implemented during docking to generate 10 best scoring poses per each molecule.

### **Results and Discussion:**

The NAD<sup>+</sup> binding site is present in a cleft between the small and large domains of catalytic core. Based on the binding of NAD<sup>+</sup> cofactor, the receptor's active site can be sub divided in to the adenine nucleotide binding site and nicotinamide binding site. The adenine binding site

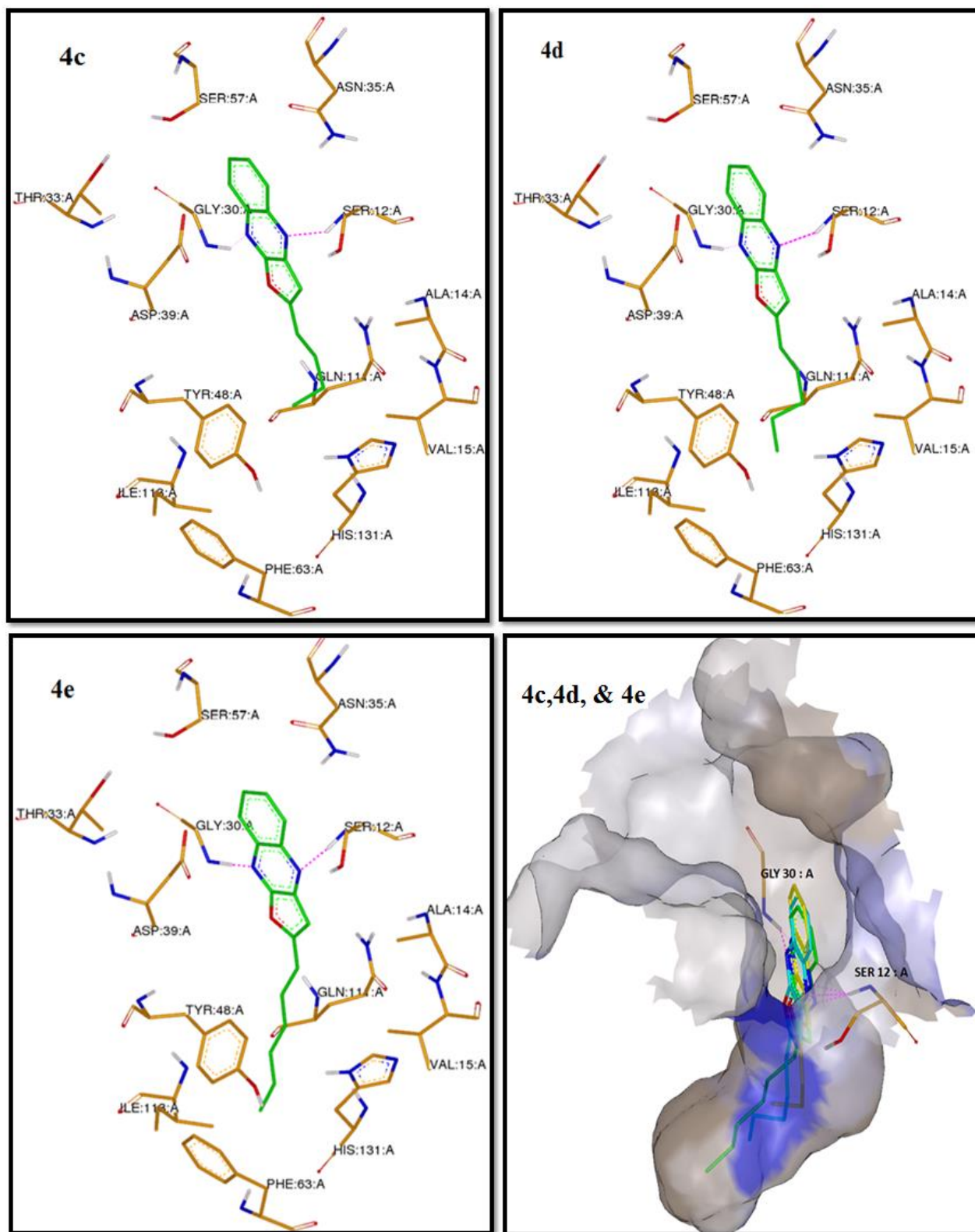
is present at the surface of the protein and leads to the nicotinamide binding site that appears as a tunnel passing deep in to the protein.

Interpretation of the docking results of the molecules **4c**, **4d** and **4e** reveal that these molecules binds in an orientation by positioning their furo[2,3-*b*]quinoxaline moiety near adenine binding site and thereby passing aliphatic chain into nicotinamide binding site. All three compounds made conserved hydrogen bonding with two nitrogen of the quinoxaline moiety and the backbone amino groups of SER12 and GLY 30 respectively (Fig.2). There is a significant change in dock scores with increase in the length of the hydrophobic tail (Table S1). This could be due to the van der waals interactions made by this hydrophobic tail with TYR 48, ILE 118 and PHE 63. The hydrophobic surface view of the protein receptor based on Eisenberg potential shows the hydrophobic tail of **4c**, **4d** and **4e** (see main draft) aligning well with the hydrophobic surface area of the receptor (Fig 2).

**Table S-1:**

Molecules	Dock Score <sup>a</sup>	Steric	Protein Desolvation	Ligand Desolvation	Clash	Ligand Desolvation	Hydrogen bond
<b>4e</b>	-10.2	-15.7	9.0	-4.4	0.5	2.9	-2.6
<b>4d</b>	-8.7	-14.5	8.6	-4.1	0.4	2.9	-2.1
<b>4c</b>	-7.8	-13.4	8.0	-4.0	0.4	2.9	-1.8

<sup>a</sup> FRED Chemgauss4 score



**Figure S-2.** The binding mode of **4c**, **4d** and **4e** in the catalytic site. **4c**, **4d** and **4e** along binding mode of **4c** (yellow), **4d** (cyan) and **4e** (green) at the catalytic site represented as surface and mapped on Eisenberg potential hydrophobicity.

## Zebrafish embryo toxicity evaluation:

### Materials & Methods:

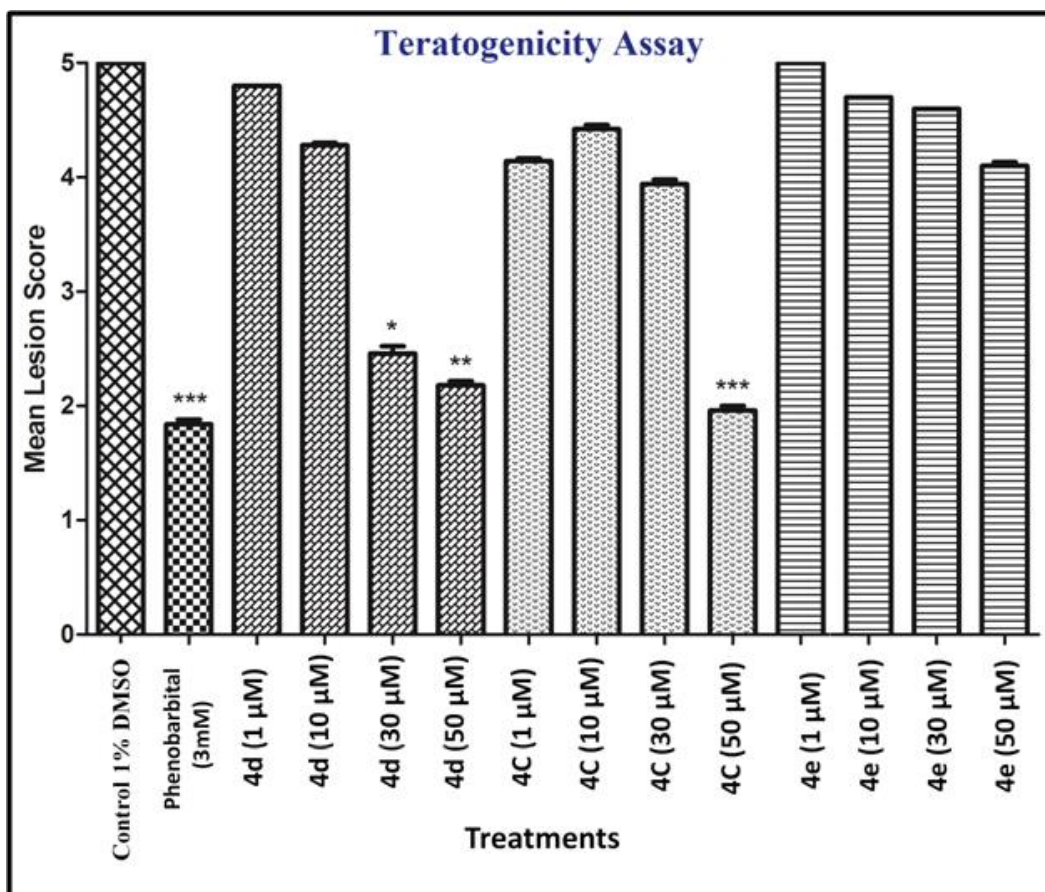
#### *Aquaculture:*

All procedures for experimentation were as per Guidelines for Use of Zebrafish in the NIH Intramural Research Program (<http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf>) and the Zebrafish Book (Westerfield, 2000).<sup>12</sup> Indigenous wild type zebrafish strains were obtained from Vikrant Aquaculture, Mumbai, India. Fish were maintained in a recirculation system with polysulphone housing tanks containing purified water (Millipore ELIX system grade) with 0.2% sea salt at 28°C under a 14:10 h light and dark cycle (Westerfield M, 2000). Fish were fed three times daily with live hatched brine shrimp and dry food. Males and females were separated for four days before they were allowed to spawn. On day five they were allowed to span at optimal conditions at the ratio of two males to one female. Collected embryos were grown in E3 medium at 28.5°C.

#### *Toxicity Evaluation:*

Preliminary safety evaluation of test compounds **4c**, **4d** and **4e** was carried out in a zebrafish embryo model based on the protocol and morphological score assessment by Panzica-Kelly et al, 2010.<sup>13</sup> Five embryos/treatment/concentration were exposed in a twenty-four well plate to the vehicle, positive control (3mM Phenobarbital) and test compounds (each test compound at four concentrations viz. 1, 10, 30 and 50 µM) from day 1 post fertilization to day 5 post fertilization and observed on day 5. Each embryo was assigned a lesion score (5-0, 5 being non-toxic) for various parameters and mean morphological assessment scores were used to conduct the statistical evaluation using the Kruskal–Wallis one-way analysis of variance by ranks followed by Dunn’s post hoc test. The results of the statistical analysis of mean morphological scores are depicted in Figure 1. However, the determination of No Observed Adverse Effect Level (NOAEL) and Minimum Toxic Concentration (MTC) were based on both statistical and biological significance. The details of the NOAEL, MTC and toxic effects have been mentioned in Table 1. All embryos survived the entire duration of the study and the toxic effects observed in the positive control group i.e. Phenobarbital (3mM) were consistent with our in-house control. Overall assessment suggests that compound **4e** was the safest amongst the three in the present assay.





**Figure S-3.** Evaluation of teratogenic effects of the compounds in Zebrafish embryos.

(\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ )

**Table S-2:** Results of zebrafish embryo toxicity study with toxicological indices and major organs/systems affected in positive control and at MTC in test compounds. (- no effect, x- slightly toxic, xx-moderately toxic, xxx-severely toxic)

	<b>4c</b>	<b>4d</b>	<b>4e</b>	<b>Phenobarbital</b>
<b>Test Concentrations (µM)</b>	1, 10, 30, 50	1, 10, 30, 50	1, 10, 30, 50	3000
<b>Statistically Significant Toxic Concentration (µM)</b>	50	30, 50	> 50	Positive Control
<b>No Observed Adverse Effect Level (NOAEL) (µM)</b>	< 1	1	30	
<b>Minimal Toxic Concentration (MTC) (µM)</b>	1	10	50	
<b>Parameters of toxicity at MTC</b>				
Body Shape	-	-	-	xxx
Motility	-	-	-	xxx
Pigmentation	-	-	-	-
Swim Bladder	x	x	x	xxx
Intestine	xx	xxx	x	xxx
Liver	xx	-	-	xxx
Yolk	xx	xx	x	xxx
Somites	-	-	-	xxx
Notocord	-	-	-	xxx
Tail	-	-	x	xx
Caudal & Tail Fin	-	-	-	xx
Pectoral Fin	-	-	-	xx
Heart	xx	-	-	xxx
Brain	xx	x	x	xxx
Upper Facial Structures	-	-	x	xxx
Pharangeal Arches	x	x	x	xxx
Upper & Lower Jaw	-	-	x	Xxx

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