Electronic Supplementary Information

Zebrafish based strategy for the identification of potential pharmacophore for apoptosis:A greener CuAAC approach to novel 1,2,3-triazoles derived from mefenamic acid

P. Vijaya Babu[a,b], Soumita Mukherjee[a], Dhilli Rao Gorja[a], Swapna Yellanki[a,c], Raghavender Medisetti[a,c], Pushkar Kulkarni,[a,c] K. Mukkanti[b] and Manojit Pal[a,*]

[a]*Dr Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India.*

[b]*Chemistry Division, Institute of Science and Technology, JNT University, Kukatpally, Hyderabad 500072, India.*

[c]*Zephase Therapeutics (an incubated company at the Dr Reddy's Institute of Life Sciences), University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India.*

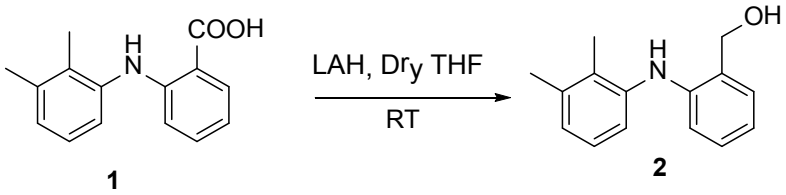
E-mail: manojitpal@rediffmail.com

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 and ^{13}C NMR spectra were determined in $CDCl_3$ solution by using a 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), td (triplet of doublet) 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 (Agilent 6430 Triple Quadrupole LC/MS).

Preparation of (2-(2,3-dimethylphenylamino)phenyl)methanol (2):

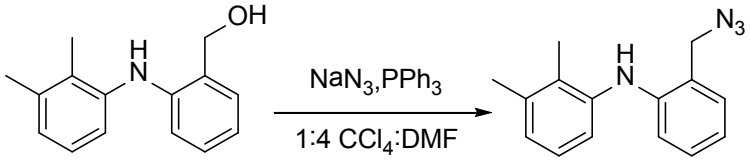


1 **2**

To a mixture of compound **1** (8.0 g, 33.1 mmol) in dry THF (50 mL), was added lithium aluminium hydride (LAH) (2.0 g, 49 mmol) at 0 °C under a nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. After completion of the reaction (indicated by TLC), the excess of LAH was quenched by adding ice (2 g) portion wise. The mixture was then extracted with ethyl acetate (3 × 15 mL), washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using 20% ethyl acetate/*n*-hexane to give desired compound **2**.

Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21-7.17 (m, 2H), 7.11-7.04 (m, 2H), 6.99 (d, J = 8.8 Hz, 1H), 6.89 (d, J = 6.8 Hz, 1H), 6.82 (t, J = 7.6 Hz, 1H), 4.75 (s, 2H), 2.34 (s, 3H), 2.16 (s, 3H); MS (CI): 227.8 (M+1).

Preparation of *N*-(2-(azidomethyl)phenyl)-2,3-dimethylaniline (3):



2 **3**

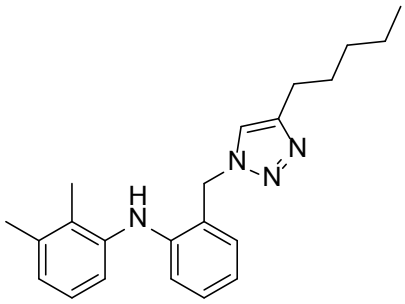
Sodium azide (1.03 g, 15.8 mmol) was added to a solution of the alcohol **2** (3.0 g, 13.2 mmol) and PPh_3 (7.0 g, 26.4 mmol) in 1:4 CCl_4-DMF (30 mL) under a nitrogen atmosphere and then heated at 75 °C for 2 h. After completion of the reaction, the mixture was cooled to room temperature, diluted with ethyl acetate (3 × 15 mL) and washed with cold water (2 × 10 mL). The combined organic layer was washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using 10% ethyl acetate/ *n*-hexane to give the desired compound **3**; yield: 95%; yellow liquid; IR (KBr): ν_{max} 3400, 2927, 2102, 1588, 1473, 1305 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.19 (m, 2H), 7.06 (t, J = 7.6 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.98-6.82 (m, 3H), 5.84 (bs, 1H), 4.39 (s, 2H), 2.33 (s, 3H), 2.15 (s, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.9, 140.5, 137.9, 130.4, 129.7, 128.3, 125.9, 124.6, 122.8, 119.8, 118.7, 116.7, 53.3, 20.6, 13.5; MS (CI): 253.1 (M+1).

General procedure for preparation of triazole derivatives (5)

A mixture of *N*-(2-azide methyl) phenyl)-2,3-dimethylaniline (**3**) (1.0 mmol), an appropriate alkyne (**4**) (1.0 mmol), K_2CO_3 (3 mmol) and CuI (0.05 mmol) in 1:1 PEG:H_2O (5 mL) was stirred at 80 °C for 1.5 h under a nitrogen atmosphere. After completion of the reaction (indicated by TLC), the mixture was cooled to room temperature and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with water (2 × 10 mL), brine (4 mL), dried over anhydrous Na_2SO_4 and filtered. The solution was then concentrated under vacuum

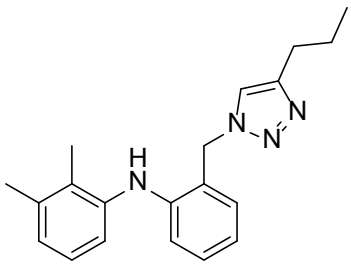
and the residue was purified by column chromatography on silica gel (230-400 mesh) using 30% ethylacetate/ *n*-hexane to give the desired product **5**.

1. 2,3-Dimethyl-*N*-(2-((4-pentyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5a)



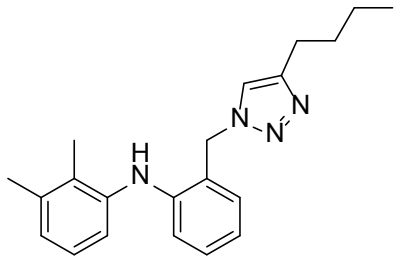
Yield: 88%; Brown liquid; IR (KBr) ν_{max}: 3334, 2927, 1722, 1587, 1468, 1301 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.28 (m, 2H), 7.20 (td, J = 7.8, 1.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.88 (td, J = 6.4, 2.0 Hz, 2H), 6.66 (bs, 1H), 5.48 (s, 2H), 2.66 (t, J = 7.6 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 1.68-1.54 (m, 4H), 1.38-1.29 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.4, 140.7, 137.9, 130.8, 130.6, 130.0, 128.4, 125.7, 124.4, 122.8, 120.6, 119.9, 118.4, 117.8, 51.7, 31.4, 29.1, 25.6, 22.3, 20.6, 13.9, 13.6; MS (CI): 349.2 (M+1); HPLC: 97.1%, 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, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210.5 nm, retention time 5.0 min; Elemental analysis found C, 75.59; H, 8.05; N, 16.23; $C_{22}H_{28}N_4$ requires C, 75.82; H, 8.10; N, 16.08.

2. 2,3-Dimethyl-*N*-(2-((4-propyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5b)



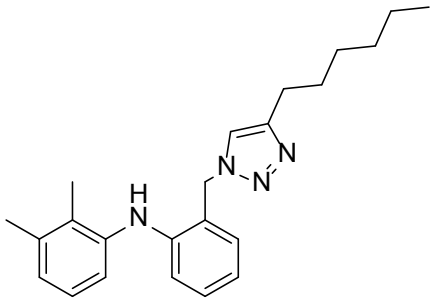
Yield: 98%; white solid; mp: 62-64 °C; IR (KBr) ν_{max}: 3331, 2958, 1583, 1468, 1309 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.33-7.28 (m, 2H), 7.20 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.90-6.85 (m, 2H), 6.66 (s, 1H), 5.48 (s, 2H), 2.67 (t, J = 7.6 Hz, 2H), 2.31 (s, 3H), 2.11 (s, 3H), 1.70-1.65 (m, 2H), 0.95 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.6, 144.4, 140.7, 137.9, 130.6, 130.0, 128.4, 125.7, 124.4, 122.7, 120.7, 119.9, 118.4, 117.8, 51.7, 27.6, 22.6, 20.6, 13.7, 13.6; MS (CI): 320.7 (M^+, 100%); HPLC: 93.4%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water mobile phase B: ACN T/%B: 0/20, 3/20, 8/98, 16/98, 18/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 9.1 min; Elemental analysis found C, 74.69; H, 7.57; N, 17.61; $C_{20}H_{24}N_4$ requires C, 74.97; H, 7.55; N, 17.48.

3. *N*-(2-((4-Butyl-1*H*-1, 2,3-triazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5c)



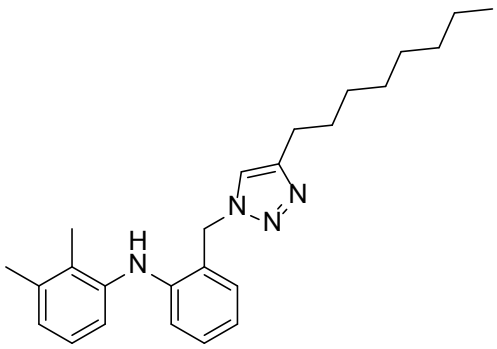
Yield: 90%; Brown liquid; IR (KBr) ν_{max}: 3335, 2931, 1585, 1470, 1301 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.27 (m, 2H), 7.20 (t, J = 7.8 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.95 (dd, J = 6.8, 2.8 Hz, 2H), 6.88 (t, J = 6.8 Hz, 2H), 6.65 (bs, 1H), 5.48 (s, 2H), 2.69 (t, J = 7.6 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 1.68-1.58 (m, 2H), 1.42-1.30 (m, 2H), 0.92 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.7, 144.4, 140.7, 137.9, 130.7, 130.0, 128.4, 125.7, 124.4, 122.7, 120.7, 120.0, 118.4, 117.9, 51.8, 31.4, 25.3, 22.3, 20.6, 13.7, 13.6; MS (CI): 335.1 (M+1, 100%). HPLC: 95%, column: X-Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (gradient) T/%B: 0/20, 2/20, 9/98, 16/98, 17/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 10.9 min; Elemental analysis found C, 75.58; H, 7.86; N, 16.52; $C_{21}H_{26}N_4$ requires C, 75.41; H, 7.84; N, 16.75.

4. *N*-(2-((4-Hexyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5d)



Yield: 85%; Brown liquid; IR (KBr) ν_{max} 3334, 2927, 1585, 1469, 1301 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-7.27 (m, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.92-6.84 (m, 2H), 6.66 (s, 1H), 5.47 (s, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 1.70-1.52 (m, 3H), 1.45-1.21 (m, 5H), 0.92-0.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.9, 144.4, 140.7, 137.9, 130.6, 130.0, 128.4, 125.7, 124.4, 122.8, 120.6, 119.9, 118.4, 117.8, 51.7, 31.5, 29.4, 28.9, 25.6, 22.5, 20.7, 14.0, 13.6; MS (CI): 362.7 (M+1, 100%); HPLC: 95.2%, 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 210.4 nm, retention time 5.7 min; Elemental analysis found C, 76.40; H, 8.36; N, 15.23; $C_{23}H_{30}N_4$ requires C, 76.20; H, 8.34; N, 15.46.

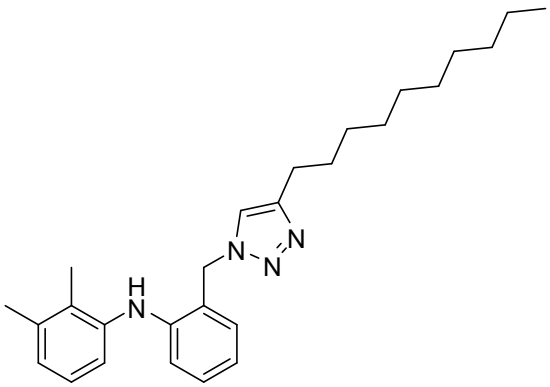
5. 2,3-Dimethyl-*N*-(2-((4-octyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5e)



Yield: 98%; Colorless liquid; IR (KBr) ν_{max}: 3342, 2926, 2849, 1583, 1468, 1298 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.33-7.28 (m, 2H), 7.20 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 2H), 6.91-6.85 (m, 2H), 6.67 (s, 1H), 5.48 (s, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.31

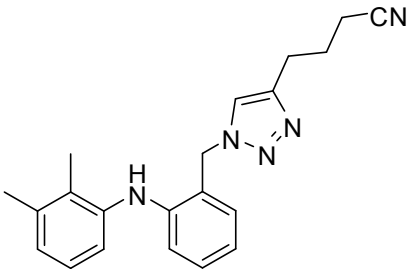
(s, 3H), 2.10 (s, 3H), 1.73-1.57 (m, 4H), 1.32-1.22 (m, 8H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.9, 144.4, 140.8, 138.0, 130.7, 130.0, 128.4, 125.8, 124.4, 122.8, 120.6, 119.9, 118.4, 117.8, 51.7, 31.8, 29.4, 29.3, 29.2, 29.1, 25.7, 22.6, 20.7, 14.1, 13.6; MS (CI): 390.8 (M^+, 100%); HPLC: 94.9%, column: symmetry C-18 75*4.6 mm 5µm, mobile phase A: 0.1 % Formic acid in water B: ACN (gradient) T/%B: 0/70, 2/70, 7/98, 12/98, 13/70, 20/70; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 9.3 min; Elemental analysis found C, 76.65; H, 8.76; N, 14.51; $C_{25}H_{34}N_4$ requires C, 76.88; H, 8.77; N, 14.35.

6. *N*-(2-((4-Decyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5f)



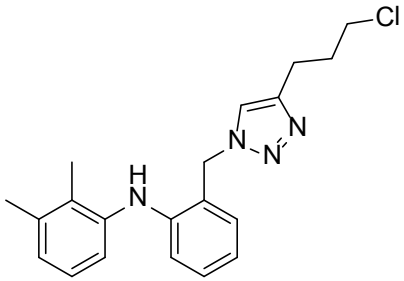
Yield: 80%; Brown liquid; IR (KBr) ν_{max}: 3325, 2950, 1590, 1471, 1295 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (d, J = 8.8 Hz, 2H), 7.20 (t, J = 8.0 Hz, 1H), 7.02 (t, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.91-6.84 (m, 2H), 6.67 (s, 1H), 5.47 (s, 2H), 2.68 (t, J = 8.0 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 1.68-1.60 (m, 2H), 1.34-1.22 (m, 14H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.9, 144.4, 140.7, 137.9, 130.6, 130.0, 128.4, 125.7, 124.4, 122.8, 120.6, 119.9, 118.4, 117.8, 51.7, 31.8, 29.5 (2C), 29.4, 29.3 (2C), 29.2, 25.6, 22.6, 20.6, 14.1, 13.6; MS (CI): 419.3 (M+1, 100%); HPLC: 96.5%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water 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 210.4 nm, retention time 7.6 min; Elemental analysis found C, 77.25; H, 9.16; N, 13.49; $C_{27}H_{38}N_4$ requires C, 77.47; H, 9.15; N, 13.38.

7. 4-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)butanenitrile (5g)



Yield: 95%; ash colored solid; mp: 173-175 °C; IR (KBr) ν_{max}: 3321, 1589, 1472, 1310 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (s, 1H), 7.30 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.99-6.85 (m, 4H), 6.56 (s, 1H), 5.49 (s, 2H), 2.85 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 2.09-2.01 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9, 144.3, 140.8, 137.9, 130.7, 130.2, 128.3, 125.8, 124.5, 122.7, 121.3, 120.2, 119.2, 118.3, 118.1, 51.8, 24.8, 24.1, 20.6, 16.4, 13.6; MS (CI): 345.7 (M+1, 100%); HPLC: 96.1%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water 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 210.4 nm, retention time 3.8 min; Elemental analysis found C, 73.21; H, 6.76; N, 20.01; $C_{21}H_{23}N_5$ requires C, 73.02; H, 6.71; N, 20.27.

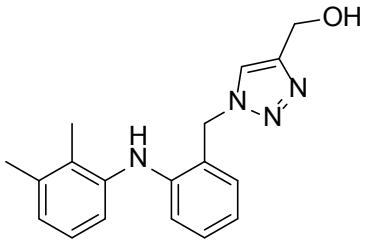
8. *N*-(2-((4-(3-Chloropropyl)-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5h)



Yield: 97%; White solid; mp: 85-88°C; IR (KBr) ν_{max}: 2931, 2849, 1589, 1441, 1326 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (s, 1H), 7.30 (d, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.98-6.92 (m, 2H), 6.90 (t, J = 7.6 Hz, 2H), 6.61 (s, 1H), 5.50 (s, 2H), 3.57 (t, J = 6.4 Hz, 2H), 2.88 (t, J = 7.2 Hz, 2H), 2.32 (s, 3H), 2.20-2.12 (m, 2H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.7, 144.3, 140.7, 137.9, 130.7, 130.1, 128.4, 125.8, 124.5, 122.6,

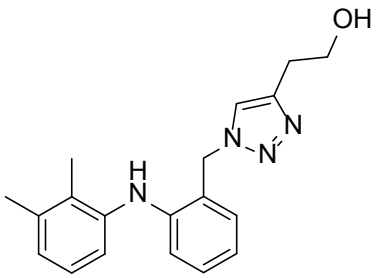
121.4, 120.2, 118.5, 118.1, 61.6, 51.9, 31.8, 21.9, 20.5, 13.6; MS (CI): 354.7 (M^+, 100%); HPLC: 97.4%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water mobile phase B: ACN T/%B: 0/20, 3/20, 10/98, 16/98, 18/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 10.4 min; Elemental analysis found C, 67.41; H, 6.56; N, 15.98; $C_{20}H_{23}ClN_4$ requires C, 67.69; H, 6.53; N, 15.79.

9. (1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)methanol (5i)



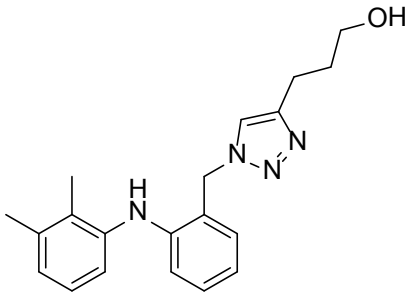
Yield: 95%; White Solid; mp: 130°C; IR (KBr) ν_{max}: 3316, 2865, 1583, 1470, 1293.0 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.30 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.98-6.85 (m, 4H), 6.57 (s, 1H), 5.51 (s, 2H), 4.78 (d, J = 4.8 Hz, 2H), 2.31 (s, 3H), 2.12 (s, 3H), 2.10-2.03 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 144.2, 140.8, 138.0, 130.7, 130.2, 128.2, 125.8, 124.4, 122.7, 121.7, 120.3, 118.8, 118.2, 56.6, 51.8, 20.7, 13.6; MS (CI): 308.7 (M^+, 100%); HPLC: 93.9%, 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: 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 4.4 min; Elemental analysis found C, 70.28; H, 6.56; N, 18.02; $C_{18}H_{20}N_4O$ requires C, 70.11; H, 6.54; N, 18.17.

10. 2-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)ethanol (5j)



Yield: 80%; Brown liquid; IR (KBr) ν_{max}: 3342, 2924, 1586, 1472, 1301 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (s, 1H), 7.29 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.02 (t, J = 8.0 Hz, 1H), 6.97-6.91 (m, 2H), 6.89 (t, J = 6.8 Hz, 2H), 6.59 (s, 1H), 5.49 (s, 2H), 3.92 (t, J = 5.8 Hz, 2H), 2.98-2.92 (m, 2H), 2.88 (s, 1H), 2.31 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9, 144.3, 140.7, 137.9, 130.7, 130.1, 128.4, 125.8, 124.5, 122.7, 121.8, 120.1, 118.4, 118.0, 61.4, 51.7, 28.7, 20.6, 13.6; MS (CI): 322.7 (M^+, 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 (gradient) T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210.5 nm, retention time 5.0 min; Elemental analysis found C, 70.99; H, 6.85; N, 17.27; $C_{19}H_{22}N_4O$ requires C, 70.78; H, 6.88; N, 17.38.

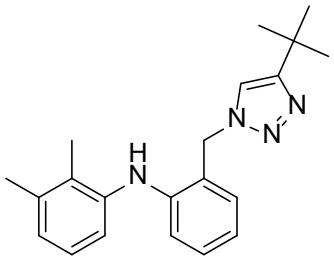
11. 3-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (5k)



Yield: 98%; white solid; mp: 82-84 °C; IR (KBr) ν_{max}: 3397, 2920, 2860, 1583, 1457, 1304 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (s, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.98-6.92 (m, 2H), 6.91-6.86 (m, 2H), 6.60 (s, 1H), 5.48 (s, 2H), 3.69 (t, J = 6.0 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 2.04 (s, 1H), 1.97-1.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 144.4, 140.7, 138.0, 130.7, 130.1, 128.4, 125.8, 124.4, 122.7, 121.0, 120.1, 118.4, 117.9, 61.8, 51.8, 31.8, 22.0, 20.7, 13.6; MS (CI): 336.7 (M^+,

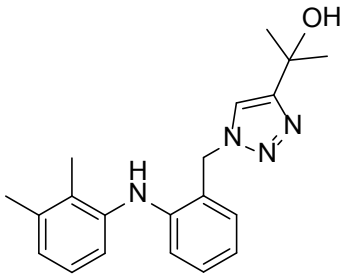
100%); HPLC: 96.0%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water B: ACN T/%B: 0/20, 3/20, 8/98, 16/98, 18/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 7.7 min; Elemental analysis found C, 71.29; H, 7.09; N, 16.83; $C_{20}H_{24}N_4O$ requires C, 71.40; H, 7.19; N, 16.65.

12. *N*-(2-((4-*tert*-Butyl-1*H*-imidazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5l)



Yield: 95%; Brown liquid; IR (KBr) ν_{max}: 3335, 2959, 1587, 1472, 1302, 1224 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-7.27 (m, 2H), 7.21 (td, J = 7.6, 1.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.94 (t, J = 8.4 Hz, 2H), 6.88 (t, J = 7.2 Hz, 2H), 6.61 (bs, 1H), 5.47 (s, 2H), 2.30 (s, 3H), 2.07 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.0, 144.5, 140.7, 137.9, 130.7, 130.0, 128.5, 125.7, 124.5, 122.7, 119.8, 118.6 (2C), 117.7, 51.7, 30.7, 30.3 (3C), 20.6, 13.6; MS (CI): 335.2 (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 (gradient) T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 5.0 min; Elemental analysis found C, 75.58; H, 7.85; N, 16.55; $C_{21}H_{26}N_4$ requires C, 75.41; H, 7.84; N, 16.75.

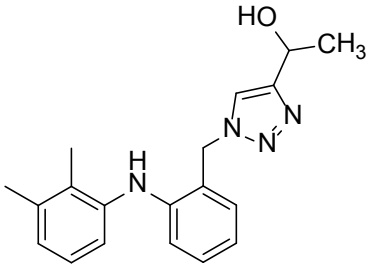
13. 2-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)propan-2-ol (5m)



Yield: 85%; Light yellow solid; mp: 133-135 °C; IR (KBr) ν_{max}: 3349, 2972, 1590, 1472, 1302 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 1H), 7.31 (d, J = 7.2 Hz, 1H), 7.22 (t, J = 8.0 Hz,

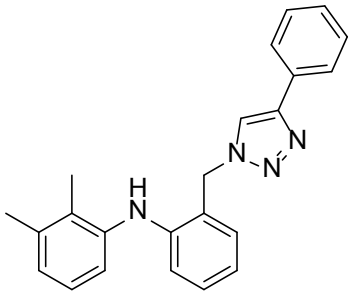
1H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.99-6.86 (m, 4H), 6.54 (s, 1H), 5.49 (s, 2H), 2.35-2.30 (m, 4H), 2.09 (s, 3H), 1.61 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 155.9, 144.3, 140.7, 137.9, 130.8, 130.2, 128.3, 125.8, 124.5, 122.6, 120.2, 119.2, 118.4, 118.1, 68.5, 51.8, 30.4, 20.6, 13.6; MS (CI): 336.7 (M^+, 100%); HPLC: 94.7%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: MeOH T/B%: 0/60, 0.5/60, 3/98, 10/98, 10.5/60, 12/60; flow rate: 1.0 mL/min, UV 210.8 nm, retention time 6.4 min; Elemental analysis found C, 71.59; H, 7.15; N, 16.43; $C_{20}H_{24}N_4O$ requires C, 71.40; H, 7.19; N, 16.65.

14. 1-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)ethanol (5n)



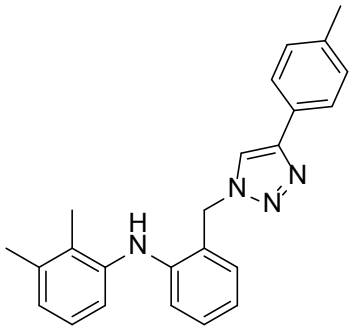
Yield: 80%; Yellow liquid; IR (KBr) ν_{max}: 3368, 1588, 1298, 1062 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (s, 1H), 7.30 (dd, *J* = 6.8, 1.2 Hz, 1H), 7.21 (td, *J* = 7.6, 1.2 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.97-6.85 (m, 4H), 6.53 (s, 1H), 5.50 (s, 2H), 5.06 (q, *J* = 6.4 Hz, 1H), 2.31 (s, 3H), 2.10 (s, 3H), 1.62-1.54 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.5, 144.2, 140.6, 137.8, 130.6, 130.0, 128.2, 125.7, 124.3, 122.6, 120.1, 119.9, 118.2, 118.0, 63.0, 51.7, 23.0, 20.5, 13.4; MS (CI): 322.7 (M^+, 100%); HPLC: 95.7%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: MeOH T/B%: 0/60, 0.5/60, 3/98, 10/98, 10.5/60, 12/60; flow rate: 1.0 mL/min, UV 210.8 nm, retention time 6.4 min; Elemental analysis found C, 70.69; H, 6.86; N, 17.62; $C_{19}H_{22}N_4O$ requires C, 70.78; H, 6.88; N, 17.38.

15. 2,3-Dimethyl-*N*-(2-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5o)



Yield: 98%; Light yellow solid; mp: 165-168 °C; IR (KBr) ν_{max}: 3336, 2925, 1589, 1468, 1302 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.81-7.78 (m, 3H), 7.41 (t, J = 7.6 Hz, 2H), 7.38-7.30 (m, 2H), 7.28-7.19 (m, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.94 (d, J = 8.0 Hz, 2H), 6.89 (t, J = 7.2 Hz, 2H), 6.55 (s, 1H), 5.56 (s, 2H), 2.31 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.2, 144.3, 140.7, 138.0, 130.7, 130.3, 130.2, 128.8 (2C), 128.4, 128.2, 125.8, 125.7 (2C), 124.5, 122.7, 120.2, 119.7, 118.5, 118.1, 51.9, 20.7, 13.7; MS (CI): 355.2 (M+1, 100%). HPLC: 93.7%, 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 210.4 nm, retention time 5.0 min; Elemental analysis found C, 77.71; H, 6.27; N, 15.92; $C_{23}H_{22}N_4$ requires C, 77.94; H, 6.26; N, 15.81.

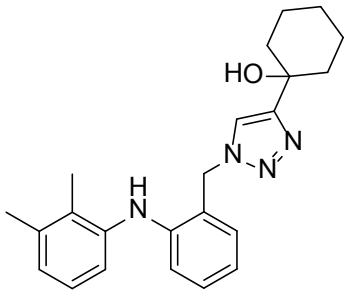
16. 2, 3-Dimethyl-*N*-(2-((4-*p*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5p)



Yield: 97%; light Brown solid; mp: 170-173 °C; IR (KBr) ν_{max}: 3344, 2919, 1586, 1306 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 7.2 Hz, 1H), 7.26-7.19 (m, 3H), 7.03 (t, J = 7.6 Hz, 1H), 6.99-6.93 (m, 2H), 6.92-6.87 (m, 2H), 6.58 (s, 1H), 5.57 (s, 2H), 2.38 (s, 3H), 2.32 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.3, 144.3, 140.7, 138.1, 137.9, 130.7, 130.2, 129.4 (2C), 128.4, 127.5, 125.8, 125.6 (2C), 124.5, 122.7, 120.2, 119.3, 118.5, 118.1, 51.9, 21.2, 20.7, 13.7; MS (CI): 369.1 (M+1, 100%); HPLC:

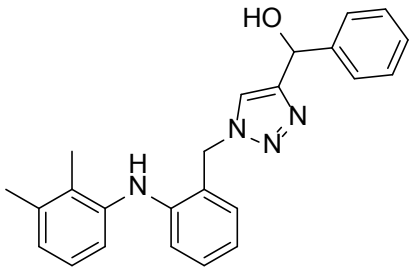
95.3%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: ACN (gradient) T/%B: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 5.3 min; Elemental analysis found C, 78.41; H, 6.56; N, 15.02; $C_{24}H_{24}N_4$ requires C, 78.23; H, 6.57; N, 15.21.

17. 2-(1-(2-(2,3-Dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)propan-2-ol (5q)



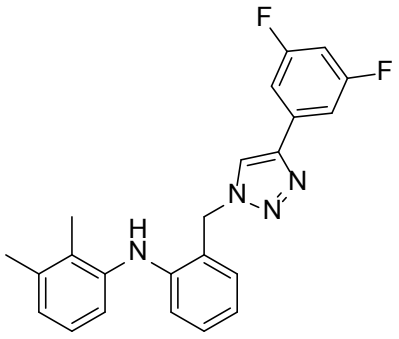
Yield: 91%; White solid; mp: 132-135 °C; IR (KBr) ν_{max}: 3316, 2931, 1589, 1466, 1306 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 1H), 7.30 (dd, J = 6.4, 1.2 Hz, 1H), 7.21 (td, J = 8.0, 1.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.99-6.85 (m, 4H), 6.53 (s, 1H), 5.49 (s, 2H), 2.31 (s, 3H), 2.08 (s, 3H), 1.99-1.89 (m, 2H), 1.88-1.80 (m, 2H), 1.78-1.69 (m, 2H), 1.66-1.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0, 144.3, 140.7, 137.9, 130.8, 130.1, 128.3, 125.8, 124.4, 122.7, 120.1, 119.6, 118.4, 118.0, 109.9, 69.6, 51.8, 38.0 (2C), 25.2, 21.8, 20.7, 13.6; MS (CI): 376.4 (M^+, 100%); HPLC: 93.3%, 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 210.4 nm, retention time 4.2 min; Elemental analysis found C, 73.58; H, 7.48; N, 14.72; $C_{23}H_{28}N_4O$ requires C, 73.37; H, 7.50; N, 14.88.

18. (1-(2-(2,3-dimethylphenylamino)benzyl)-1*H*-1,2,3-triazol-4-yl)(phenyl)methanol (5r)



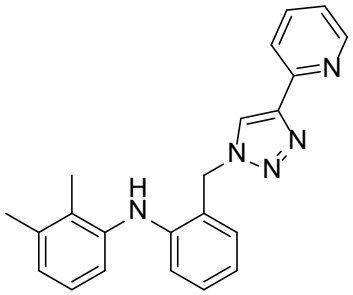
Yield: 90%; Yellow solid; mp: 120-125 °C; IR (KBr) ν_{max}: 3345, 2915, 2861, 1589, 1474, 1299 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, J = 7.2 Hz, 2H), 7.39-7.27 (m, 3H), 7.26-7.20 (m, 2H), 7.18 (dd, J = 8.0, 1.2 Hz, 1H), 7.00 (t, J = 8.0 Hz, 1H), 6.95-6.90 (m, 2H), 6.67 (d, J = 7.6 Hz, 2H), 6.56 (s, 1H), 6.00 (s, 1H), 5.44 (s, 2H), 3.07 (s, 1H), 2.30 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.7, 144.3, 141.7, 140.8, 137.9, 130.8, 130.2, 128.5 (2C), 128.3, 128.0, 126.3 (2C), 125.8, 124.4, 122.6, 121.3, 120.3, 118.3, 118.2, 69.0, 51.9, 20.7, 13.6; MS (CI): 384.6 (M^+, 100%); HPLC: 96.6%, column: symmetry C-18 75*4.6 mm 3.5 µm, 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 210.5 nm, retention time 5.0 min; Elemental analysis found C, 74.69; H, 6.30; N, 14.72; $C_{24}H_{24}N_4O$ requires C, 74.97; H, 6.29; N, 14.57.

19. *N*-(2-((4-(3,5-Difluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl)-2,3-dimethylaniline (5s)



Yield: 96%; yellow solid; mp: 120-122 °C; IR (KBr) ν_{max}: 3265, 2926, 2860, 1605, 1506, 1468, 1309 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.33 (t, J = 7.2 Hz, 3H), 7.22 (d, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.98-6.87 (m, 4H), 6.76 (t, J = 8.8 Hz, 1H), 6.44 (s, 1H), 5.57 (s, 2H), 2.31 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3 (C-F, J = 246.8 Hz), 163.1 (C-F, J = 246.8), 146.2, 144.2, 140.7, 138.0, 133.4, 130.5 (C-F, J = 33.3 Hz), 128.3, 125.9 (2C), 124.6 (2C), 122.5, 120.4 (2C), 118.4 (C-F, J = 3.9 Hz, 2C), 108.5 (C-F, J = 26.6 Hz), 103.3 (C-F, J = 25.3 Hz), 52.0, 20.6, 13.6; MS (CI): 390.7 (M^+, 100%); HPLC: 97.8%, column: symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water mobile phase B: ACN T/%B: 0/20, 3/20, 8/98, 16/98, 18/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 9.7 min; Elemental analysis found C, 70.49; H, 5.18; N, 14.52; $C_{23}H_{20}F_2N_4$ requires C, 70.75; H, 5.16; N, 14.35.

20. 2,3-Dimethyl-*N*-(2-((4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)methyl)phenyl)aniline (5t)



Yield: 95%; Light green solid; mp: 110-112 °C; IR (KBr) ν_{max}: 3315, 2915, 2854, 1517, 1463, 1304 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.23-8.14 (m, 2H), 7.78 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.21 (t, J = 7.2 Hz, 2H), 7.02 (t, J = 7.6 Hz, 1H), 6.98-6.87 (m, 4H), 6.51 (s, 1H), 5.59 (s, 2H), 2.31 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.9, 149.3, 144.2, 140.8, 137.9, 136.9, 130.9, 130.2, 128.3, 125.8 (2C), 124.4 (2C), 122.7, 122.1, 120.4 (2C), 118.4, 118.2, 51.9, 20.7, 13.7; MS (CI): 355.7 (M^+, 100%); HPLC: 98.2%, column: symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water mobile phase B: ACN T/%B: 0/20, 3/20, 8/98, 16/98, 18/20, 20/20; flow rate: 1.0 mL/min, UV 210.4 nm, retention time 7.6 min; Elemental analysis found C, 74.59; H, 5.94; N, 19.42; $C_{22}H_{21}N_5$ requires C, 74.34; H, 5.96; N, 19.70.

Biology

Husbandry: Zebrafish obtained from a local vendor were maintained in in-house built recirculatory system under 14-10 h light dark cycle and at 28°C temperature as described in Banote *et al.*, 2013.[1] Breeding was carried out using females and males in ratio of 2:3 and the embryos obtained were collected in petridishes and maintained at 28°C (Westerfield *et al.*, 2000, Nakhi *et al.*, 2013).[2,3]

Apoptosis Assay: 24hpf embryos were de-chorinated manually. Among them 6 embryos were distributed as two sets in each well of 24 well plates with 250 µl of 0.1% DMSO. The working stock solutions were prepared by serial dilution as described earlier. Each well was added with

250µl of respective concentration to obtain final working concentration. Embryos were incubated at 28°C for 24 h and 48 h.

Check apoptotic effect at 24 h and 48 h by washing drug exposed embryos thrice with E3 medium. Acridine orange (2µg/ml) solution of dye in E3 medium was added and incubated for 30 min. The embryos were rinsed thoroughly twice in fresh E3 medium to wash the acridine orange solution. Stained embryos were anesthetized with tricaine and photographed under UV illumination using Zeiss AxioCamMR camera attached to a Zeiss florescence microscope (GFP filter set:excitation 473, emission 520) under 5× magnification. The Images were taken and analyzed using Image J software.

Teratogenicity assay: 1dpf embryos at same developmental stage were sorted out and dechorionated using protease K. Test compounds stock solutions were prepared by dissolving in 100% DMSO. By serial dilution from stock solutions various concentrations were prepared and the final concentration of DMSO becomes 0.1%. The embryos were distributed in 24 well plate (3/well) and concentrations of test compounds starting from 1µM to 30µM compound was added to each well accordingly where n=6.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).[4]

Table S-1. Results of Zebrafish embryo toxicity study with toxicological indices and major organs/systems affected in positive control and at MTC (maximum tolerated concentration) in test compounds.

	5d	**5p**	**5q**	**Phenobarbital**
Test Concentrations (µM)	1, 3, 10, 30	1, 3, 10, 30	1, 3, 10, 30	3000
Statistically Significant Toxic Concentration (µM)	-	-	1	Positive Control
No Observed Adverse Effect Level (NOAEL) (µM)	≥ 10	≥ 10	≥ 3	
Parameters of toxicity at MTC				

Body Shape	-	-	xx	xxx
Somites	-	-	xx	xxx
Notochord	-	-	xxx	xxx
Tail	-	-	xx	xxx
Fins	-	-	xxx	xxx
Brain	-	-	xxx	xxx
Upper jaw	-	-	xxx	xxx
Heart	x	xx	xx	xx
Intestine	xx	xx	xx	xxx
Lower jaw	-	-	xxx	xx
Liver	xx	xx	xxx	xxx
Swim Bladder	-	xx	xx	xxx

(-) No effect; (x) Slightly toxic; (xx) Moderately toxic; (xxx)-Severely toxic.

Hepatotoxicity assay: In this assay 4dpf embryos were exposed to various concentration of test compound prepared from stock solutions as described above. The embryos were distributed in 24 well plates along with 250µl of 0.1% DMSO with 6 embryos in each well. Respective working stock solutions was added each well 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 anesthetized using tricaine. The images of embryos treated with different compounds of various concentrations are analyzed using Image J software for their liver size, liver degeneration and yolk sac retention and percentages were calculated.

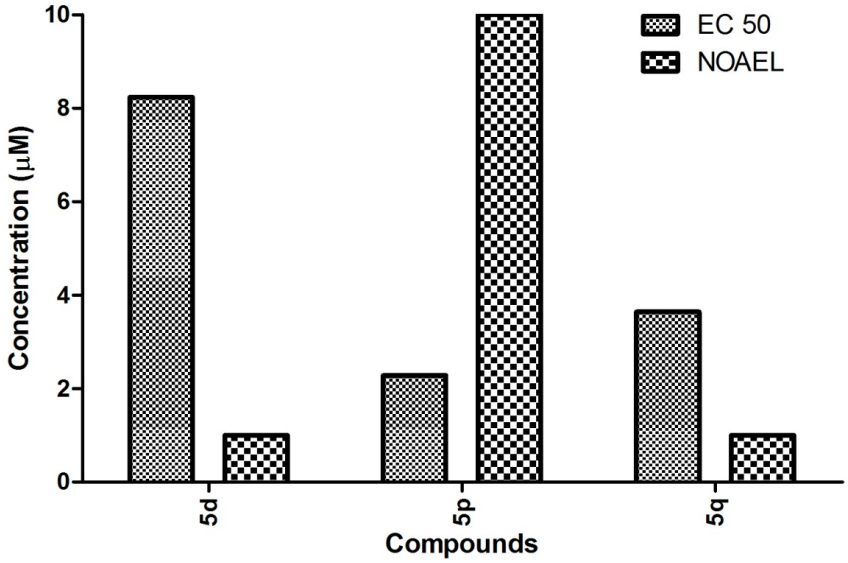


Fig. S-1. The graphical representation of the EC_{50} and NOAEL of compounds **5d, 5p** and **5q**

References

1. Banote, R. K.; Koutarapu, S.; Chennubhotla, K. S.; Chatti, K.; Kulkarni, P. *Epilepsy Behav*. 2013, *27*, 212-219.
2. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish* (Danio rerio); 4th Edition, Eugene, University of Oregon Press, 2000.
3. Nakhi, A.; Archana, S.; Seerapu, G. P.; Chennubhotla, K. S.; Kumar, K. L.; Kulkarni, P.; Haldar, D.; Pal, M. *Chem. Commun.* 2013, *49*, 6268-6270.
4. Panzica-Kelly, J. M.; Zhang, C. X.; Danberry, T. L.; Flood, A.; DeLan, J. W.; Brannen, K. C.; Augustine-Rauch, K. A. 2010, *Birth Defects Res. B*, 89, 382–395.