Electronic Supplementary Information

1,2,3-Triazoles derived from olanzapine: their synthesis *via* ultrasound assisted CuAAC method and evaluation as inhibitors of PDE4B

Suresh Babu Nallapati,[a,b] B. Yogi Sreenivas,[a] Ramudu Bankala,[a] Kishore V. L. Parsa,[a] Shivashankar Sripelly,[a] K. Mukkanti,[b] and Manojit Pal[a,*]

[a]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India*

[b]*Institute of Science and Technology, JNT University Hyderabad, Hyderabad-500085, India*

E-mail: manojitpal@rediffmail.com

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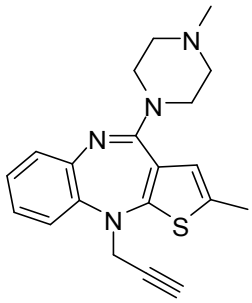
Chemistry

General methods: The reactions were performed using a standard ultrasonic bath instrument (SONOREX SUPER RK 510H model) producing irradiation of 35 KHz. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualized with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane, methanol. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solution by using 400 and 100 MHz spectrometers, respectively. 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), q (quartet) and m (multiplet) as well as bm (broad multiplet). Coupling constants (*J*) are given in hertz. 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). 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. The propargyl bromide used was purchased from Avra Synthesis Pvt. Ltd., Hyderabad, India.

General procedure for the preparation of compound 1[1]:



Propargyl bromide solution (80% in toluene, 19.2 mmol) was added to a solution of olanzapine (16 mmol) and sodium hydride (32 mmol) in THF (20 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 7h. After completion of the reaction (confirmed by TLC), the mixture was diluted with ice-water (60 mL) and extracted with ethyl acetate (3 x 15 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue was purified by column chromatography using

DCM/MeOH as eluent to afford the title compound; pale yellow solid, 154-156 °C (lit[1] mp 155-156 °C).

Experimental procedure for the preparation of azide 2:

General procedure A: preparation of azides 2a-g, 2p, 2q, 2r[2]

To a stirred solution of the corresponding bromide (1.0 equiv) in a 50 mL water/acetone mixture (1:4) was added NaN_3 (1.5 equiv). The resulting suspension was stirred at room temperature for 24 h. DCM was added to the mixture and the organic layer was separated. The aqueous layer was extracted with 3 x 10 mL aliquots of DCM and the combined organic layers were dried over Na_2SO_4. Solvent was removed under reduced pressure, and the azide was sufficiently pure to use without further purification.

In case of ethyl 4-azidobutanoate (**2r**) the reaction mixture was heated under reflux conditions for 7 h.

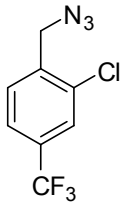
Spectral data of all these compounds e.g. **2a-g, 2p, 2q, 2r** are compared with that reported in the literature.

General procedure B: preparation of azides 2h-o [3]

To a mixture of substituted phenyl amine (0.06 mol) and 15% HCl (60 mL), $NaNO_2$ (5 g, 0.072 mol) in H_2O (200 mL) was added drop-wise at 0 °C. After the completion of addition, the reaction mixture was stirred at the same temperature for 30 min. A solution of sodium azide [7.8 g (0.12 mol) in 30 mL of H_2O] was added in a drop wise manner to the reaction mixture at 0 °C. After addition was over the reaction was maintained at 0 °C for 1 h, the progress of the reaction was monitored by TLC. The mixture was then extracted with ethyl acetate followed by washing with water up to neutral pH. The organic layer was collected, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the desired product.

All compounds are known except compound **2f** and spectral data of all these compounds were compared with that reported in the literature.[3]

1-(Azidomethyl)-2-chloro-4-(trifluoromethyl)benzene (2f)

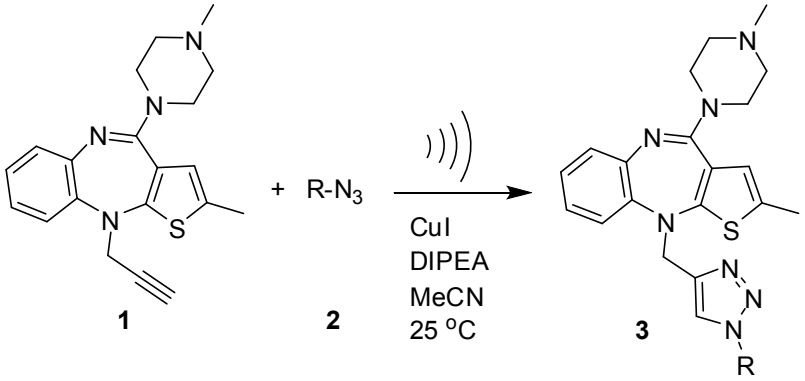


Oily liquid; Yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 7.2 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 4.60 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.9, 132.7, 131.6 (C-F, J = 1.6 Hz), 129.3 (C-F, J = 30.0 Hz), 127.3 (q), 126.9, 122.7 (C-F, J = 272.0 Hz), 52.0.

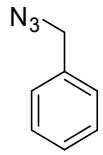
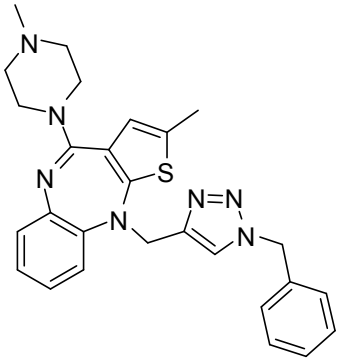
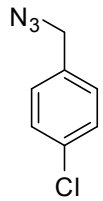
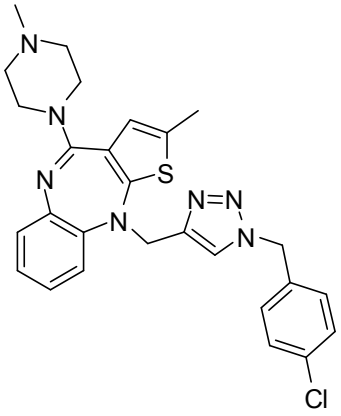
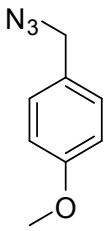
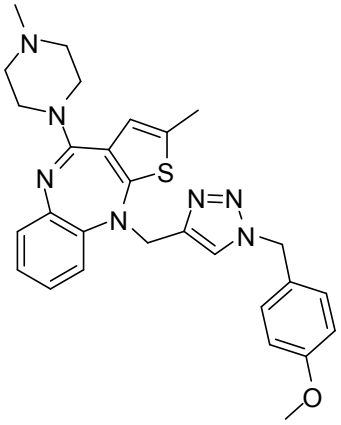
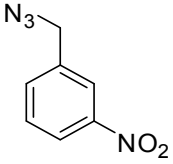
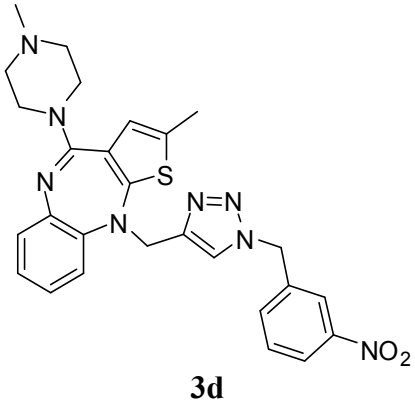
General procedure for the preparation of compound 3

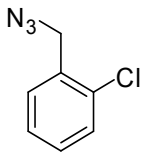
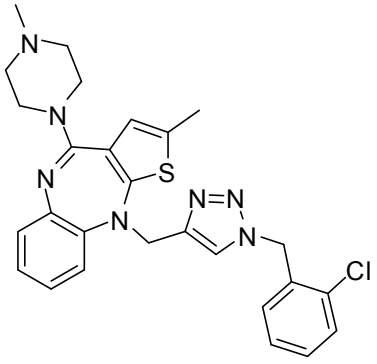
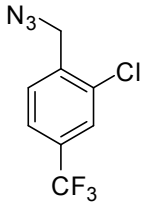
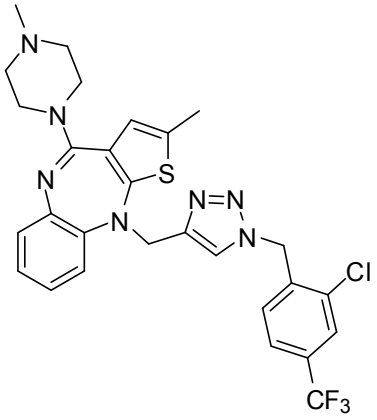
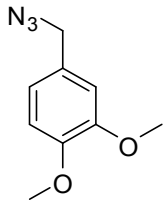
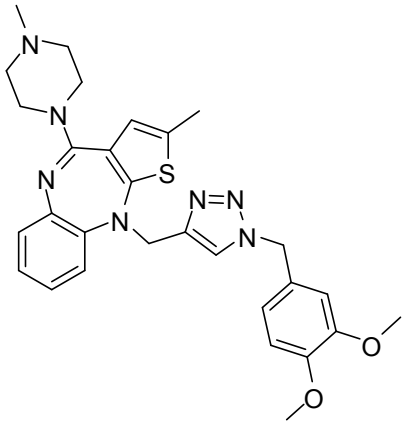
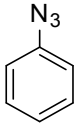
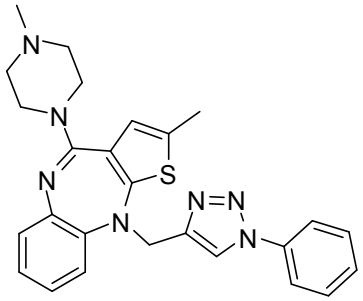
A mixture of compound **1** (1.0 mmol), azide **2** (1.2 mmol), CuI (10 mole %) and *N,N*-diisopropylethylamine (2.0 mmol) in acetonitrile (10 mL) was irradiated with ultrasound (35 KHz) continuously at room temperature for the time indicated in Table S-1. After completion of the reaction (indicated by TLC) the reaction mixture is diluted with EtOAc (50 mL) and filtered through celite bed. The organic layer was collected, combined, washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The crude residue was purified by column chromatography on silica gel using methanol/dichloromethane to afford the desired product. All the products prepared (**3a–r**) were characterized by MS, NMR spectra and purity was determined by HPLC method.

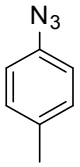
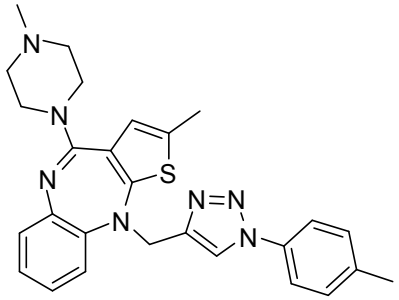
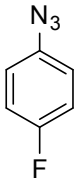
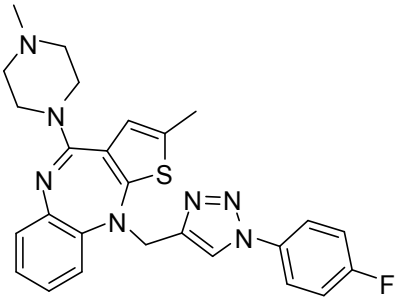
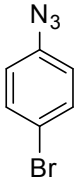
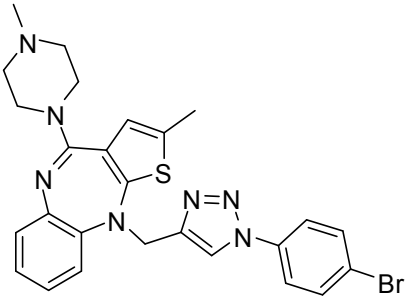
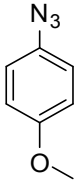
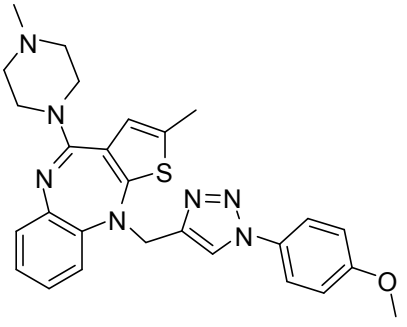
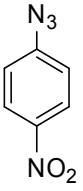
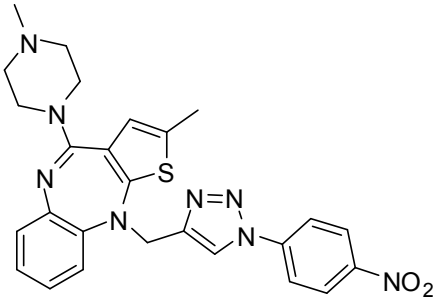
Table S-1. Synthesis of olanzapine based 1,2,3-triazole derivatives (**3**) *via* CuAAC method.[a]

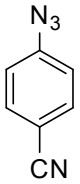
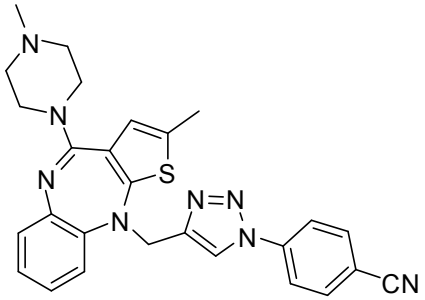
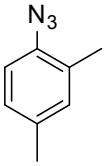
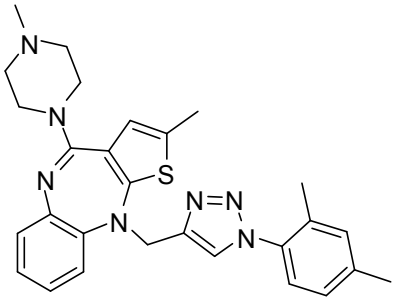
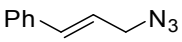
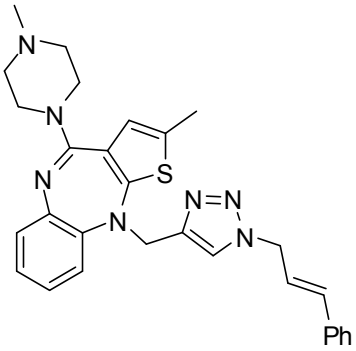
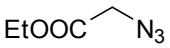
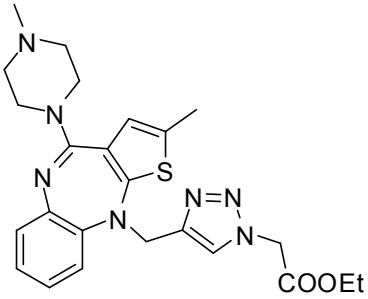
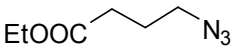
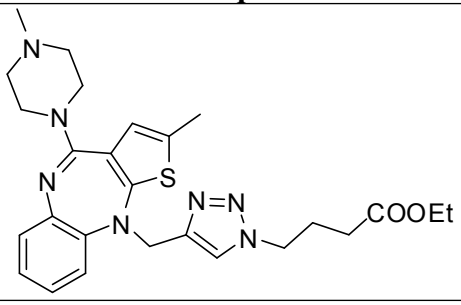


Entry	Azide **(2)**	Product **(3)**	Time (min)	Yield[b] (%)

1	 **2a**	 **3a**	20	89
2	 **2b**	 **3b**	25	83
3	 **2c**	 **3c**	20	85
4	 **2d**	 **3d**	25	77

5	 **2e**	 **3e**	30	79
6	 **2f**	 **3f**	35	73
7	 **2g**	 **3g**	30	84
8	 **2h**	 **3h**	25	78

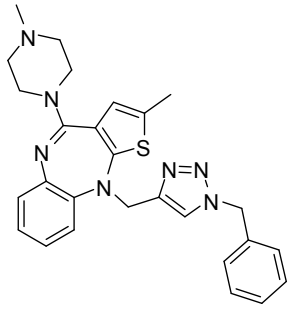
9	 **2i**	 **3i**	25	81
10	 **2j**	 **3j**	30	70
11	 **2k**	 **3k**	30	68
12	 **2l**	 **3l**	30	82
13	 **2m**	 **3m**	30	72

14	 **2n**	 **3n**	35	78
15	 **2o**	 **3o**	35	69
16	 **2p**	 **3p**	30	74
17	 **2q**	 **3q**	25	81
18	 **2r**		30	87

		3r		

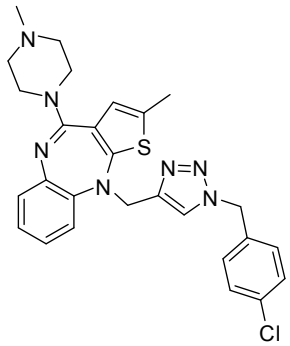
[a]All the reactions were carried out using alkyne **1** (1.0 mmol), azide **2** (1.2 mmol), CuI (10 mole %) and DIPEA (2.0 mmol) under ultrasound irradiation (35 KHz) at room temperature. [b]Isolated yield.

(*E*)-10-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3a)



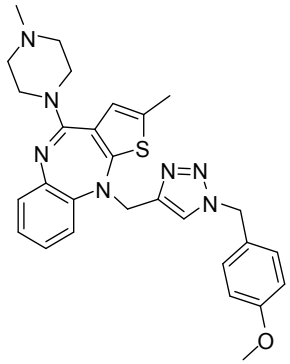
Off-white solid; Yield: 89%; mp 99-101 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (s, 1H), 7.29-7.27 (m, 3H), 7.08-7.05 (m, 2H), 7.03-6.92 (m, 3H), 6.86 (dd, J = 7.6, 1.4 Hz, 1H), 6.25 (s, 1H), 5.49 (d, J = 14.8 Hz, 1H), 5.38 (d, J = 15.2 Hz, 1H), 4.88 (d, J = 15.2 Hz, 1H), 4.77 (d, J = 14.4 Hz, 1H), 3.57-3.42 (bm, 4H), 2.60-2.52 (bm, 2H), 2.49-2.42 (bm, 2H), 2.38 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.2, 151.8, 145.4, 144.2, 134.8, 132.4, 129.5, 128.9 (2C), 128.5, 127.5 (2C), 127.2, 124.8, 124.0, 122.8, 121.2, 120.5, 118.0, 54.7, 54.0 (2C), 48.8, 47.3 (2C), 45.9, 15.8; MS (ES mass): m/z 484.3 (M+1); HPLC: 98.4%; column: Symmetry C-18 75*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN:WATER (80:20) UV 210 nm, retention time 5.7 min.

(*E*)-10-((1-(4-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3b)



Off-white solid; Yield: 83%; mp 152-154 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (s, 1H), 7.25-7.23 (m, 2H), 7.03-6.91 (m, 5H), 6.84 (d, J = 7.6 Hz, 1H), 6.26 (s, 1H), 5.45 (d, J = 15.2 Hz, 1H), 5.34 (d, J = 15.2 Hz, 1H), 4.89 (d, J = 14.8 Hz, 1H), 4.80 (d, J = 14.4 Hz, 1H), 3.51-3.37 (bm, 4H), 2.56-2.49 (bm, 2H), 2.46-2.39 (bm, 2H), 2.35 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.0, 145.7, 144.0, 143.0, 134.4, 133.3, 132.2, 129.1 (2C), 128.8 (2C), 127.3, 124.8, 123.8, 122.7, 121.3, 120.6, 117.9, 55.1, 53.2 (2C), 47.2 (2C), 46.4, 46.1, 15.8; MS (ES mass): m/z 518.0 (M+1); HPLC: 98.7%; column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 210 nm, retention time 3.5 min.

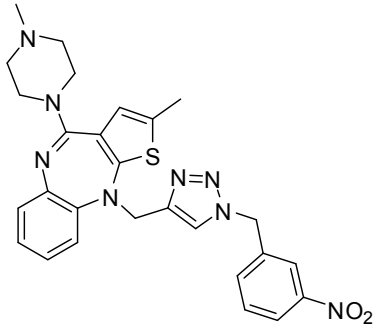
(*E*)-10-((1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3c)



Pale yellow solid; Yield: 85%; mp 194-196 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (s, 1H), 7.06-6.91 (m, 5H), 6.85-6.79 (m, 3H), 6.25 (s, 1H), 5.40 (d, J = 14.4 Hz, 1H), 5.30 (d, J = 14.4 Hz, 1H), 4.85 (d, J = 14.4 Hz, 1H), 4.78 (d, J = 14.8 Hz, 1H), 3.77 (s, 3H), 3.49-3.36 (bm, 4H), 2.58-2.46 (bm, 2H), 2.44-2.38 (bm, 2H), 2.34 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 157.3, 155.0, 145.4, 144.2, 143.0, 132.2,

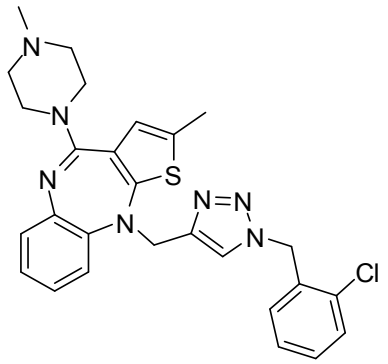
129.1 (2C), 127.2, 126.7, 124.7, 123.8, 122.5, 121.2, 120.6, 117.9, 114.2 (2C), 55.2, 54.9 (2C), 53.5, 47.3, 46.3 (2C), 46.1, 15.8; MS (ES mass): *m*/*z* 514.1 (M+1); HPLC: 99.8%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 230 nm, retention time 2.5 min.

(*E*)-2-Methyl-4-(4-methylpiperazin-1-yl)-10-((1-(3-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3d)



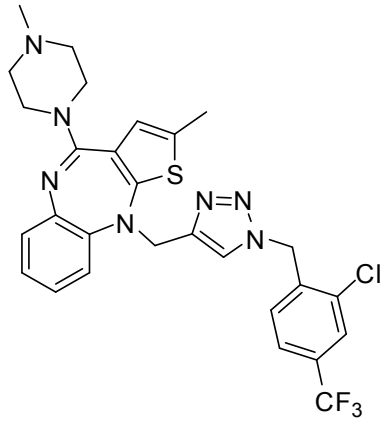
Light yellow solid; Yield: 77%; mp 183-185 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 7.6 Hz, 1H), 7.95 (s, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.42 (s, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.03-6.91 (m, 3H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.28 (s, 1H), 5.59-5.49 (m, 2H), 4.94 (d, *J* = 14.8 Hz, 1H), 4.80 (d, *J* = 13.6 Hz, 1H), 3.52-3.44 (bm, 4H), 2.58-2.51 (bm, 2H), 2.47-2.41 (bm, 2H), 2.36 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.3, 148.4, 145.9, 143.8, 142.8, 136.9, 133.5, 132.4, 130.1, 127.3, 124.9, 123.9, 123.4, 123.0, 122.3, 121.3, 120.4, 118.1, 55.0 (2C), 52.9, 47.2, 46.4 (2C), 46.0, 15.8; MS (ES mass): *m*/*z* 529.0 (M+1); HPLC: 97.8%; column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 210 nm, retention time 3.4 min.

(*E*)-10-((1-(2-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3e)



Pale yellow solid; Yield: 79%; mp 145-147 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (s, 1H), 7.38-7.36 (m, 1H), 7.29-7.27 (m, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.04-6.93 (m, 3H), 6.89-6.83 (m, 2H), 6.26 (s, 1H), 5.56 (s, 2H), 4.88 (d, J = 14.8 Hz, 1H), 4.82 (d, J = 14.4 Hz, 1H), 3.59-3.46 (bm, 4H), 2.56-2.53 (bm, 2H), 2.51-2.44 (bm, 2H), 2.38 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.0, 145.7, 144.0, 143.0, 134.4, 133.3, 132.2, 129.1 (2C), 128.8 (2C), 127.3, 124.8, 123.8, 122.7, 121.3, 120.6, 117.9, 55.1 (2C), 53.2, 47.2 (2C), 46.4, 46.1, 15.8; MS (ES mass): m/z 518.2 (M+1); HPLC: 99.0%; column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 210 nm, retention time 3.45 min.

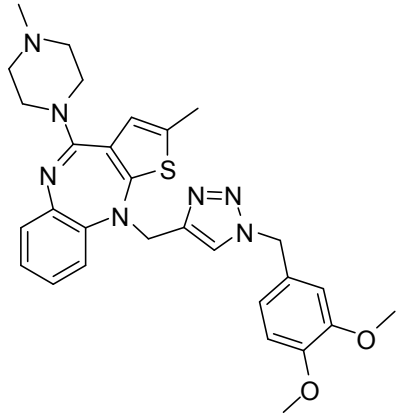
(*E*)-10-((1-(2-Chloro-4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3f)



Off-white solid; Yield: 73%; mp 151-153 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 7.6 Hz, 1H), 7.47 (s, 1H), 7.25-7.21 (m, 1H), 6.99-6.90 (m, 4H), 6.84-6.82 (m, 1H), 6.24 (s, 1H), 5.58 (s, 2H), 4.88 (d, J = 15.2 Hz, 1H), 4.75 (d, J =

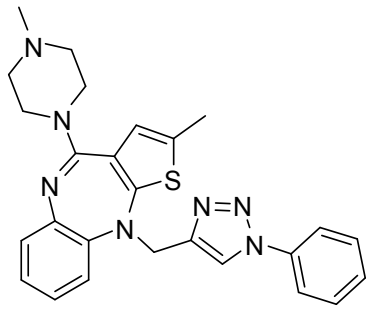
14.8 Hz, 1H), 3.63-3.47 (bm, 4H), 2.58-2.51 (bm, 2H), 2.49-2.41 (bm, 2H), 2.35 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.8, 154.6, 144.7, 143.0, 136.8, 134.1, 132.7, 130.5, 128.3, 128.2, 128.1, 128.0, 127.1, 125.2, 124.9, 124.5, 123.9, 121.6, 120.8, 118.9, 54.1 (2C), 50.9, 47.0, 46.1, 45.5, 31.9, 15.8; MS (ES mass): *m*/*z* 586.0 (M+1); HPLC: 98.3%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 200 nm, retention time 2.6 min.

(*E*)-10-((1-(3,4-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4 methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3g)



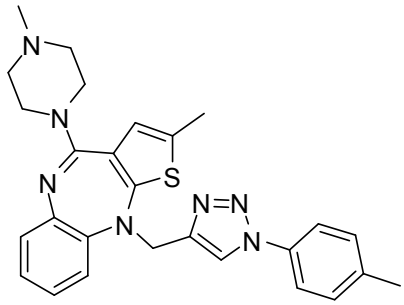
Off-white solid; Yield: 84%; mp 169-171 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (s, 1H), 7.02-6.92 (m, 3H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 6.25 (s, 1H), 5.42 (d, *J* = 15.2 Hz, 1H), 5.29 (d, *J* = 14.8 Hz, 1H), 4.88 (d, *J* = 14.4 Hz, 1H), 4.77 (d, *J* = 14.0 Hz, 1H), 3.86 (s, 3H), 3.74 (s, 3H), 3.54-3.38 (bm, 4H), 2.62-2.52 (bm, 2H), 2.50-2.42 (bm, 2H), 2.38 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 155.3, 149.3, 149.1, 145.4, 144.2, 132.3, 127.2, 127.1, 124.8 (2C), 123.9, 122.5, 121.2, 120.4, 120.3, 117.9, 111.0, 110.5, 55.8 (2C), 54.7 (2C), 53.9, 47.3, 46.1 (2C), 45.8, 15.8; MS (ES mass): *m*/*z* 544.1 (M+1); HPLC: 98.6%; column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 210 nm, retention time 3.3 min.

(*E*)-2-Methyl-4-(4-methylpiperazin-1-yl)-10-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3h)



Off-white solid; Yield: 78%; mp 144-146 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.41-7.37 (m, 1H), 7.07-6.91 (m, 4H), 6.30 (s, 1H), 4.99 (d, J = 14.8 Hz, 1H), 4.88 (d, J = 14.8 Hz, 1H), 3.60-3.52 (bm, 4H), 2.58-2.51 (bm, 2H), 2.49-2.41 (bm, 2H), 2.35 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 155.2, 144.3, 142.8, 132.4, 130.3, 130.1, 128.9, 127.3, 124.9, 124.0, 121.8 (2C), 121.3, 120.7, 120.5, 117.9, 114.7 (2C), 55.0 (2C), 47.3, 46.4 (2C), 46.1, 15.8; MS (ES mass): m/z 470.3 (M+1); HPLC: 99.5%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 3/20, 8/40, 15/95, 20/95, 25/20, 30/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (80:20) UV 240 nm, retention time 9.3 min.

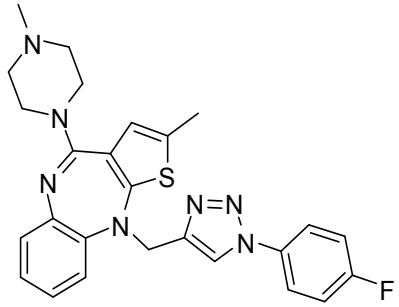
(*E*)-2-Methyl-4-(4-methylpiperazin-1-yl)-10-((1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3i)



Pale yellow solid; Yield: 81%; mp 144-146 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.29-7.26 (m, 2H), 7.06-7.04 (m, 1H), 7.02-6.95 (m, 2H), 6.93-6.91 (m, 1H), 6.29 (s, 1H), 4.98 (d, J = 14.8 Hz, 1H), 4.86 (d, J

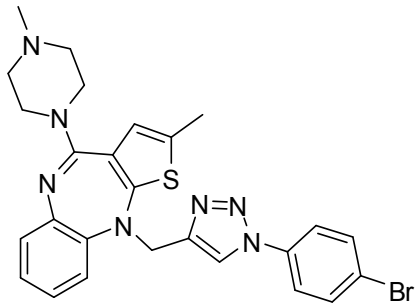
= 13.2 Hz, 1H), 3.59-3.53 (bm, 4H), 2.57-2.53 (bm, 2H), 2.49-2.43 (bm, 2H), 2.39 (s, 3H), 2.35 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 155.0, 145.6 (2C), 144.2, 143.0, 138.7, 134.6, 132.4, 130.1 (2C), 127.3, 124.8, 123.8, 121.3, 120.5, 120.1 (2C), 117.8, 55.1 (2C), 47.3, 46.4 (2C), 46.1, 21.0, 15.8; MS (ES mass): *m*/*z* 484.3 (M+1); HPLC: 99.2%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN:WATER (80:20) UV 210 nm, retention time 6.0 min.

(*E*)-10-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3j)



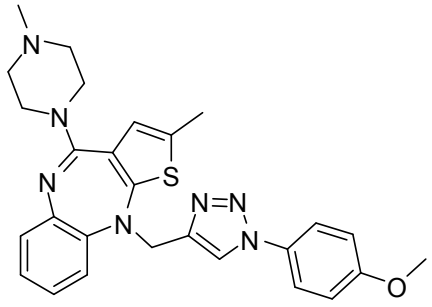
Off-white solid; Yield: 70%; mp 218–219 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (s, 1H), 7.65-7.60 (m, 2H), 7.16 (t, *J* = 8.4 Hz, 2H), 7.06 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.03-6.96 (m, 2H), 6.93-6.90 (m, 1H), 6.30 (s, 1H), 5.00 (d, *J* = 14.8 Hz, 1H), 4.86 (d, *J* = 14.8 Hz, 1H), 3.68-3.55 (bm, 4H), 2.67-2.58 (bm, 2H), 2.56-2.48 (bm, 2H), 2.39 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.5 (C-F *J* = 247.9 Hz), 157.3, 145.6, 144.3, 133.1 (C-F *J* = 4.4 Hz), 132.7, 127.3 (2C), 126.9, 125.0, 124.4, 122.3 (C-F *J* = 8.5 Hz), 121.3, 120.9, 120.1, 118.0, 116.8 (C-F *J* = 23.1 Hz), 114.2 (C-F *J* = 5.6 Hz), 54.6 (2C), 47.1, 46.3 (2C), 45.7, 15.9; MS (ES mass): *m*/*z* 488.2 (M+1); HPLC: 99.3%; column: Agilent Eclipse Plus C-18 250*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/5, 3/5, 10/95, 20/95, 22/5, 25/5; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20) UV 240 nm, retention time 9.6 min.

(*E*)-10-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3k)



Light brown solid; Yield: 68%; mp 149-151 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.10-6.89 (m, 4H), 6.31 (s, 1H), 5.02 (d, J = 15.2 Hz, 1H), 4.87 (d, J = 15.2 Hz, 1H), 3.69-3.54 (bm, 4H), 2.68-2.57 (bm, 2H), 2.54-2.48 (bm, 2H), 2.39 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 154.8, 145.4, 144.9, 136.1, 133.3, 133.2 (2C), 132.9, 127.1, 125.0, 124.3, 122.5, 122.1 (2C), 121.7, 121.6, 120.5, 118.9, 55.3 (2C), 53.0, 47.0 (2C), 45.0, 15.9; MS (ES mass): *m*/*z* 548.1 (M+1); HPLC: 97.8%; column: Agilent Eclipse Plus C-18 250*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/5, 3/5, 10/95, 20/95, 22/5, 25/5; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20) UV 254 nm, retention time 9.6 min.

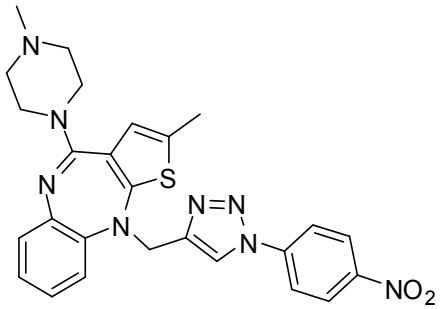
(*E*)-10-((1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3l)



Off-white solid; Yield: 82%; mp 124–126 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 7.2 Hz, 1H), 7.02-6.91 (m, 5H), 6.29 (s, 1H), 4.97 (d, J = 14.4 Hz, 1H), 4.86 (d, J = 14.8 Hz, 1H), 3.84 (s, 3H), 3.65-3.52 (bm, 4H), 2.65-2.55 (bm, 2H), 2.53-2.42 (bm, 2H), 2.38 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 157.3, 155.2, 144.3, 142.8, 132.4, 130.3, 128.9, 127.3, 124.9, 124.0, 121.8 (2C), 121.3, 120.7, 120.5, 117.9, 114.7 (2C), 55.6, 55.0 (2C), 47.3, 46.4 (2C), 46.0,

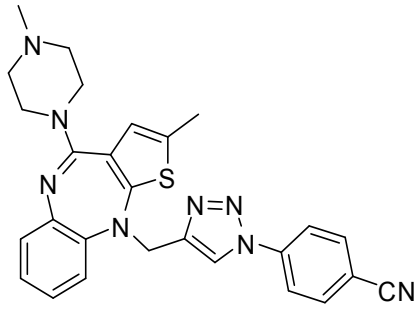
15.8; MS (ES mass): *m*/*z* 500.1 (M+1); HPLC: 97.8%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 260 nm, retention time 2.5 min.

(*E*)-2-Methyl-4-(4-methylpiperazin-1-yl)-10-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3m)



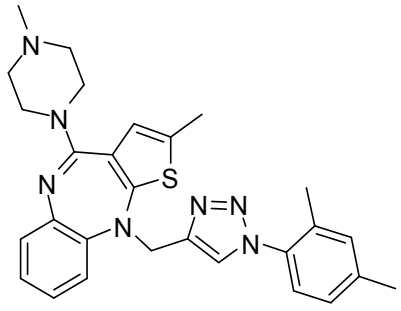
Pale yellow solid; Yield: 72%; mp 148-150 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (d, J = 9.2 Hz, 2H), 7.95 (s, 1H), 7.89 (d, J = 9.2 Hz, 2H), 7.09-6.89 (m, 4H), 6.32 (s, 1H), 5.03 (d, J = 15.2 Hz, 1H), 4.88 (d, J = 14.4 Hz, 1H), 3.65-3.48 (bm, 4H), 2.62-2.50 (bm, 2H), 2.48-2.42 (bm, 2H), 2.37 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.0, 147.0, 146.7, 143.8, 141.0, 132.4, 127.4, 125.4 (2C), 125.0, 123.9, 121.5, 120.6, 120.5, 120.3 (2C), 117.7, 109.9, 55.1 (2C), 47.0, 46.6 (2C), 46.2, 15.8; MS (ES mass): *m*/*z* 515.1 (M+1); HPLC: 99.2%; column: Agilent Eclipse Plus C-18 250*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/5, 3/5, 10/95, 20/95, 22/5, 25/5; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20) UV 265 nm, retention time 9.4 min.

(*E*)-4-(4-((2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepin-10-yl)methyl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (3n)



Off-white solid; Yield: 78%; mp 182-184 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.10-6.89 (m, 4H), 6.31 (s, 1H), 5.02 (d, J = 15.2 Hz, 1H), 4.87 (d, J = 15.2 Hz, 1H), 3.69-3.54 (bm, 4H), 2.68-2.57 (bm, 2H), 2.54-2.48 (bm, 2H), 2.39 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 155.8, 146.2, 144.2, 139.5, 133.8 (2C), 132.8, 127.4, 125.1, 124.4, 121.3, 120.6, 120.4 (2C), 120.1, 118.0, 117.7, 112.2, 54.6 (2C), 47.0, 46.3 (2C), 45.6, 15.8; MS (ES mass): m/z 495.1 (M+1); HPLC: 97.7%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 260 nm, retention time 2.5 min.

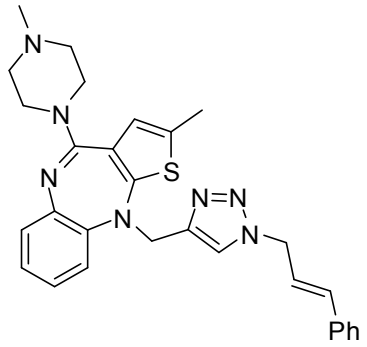
(*E*)-10-((1-(2,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3o)



Light brown solid; Yield: 69%; mp 152-154 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.12-6.98 (m, 6H), 6.94-6.91 (m, 1H), 6.30 (s, 1H), 4.99 (d, J = 13.2 Hz, 1H), 4.86 (d, J = 13.2 Hz, 1H), 3.80-3.54 (bm, 4H), 2.78-2.55 (bm, 4H), 2.43 (s, 3H), 2.36 (s, 3H), 2.33 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.1, 145.7, 145.6, 144.3, 143.1, 138.7, 134.6, 132.4, 130.1 (2C), 127.3, 124.9, 123.9, 121.3,

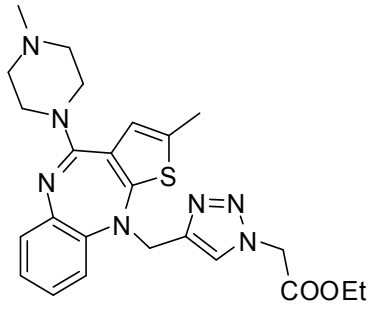
120.6, 120.1 (2C), 117.8, 55.1 (2C), 47.3, 46.4 (2C), 46.1, 21.0, 18.1, 15.9; MS (ES mass): *m*/*z* 497.3 (M+1); HPLC: 96.2%; column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 210 nm, retention time 3.5 min

(*E*)-10-((1-Cinnamyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine (3p)



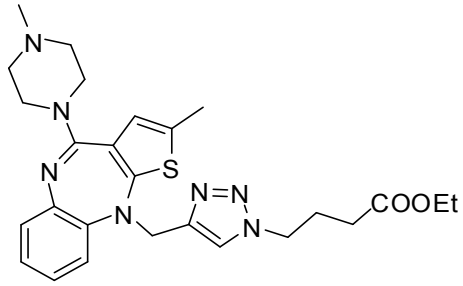
Off-white solid; Yield: 74%; mp 210–211 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 1H), 7.32-7.25 (m, 5H), 7.04 (dd, J = 7.6, 2.0 Hz, 1H), 7.00-6.93 (m, 2H), 6.89 (dd, J = 7.6, 1.8 Hz, 1H), 6.41 (d, J = 15.6 Hz, 1H), 6.27-6.21 (m, 2H), 5.07-4.99 (m, 2H), 4.92 (d, J = 15.2 Hz, 1H), 4.80 (d, J = 14.0 Hz, 1H), 3.56-3.45 (bm, 4H), 2.56-2.46 (bm, 2H), 2.42-2.34 (bm, 2H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 155.4, 145.3, 144.3, 142.7, 135.4, 134.5, 132.4, 128.6 (2C), 128.4, 127.2, 126.6 (2C), 124.8, 123.9, 122.5, 122.0, 121.3, 120.5, 118.0, 54.7 (2C), 52.1, 47.3, 46.3 (2C), 45.8, 15.8; MS (ES mass): *m*/*z* 510.1 (M+1); HPLC: 99.7%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 250 nm, retention time 2.5 min.

(*E*)-Ethyl-2-(4-((2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepin-10-yl)methyl)-1*H*-1,2,3-triazol-1-yl)acetate (3q)



Off-white solid; Yield: 81%; mp 170-172 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.04 (dd, J = 7.6, 1.2 Hz, 1H), 7.00-6.91 (m, 2H), 6.87 (d, J = 7.6 Hz, 1H), 6.29 (s, 1H), 5.06 (d, J = 16.8 Hz, 1H), 5.00 (d, J = 16.4 Hz, 1H), 4.96 (d, J = 14.4 Hz, 1H), 4.80 (d, J = 14.4 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.65-3.54 (bm, 4H), 2.67-2.55 (bm, 2H), 2.60-2.48 (bm, 2H), 2.39 (s, 3H), 2.31 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 157.4, 155.4, 145.4, 144.1, 142.9, 132.3, 127.2, 124.8, 124.1, 123.9, 121.3, 120.4, 117.9, 62.3, 54.9 (2C), 50.8, 47.1 (2C), 45.9, 22.6, 15.8, 14.0; MS (ES mass): m/z 480.0 (M+1); HPLC: 96.8%; column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/%B: 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN:WATER (90:10) UV 205 nm, retention time 2.3 min.

(*E*)-Ethyl-4-(4-((2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepin-10-yl)methyl)-1*H*-1,2,3-triazol-1-yl)butanoate (3r)



Off-white solid; Yield: 87%; mp 112-114 °C; R_f (10% Methanol/Dichloromethane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (s, 1H), 7.03 (d, J = 7.6 Hz, 1H), 7.01-6.92 (m, 2H), 6.85 (d, J = 7.84 Hz, 1H), 6.30 (s, 1H), 4.92 (d, J = 14.8 Hz, 1H), 4.78 (d, J = 14.8 Hz, 1H), 4.31 (t, J = 6.8 Hz, 2H), 4.10 (q, J = 7.2 Hz, 6.8 Hz, 2H), 3.61-3.52 (bm, 4H), 2.59-2.55 (bm, 2H), 2.50-2.47 (bm, 2H), 2.39 (s, 3H), 2.32 (s, 3H), 2.21 (t, J = 6.7 Hz, 2H), 2.16-2.10 (m, 2H), 1.24 (t, J = 7.2

Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 157.1, 151.5, 144.2, 139.7, 138.6, 132.9, 128.2, 127.5, 125.3, 124.4, 121.1, 120.0 (2C), 118.0, 117.3, 114.1 (2C), 113.1, 109.9, 54.3, 48.0, 42.1, 29.6, 22.6, 15.9, 14.0; MS (ES mass): *m*/*z* 508.3 (M+1); HPLC: 97.1%; column: Agilent Eclipse Plus C-18 250*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/5, 3/5, 10/95, 20/95, 22/5, 25/5; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20) UV 240 nm, retention time 9.1 min.

References

1 Gorja, D. R.; Mukherjee, S.; Meda, C. L. T.; Deora, G. S.; Kumar, K. L.; Jain, A.; Chaudhari, G. H.; Chennubhotla, K. S.; Banote, R. K.; Kulkarni, P.; Parsa, K. V. L.; Mukkanti, K.; Pal, M. *Org. Biomol. Chem*. **2013**, *11*, 2075.

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3 Zhou, S.; Liao, H.; Liu, M.; Feng, G.; Fu, B.; Li, R.; Cheng, M.; Zhao, Y.; Gong, P. *Bioorg. Med. Chem. Lett*. **2014**, *22*, 6438.

Pharmacology

Cells and Reagents: HEK 293T and Sf9 cells were obtained from ATCC (Washington D.C., USA). HEK 293T cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC and routinely cultured in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc.). cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDE4D2 enzyme was purchased from BPS Bioscience (San Diego, CA, USA). Lipopolysaccharide (LPS) was from *Escherichia coli* strain 0127:B8 obtained from Sigma (St. Louis, MO, USA). Mouse TNF-α ELISA kit was procured from R&D Systems (Minneapolis, MN, USA).

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 ºC 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_{50}s were computed using GraphPad Prism Version 5.04 software.

$$\%\ inhibition = \frac{(RLU\ of\ vehicle\ control - RLU\ of\ inhibitor)}{RLU\ of\ vehicle\ control} X\ 100$$

PDE4D enzymatic assay

This assay was performed following a similar method as described above using 0.5 ng commercially procured PDE4D2 enzyme instead of 10 ng of in house purified PDE4B without changing any other factors or conditions.

Reference:

1. Wang, P.; Myers, J. G.; Wu, P.; Cheewatrakoolpong, B.; Egan, R. W.; Billah, M. M. *Biochem. Biophys. Res. Commun.* **1997**, *19*, 320.