Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

Novel benzoxepine–1,2,3-triazole hybrids: Synthesis and pharmacological evaluation as potential antibacterial and anticancer agents

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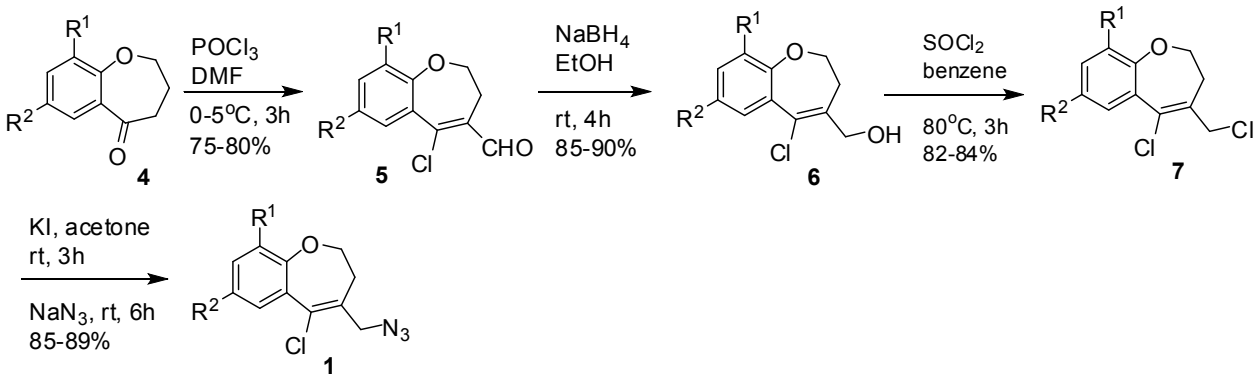
General methods

Melting points were determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker – alpha t spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 or 400 MHz spectrometer using $CDCl_3$, with reference to tetramethylsilane as an internal reference. Mass spectra were recorded on a Jeol JMC D-300 instrument by using Electron ionization at 70 eV. All reactions were monitored by TLC (thin layer chromatography) on pre-coated silica gel plates. Column chromatography was performed by using silica gel (100–200 mesh, SRL, India) [10–20 times (by weight) of the crude product.

Synthesis of azide 1



Preparation of aldehyde 5

The aldehyde **5** was prepared via Vilsmeier-Haack-Arnold reaction of ketone **4** according to the reported method.[1,2]

References

1. Z. Arnold, and J. Zemlicka, *Collect. Czech. Chem. Commun.* 1959, **24**, 2385.
2. R. Bera, N. K. Swamy, G. Dhananjaya, J. M. Babu, P. R. Kumar, K. Mukkanti, and M. Pal, *Tetrahedron* 2007, **63**,13018.

Preparation of alcohol 6

To a stirred solution of aldehyde **5** (20.0 mmol) in EtOH (20 mL), was added sodium borohydride (378.3 mg, 10.0 mmol) slowly at 0-5 °C. Then the temperature was allowed to increase to 25- 30 °C. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to 0-5 °C, acidified with conc. HCl and extracted with EtOAc (3 x 50 mL). The

combined organic extract was washed with brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the desired alcohol **6**. This compound was used for the next step without further purification.

Preparation of chloro compound 7

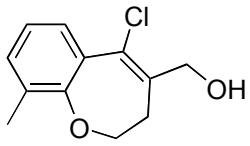
A solution of alcohol **6** (18.0 mmol) and pyridine (0.2 mL) in benzene (7 mL) was placed in a RB flask. The flask was immersed in an ice bath and the solution was vigorously stirred. To this was added thionyl chloride (16 mL, 22.0 mmol) in benzene (10 mL) slowly. The ice bath was removed and the solution was stirred at room temperature for 30 min and then at 80 °C for 3 h. After completion of the reaction the solvent was removed by distillation to give desired chloro compound **7** that was used in the next step without any further purification.

Preparation of azide 1

A mixture of chloro compound **7** (8 mmol) and potassium iodide (1658.7 mg, 10.0 mmol) in dry acetone (15 mL) was stirred for 2h (the progress of the reaction was monitored by TLC). After completion of the reaction a solution of sodium azide (572.08 mg, 8.8 mmol) dissolved in water (10 mL) was added to the residue. The progress of the reaction was monitored by TLC. After completion of the reaction the mixture was poured into crushed ice and extracted with EtOAc (3 x 50 mL). The combined organic extract was washed with brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the expected azide **1**.

Spectral data of alcohols (6), chloro compounds (7) and azides (1)

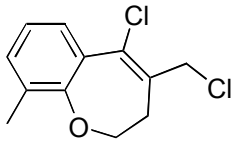
(5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methanol (**6a**):



Colourless liquid, Yield 82%; R_f 0.5 (Pet ether : EtOAc 1:9); MS *m/z* 225.1 (M+1, 100%); IR: 3465, 3025, 2886, 1627, 1491, 1411, 1295, 1046, 1029, 837; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (d, 1H, *J* = 7.8 Hz), 7.20 (d, 1H, *J* = 7.9 Hz), 7.16 (t, 1H, *J* = 7.6 Hz), 4.58 (s, 2H), 4.54 (t, 2H, *J*

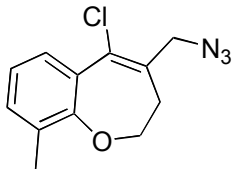
= 6.2 Hz), 2.48 (t, 2H, *J* = 6.2 Hz), 2.30 (s, 3H), 1.91 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.8, 136.4, 132.3, 131.3, 127.0, 126.2, 123.4, 77.4, 63.5, 30.2, 16.2.

5-Chloro-4-(chloromethyl)-9-methyl-2,3-dihydrobenzo[b]oxepine (**7a**)**:**



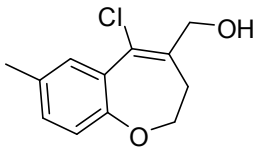
Colourless liquid, Yield 75%; R_f 0.85 (Pet ether : EtOAc 1:9); MS *m/z* 243.0 (M+1, 100%); IR: 2928, 2878, 1627, 1491, 1438, 1273, 1209, 1034, 830; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42 (d, 1H, *J* = 7.8 Hz), 7.20 (d, 1H, *J* = 7.9 Hz), 7.16 (t , 1H, *J* = 7.8 Hz), 4.58 (t, 2H, *J* = 6.2 Hz), 4.53 (s, 2H), 2.48 (t, 2H, *J* = 6.2 Hz), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.4, 131.7, 130.4, 128.4, 126.1, 125.9, 124.0, 120.1, 66.8, 38.0, 32.9, 15.8.

4-(Azidomethyl)-5-chloro-9-methyl-2,3-dihydrobenzo[b]oxepine (**1a**):



Yellow liquid, Yield 84%; R_f 0.75 (Pet ether : EtOAc 1:9); MS *m/z* 250.1 (M+1, 100%); IR: 3060, 2925, 2870, 2226, 2120, 1625, 1495, 1320, 1301, 1209, 1041, 820; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.40 (d, 1H, *J* = 7.8 Hz), 7.22 (d, 1H, *J* = 7.9 Hz), 7.05 (t , 1H, *J* = 7.6 Hz), 4.55 (t, 2H, *J* = 6.2 Hz), 4.30 (s, 2H), 2.42 (t, 2H, *J* = 6.2 Hz), 2.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.9, 131.8, 131.6, 131.4, 131.1, 129.3, 127.2, 123.5, 78.0, 53.8, 31.2, 16.2.

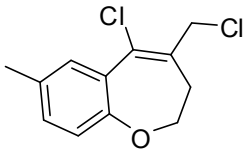
(5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methanol (**6b**)**:**



Colourless liquid, Yield 85%; R_f 0.5 (Pet ether : EtOAc 1:9); MS *m/z* 225.0 (M+1, 100%); IR: 3463, 3012, 2882, 1621, 1495, 1405, 1296, 1047, 1029, 839; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (dd, 1H, *J* = 8.0, 1.5 Hz), 7.16 (d, 1H, *J* = 7.9 Hz), 7.08 (d, 1H, *J* = 7.9 Hz), 4.60 – 4.40 (m, 4H),

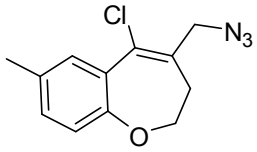
2.48 (t, 2H, *J* = 6.2 Hz), 2.35 (s, 3H), 1.83 (bs,1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.9, 136.7, 133.3, 131.4, 130.6, 129.7, 126.0, 121.8, 79.3, 63.6, 30.2, 20.8.

5-Chloro-4-(chloromethyl)-7-methyl-2,3-dihydrobenzo[b]oxepine (**7b**):



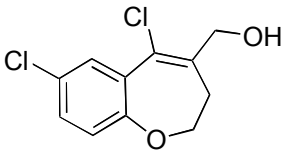
Colourless liquid, Yield 78%; R_f 0.85 (Pet ether : EtOAc 1:9); MS *m/z* 243.2 (M+1, 100%); IR: 2935, 2869, 1622, 1495, 1445, 1281, 1209, 1035, 833; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.40 (s, 1H), 7.12 (d, 1H, *J* = 7.8 Hz), 6.95 (d, 1H, *J* = 7.9 Hz), 4.56 (t, 2H, *J* = 6.2 Hz), 4.52 (s, 2H), 2.45 (t, 2H, *J* = 6.2 Hz), 2.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 152.9, 132.5, 131.8, 131.7, 131.4, 129.5, 127.3, 123.4, 78.4, 45.8, 31.5, 16.2.

4-(Azidomethyl)-5-chloro-7-methyl-2,3-dihydrobenzo[b]oxepine (**1b**)**:**



Yellow liquid, Yield 86%; R_f 0.75 (Pet ether : EtOAc 1:9); MS *m/z* 250.1 (M+1, 100%); IR: 3060, 2870, 2228, 2124, 1625, 1491, 1327, 1300, 1203, 1048, 824; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38 (s, 1H), 7.16 (d, 1H, *J* = 7.8 Hz), 6.98 (d, 1H, *J* = 7.9 Hz), 4.54 (s, 2H), 4.50 (t, 2H, *J* = 6.2 Hz), 2.42 (t, 2H, *J* = 6.2 Hz), 2.15 (s, 3H); ^{13}CNMR ($CDCl_3$, 100 MHz) 153.0, 133.5, 131.5, 131.1, 131.0, 129.9, 129.1, 121.9, 78.7, 53.9, 31.3, 20.9.

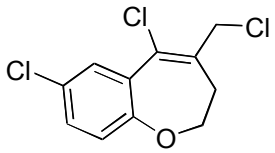
(5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methanol (**6c**):



Colourless liquid, Yield 80%; R_f 0.5 (Pet ether : EtOAc 1:9); MS *m/z* 245.1 (M+1, 100%); IR: 3467, 3013, 2886, 1630, 1467, 1401, 1287, 1039, 1029, 856; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.60 (s, 1H), 7.26 (d, 1H, *J* = 8.5 Hz), 7.03 (d, 1H, *J* = 8.6 Hz), 4.60 (s, 2H), 4.52 (t, 2H, *J* = 5.9 Hz),

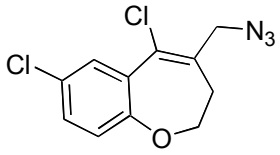
2.50 (t, 2H, *J* = 5.9 Hz), 1.82 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): □ 153.8, 136.3, 133.4, 131.5, 130.3, 129.6, 126.6, 121.7, 79.1, 65.8, 30.5.

5,7-Dichloro-4-(chloromethyl)-2,3-dihydrobenzo[b]oxepine (**7c**):



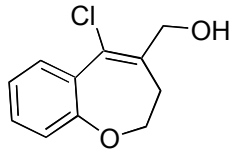
Colourless liquid, Yield 73%; R_f 0.80 (Pet ether : EtOAc 1:9); MS *m/z* 262.0 (M, 100%); IR: 2924, 2874, 1621, 1495, 1431, 1273, 1215, 1032, 856, 830; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.58 (s, 1H), 7.22 (d, 1H, *J* = 8.5 Hz), 6.97 (d, 1H, *J* = 8.6 Hz), 4.56 (s, 2H), 4.48 (t, 2H, *J* = 6.0 Hz), 2.50 (t, 2H, *J* = 6.1Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): □ 153.8, 134.3, 132.4, 130.3, 129.6, 127.9, 123.6, 115.7, 79.1, 45.7, 31.5.

4-(Azidomethyl)-5,7-dichloro-2,3-dihydrobenzo[b]oxepine (**1c**):



Colourless liquid, Yield 82%; R_f 0.73 (Pet ether : EtOAc 1:9); MS *m/z* 270.1 (M+1, 100%); IR: 3060, 2922, 2870, 2228, 2110, 1625, 1495, 1324, 1311, 1205, 1045, 820, 784; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (s, 1H), 7.29 (d, 1H, *J* = 8.5 Hz), 6.96 (d, 1H, *J* = 8.6 Hz), 4.56 (s, 2H), 4.53 (t, 2H, *J* = 6.0 Hz), 2.50 (t, 2H, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): □ 153.8, 133.2, 132.4, 130.2, 129.5, 127.5, 123.5, 115.7, 78.6, 53.8, 31.2.

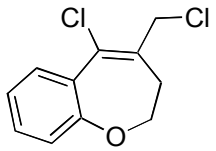
(5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methanol (**6d**):



Colourless liquid, Yield 75%; R_f 0.45 (Pet ether : EtOAc 1:9); MS *m/z* 211.2 (M+1, 100%); IR: 3469, 3022, 2883, 1621, 1496, 1410, 1292, 1036, 1021, 823; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84 (d, 1H, *J* = 7.7 Hz), 7.28 (t, 1H, *J* = 7.5 Hz), 7.17 (t, 1H, *J* = 7.5 Hz), 7.05 (d, 1H, *J* = 7.9 Hz), 4.60 – 4.40 (m, 4H), 2.50 (t, 2H, *J* = 6.0 Hz), 1.90 (bs, 1H);

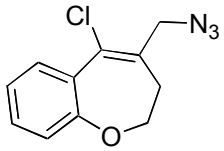
^{13}C NMR ($CDCl_3$, 100 MHz): 156.3, 131.8, 131.2, 130.3, 128.9, 128.8, 123.9, 122.2, 78.7, 63.9, 31.4.

5-Chloro-4-(chloromethyl)-2,3-dihydrobenzo[b]oxepine (**7d**):



Colourless liquid, Yield 71%; R_f 0.83 (Pet ether : EtOAc 1:9 MS *m/z* 229.2 (M+1, 100%); IR: 2934, 2876, 1620, 1492, 1445, 1271, 1212, 1029, 832; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.60 (d, 1H, *J* = 7.7 Hz), 7.28 (t, 1H, *J* = 7.5 Hz), 7.17 (t, 1H, *J* = 7.5 Hz), 7.05 (d, 1H, *J* = 7.9 Hz), 4.56 (s, 2H), 4.53 (t, 2H, *J* = 6.0 Hz), 2.50 (t, 2H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): 154.3, 133.8, 132.2, 130.3, 129.8, 126.9, 123.9, 117.2, 78.7, 43.7, 31.4.

4-(Azidomethyl)-5-chloro-2,3-dihydrobenzo[b]oxepine (**1d**):

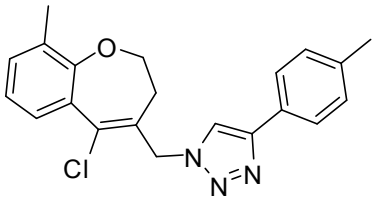


Colourless liquid, Yield 80%; R_f 0.72 (Pet ether : EtOAc 1:9); MS *m/z* 236.1 (M+1, 100%); IR: 3050, 2932, 2850, 2220, 2120, 1622, 1490, 1325, 1309, 1201, 1048, 828; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64 (d, 1H, *J* = 7.7 Hz), 7.28 (t, 1H, *J* = 7.5 Hz), 7.17 (t, 1H, *J* = 7.5 Hz), 7.05 (d, 1H, *J* = 7.9 Hz), 4.55 (t, 2H, *J* = 6.0 Hz), 4.25 (s, 2H), 2.42 (t, 2H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): 155.3, 131.8, 131.2, 130.3, 129.8, 128.9, 123.9, 122.2, 78.7, 53.9, 31.4.

General Procedure for the preparation of compound 3

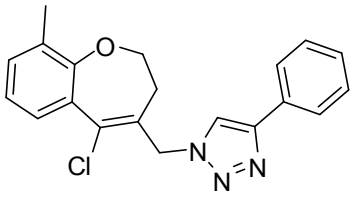
A mixture of azide **1** (2 mmol), an appropriate terminal alkyne **2** (2 mmol), copper sulphate (250 mg, 1mmol) and sodium ascorbate (198 mg, 1 mmol) in DMF (5 mL) was stirred vigorously for 5-10 min. The progress of the reaction was monitored by checking TLC at a regular interval. After completion of the reaction the mixture was poured into crushed ice (30 g). The solid separated was filtered, dried and purified by column chromatography on silica gel using chloroform and ethyl acetate to give the desired product.

*1-((5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-p-tolyl-1H-1,2,3-triazole (3**a**)*



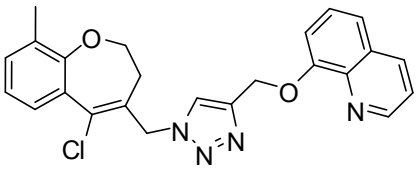
Off white solid; yield 92%; mp 124-126 ºC; R_f 0.5 ($CHCl_3$) MS *m/z* 366.1 (M+1, 100%); IR: 3086, 2923, 2853, 1634, 1591, 1459, 1438, 1199, 1028, 976, 777, 766; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89 (s, 1H), 7.74 (d, 2H, *J* = 8.0 Hz), 7.43 (d, 1H, *J* = 7.4 Hz), 7.24 (d, 2H, *J* = 7.9 Hz), 7.12 (t, 1H, *J* = 7.2), 7.07 (t, 1H, *J* = 7.6 Hz), 5.48 (s, 2H), 4.27 (t, 2H, *J* = 6.2 Hz), 2.38 (s, 3H), 2.35 (t, 2H, *J* = 6.2 Hz), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.8, 148.6, 138.2, 132.1, 131.6, 131.5, 130.7, 130.0, 129.6, 127.6, 127.2, 125.7, 123.7, 119.3, 78.2, 52.8, 30.6, 21.3, 16.2.

*1-((5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (3**b**)*



Off white solid; yield 85%; mp 114-116 ºC; R_f 0.4 ($CHCl_3$) MS *m/z* 352.1(M+1, 100%); IR: 3086, 2952, 2924, 2853, 1634, 1479, 1459, 1439, 1226, 1082, 1049, 1029, 916, 777, 767; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89 (s, 1H), 7.82 (d, 2H, *J* = 7.6 Hz), 7.45-7.32 (m, 3H) 7.24 (t, 1H, *J* = 7.6 Hz), 7.20 - 7.17 (m, 1H), 7.14 - 7.11 (m, 1H), 5.48 (s, 2H), 4.27 (t, 2H, *J* = 6.2 Hz), 2.38 (t, 2H, *J* = 6.2 Hz), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.9, 148.5, 132.1, 131.6, 131.5, 130.7, 130.4, 130.1, 128.9, 128.3, 127.2, 125.8, 123.7, 119.7, 78.2, 52.8, 30.7, 16.2.

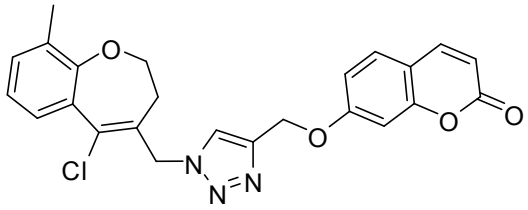
*8-((1-((5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)quinoline (3**c**)*



Off white solid; yield 90%; mp 98-100 ºC; R_f 0.5 ($CHCl_3$: EtOAc 9:1) MS *m/z* 433.1 (M+1, 100%); IR: 3109, 3056, 2953, 2879, 1623, 1502, 1468, 1262, 1192, 1103, 1026, 818, 784; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.94 (dd, 1H, *J* = 4.1, 1.6 Hz), 8.13 (dd, 1H, *J* = 8.2, 1.6 Hz), 7.94 (s, 1H) , 7.46 - 7.39 (m, 4H), 7.32 (dd, 1H, *J* = 7.3, 1.2 Hz), 7.18 (d, 1H, *J* = 7.3 Hz), 7.07 (t, 1H, *J* = 7.6 Hz), 5.59 (s, 2H), 5.42 (s, 2H), 4.24 (t, 2H, *J* = 6.2 Hz), 2.25 (s, 3H), 2.24 (t, 2H, *J* = 6.2 Hz).

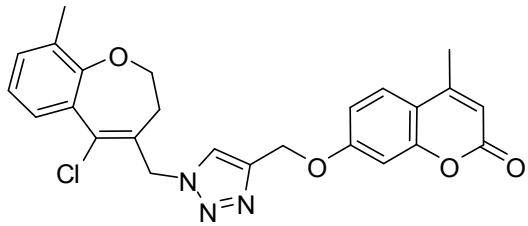
^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.8, 152.9, 149.4, 144.7, 140.3, 136.1, 132.0, 131.5, 131.4, 130.3, 129.5, 127.2, 126.7, 123.6, 121.7, 120.4, 110.1, 76.8, 62.9, 52.8, 30.7, 16.1.

*7-((1-((5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (**3d**)*



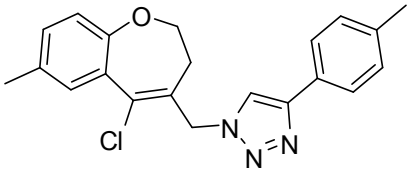
Pale yellow solid; yield 92%; mp 98-100 ºC; R_f 0.6 ($CHCl_3$: EtOAc 9:1) MS *m/z* 450.1 (M+1, 100%); IR: 3086, 2923, 2854, 1727, 1642, 1458, 1275, 1123, 1002, 836, 776; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (s, 1H), 7.64 (d, 1H, *J* = 9.4 Hz), 7.43 (d, 1H, *J* = 7.9 Hz), 7.39 (d, 1H, *J* = 9.1 Hz), 7.21 (d, 1H, *J* = 7.1 Hz), 7.10 (t, 1H, *J* = 7.6 Hz), 6.96 – 6.94 (m, 2H), 6.27 (d, 1H, *J* = 9.4 Hz), 5.47 (s, 2H), 5.28 (s, 2H), 4.25 (t, 2H, *J* = 6.4 Hz), 2.32 (t, 2H, *J* = 6.4 Hz), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 161.3, 161.1, 155.7, 152.8, 143.6, 143.3, 132.1, 131.6, 131.3, 130.5, 130.2, 128.9, 127.2, 123.2, 123.1, 113.4, 113.0, 112.8, 102.1, 78.0, 62.3, 52.9, 30.8, 16.1.

*7-((1-((5-Chloro-9-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one (**3e**)*



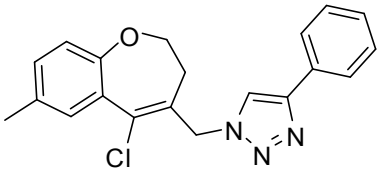
Pale yellow solid; yield 89%; mp 132-134 ºC; R_f 0.6 ($CHCl_3$: EtOAc 9:1); MS *m/z* 464 (M+1, 100%); IR: 3153, 2921, 2879, 1731, 1615, 1433, 1387, 1267, 1198, 1027, 842; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (s, 1H), 7.52 (d, 1H, *J* = 9.4 Hz), 7.40 (d, 1H, *J* = 9.3 Hz), 7.22 (d, 1H, *J* = 7.1 Hz), 7.10 (t, 1H, *J* = 7.6 Hz), 7.00 - 6.88 (m, 2H), 6.18 (s, 1H), 5.50 (s, 2H), 5.28 (s, 2H), 4.22 (t, 2H, *J* = 6.4 Hz), 2.40 (s, 3H), 2.30 (t, 2H, *J* = 6.4 Hz), 2.20 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.1, 155.1, 152.8, 152.5, 143.7, 132.1, 131.6, 131.2, 130.4, 130.2, 127.2, 125.7, 123.6, 123.2, 114.0, 112.5, 112.2, 102.1, 78.0, 62.2, 52.9, 30.8, 18.6, 16.1.

1-((5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-p-tolyl-1H-1,2,3-triazole ***(3f)***



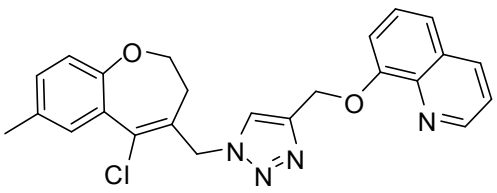
Pale yellow solid; yield 85%; mp 122-124 ºC; R_f 0.5 ($CHCl_3$); MS *m/z* 366.1 (M+1); IR: 3105, 3027, 2926, 2871, 1634, 1600, 1481, 1441, 1222, 1041, 822, 789; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84 (s, 1H), 7.72 (d, 2H, *J* = 8.2 Hz), 7.41 (s, 1H), 7.22 (d, 2H, *J* = 8.2 Hz), 7.08 (d, 1H, *J* = 7.8 Hz), 6.96 (d, 1H, *J* = 7.8 Hz), 5.46 (s, 2H), 4.24 (t, 2H, *J* = 6.4 Hz), 2.40 (s, 3H), 2.38 (s, 3H), 2.30 (t, 2H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.9, 148.5, 138.2, 133.7, 131.4, 131.0, 130.7, 129.9, 129.7, 129.6, 127.6, 125.6, 122.1, 119.3, 78.9, 52.9, 30.6, 21.3, 20.9.

*1-((5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (3****g****)*



Pale yellow solid; yield 90%; mp 112-114 ºC; R_f 0.4 ($CHCl_3$); MS *m/z* 352 (M+1, 100%); IR: 3105, 3027, 2926, 2871, 1634, 1600, 1481, 1441, 1222, 1041, 822, 789; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.94 (s, 1H), 7.86-7.84 (m, 2H), 7.45-7.41 (m, 3H), 7.34 (t, 1H, *J* = 7.4 Hz) 7.11 (dd, 1H, *J* = 8.2, 1.7 Hz), 6.93 (d, 1H, *J* = 8.1 Hz), 5.49 (s, 2H), 4.27 (t, 2H, *J* = 6.2 Hz), 2.38 (t, 2H, *J* = 6.2 Hz) 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.0, 148.6, 138.2, 133.7, 131.4, 131.0, 130.6, 129.9, 129.8, 129.6, 127.6, 125.7, 122.1, 119.3, 78.9, 52.9, 30.6, 20.7.

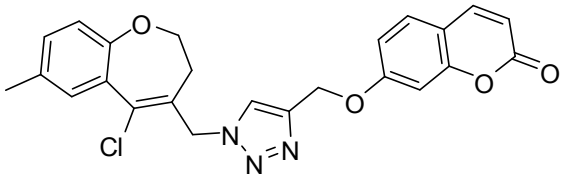
*8-((1-((5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)quinoline (3****h****)*



Pale yellow solid; yield 93%; mp 100-102 ºC; R_f 0.5 ($CHCl_3$: EtOAc 9:1); MS *m/z* 433.1 (M+1, 100%); IR: 3109, 3056, 2953, 2879, 1623, 1502, 1468, 1262, 1192, 1262, 1103, 1026, 818, 783; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.95 (d, 1H, *J* = 4.3 Hz), 8.03 (d, 1H, *J* = 7.5 Hz), 7.94 (s, 1H),

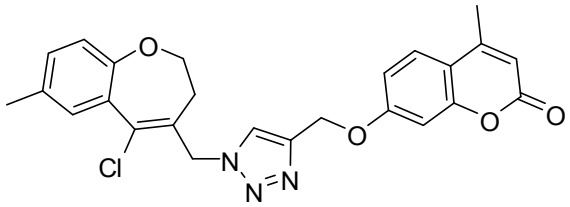
7.46 - 7.39 (m, 4H), 7.32 (d, 1H, *J* = 7.3 Hz), 7.18 (d, 1H, *J* = 7.3 Hz), 7.07 (t, 1H, *J* = 7.6 Hz), 5.59 (s, 2H), 5.42 (s, 2H), 4.24 (t, 2H, *J* = 6.4 Hz), 2.25 (s, 3H), 2.24 (t, 2H, *J* = 6.2 Hz).^{13}C NMR ($CDCl_3$,100 MHz) δ 153.8, 152.8, 149.3, 144.7, 140.3, 136.0, 132.0, 131.5, 131.3, 130.2, 129.5, 127.2, 126.7, 123.5, 121.6, 120.3, 110.1, 76.7, 62.9, 52.8, 30.6, 16.0.

7-((1-((5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one ***(3i)***



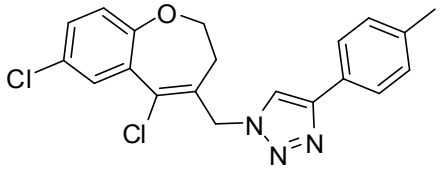
Pale yellow solid; yield 92%; mp 100-102 ºC; R_f 0.6 ($CHCl_3$: EtOAc 9:1); MS *m/z* 450.1 (M+1, 100%); IR: 3086, 2921, 2877, 1731, 1612, 1458, 1275, 1161, 1123, 1006, 836; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (s, 1H), 7.64 (d, 1H, *J* = 9.4 Hz), 7.47 (s, 1H), 7.40 (d, 1H, *J* = 9.1 Hz), 7.21 (d, 1H, *J* = 7.1 Hz), 7.10 (t, 1H, *J* = 7.6 Hz), 6.95 – 6.92 (m, 2H), 6.28 (d, 1H, *J* = 9.1 Hz), 5.47 (s, 2H), 5.28 (s, 2H), 4.25 (t, 2H, *J* = 6.1 Hz), 2.32 (t, 2H, *J* = 6.1 Hz); 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 161.3, 161.1, 155.8, 152.9, 143.7, 143.3, 133.7, 131.5, 130.5, 130.3, 129.9, 128.9, 123.2, 122.1, 113.5, 113.1, 112.9, 102.1, 78.8, 62.3, 53.1, 30.9, 20.9.

*7-((1-((5-Chloro-7-methyl-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one (3****j****)*



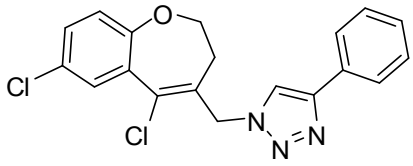
Pale yellow solid; yield 90%; mp 132-134 ºC; R_f 0.6 ($CHCl_3$: EtOAc 9:1); MS *m/z* 464 (M+1, 100%); IR: 2925, 2881, 1730, 1618, 1387, 1148, 1074, 825; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (s, 1H), 7.52 (d, 1H, *J* = 8.4 Hz), 7.41 (s, 1H), 7.12 (d, 1H, *J* = 8.5 Hz), 6.98 (s, 1H), 6.93 (d, 2H, J = 6.4 Hz), 6.16 (s, 1H), 5.46 (s, 2H), 5.28 (s, 2H), 4.26 (t, 2H, *J* = 6.4 Hz), 2.40 (s, 3H), 2.35 (t, 2H, *J* = 6.4 Hz), 1.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.2, 161.1, 155.1, 152.9, 152.5, 143.7, 133.7, 131.5, 130.6, 130.5, 130.2, 129.9, 125.7, 123.2, 122.0, 114.1, 112.5, 112.2, 102.1, 78.7, 62.2, 53.0, 30.8, 20.8, 18.6.

*1-((5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-p-tolyl-1H-1,2,3-triazole (3****k****)*



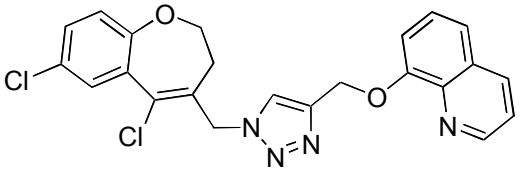
Pale yellow solid; yield 92%; mp 142-144 ºC; R_f 0.5 ($CHCl_3$); MS *m/z* 386 (M+1, 100%); IR: 3046, 2875, 2727, 1628, 1477, 1352, 1224, 1026, 874; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89 (s, 1H), 7.52 (d, 2H, *J* = 8.0 Hz), 7.43-7.44 (m, 1H), 7.12 (d, 2H, *J* = 8.0 Hz), 7.11 (d, 2H, *J* = 7.6 Hz), 5.48 (s, 2H), 4.27 (t, 2H, *J* = 6.2 Hz), 2.38 (t, 2H, *J* = 6.2 Hz), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.8, 148.6, 138.3, 132.6, 132.2, 130.6, 129.6, 129.3, 128.3, 127.4, 125.7, 123.7, 119.3, 78.9, 52.8, 30.6, 21.3.

*1-((5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (3**l**)*



Pale yellow solid; yield 90%; mp 130-132 ºC; R_f 0.5 ($CHCl_3$); MS *m/z* 372.1 (M+1 100%); IR: 3123, 2922.0, 2875, 1475, 1260, 1077, 831, 762, 691; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.92 (s, 1H), 7.86 (d, 2H, *J* = 7.3 Hz), 7.62 (d, 1H, *J* = 2.2 Hz), 7.44 (t, 2H, *J* = 7.4 Hz), 7.35 (t, 1H, *J* = 7.3 Hz), 7.28 (d, 1H, *J* = 2.5 Hz), 6.99 (d, 1H, *J* = 8.5 Hz), 5.49 (s, 2H), 4.30 (t, 2H, *J* = 6.1Hz), 2.40 (t, 2H, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.8, 148. 6, 132.5, 132.1, 130.6, 130.2, 129.5, 129.3, 128.9, 128.4, 128.3, 125.8, 123.7, 119.6, 78.9, 52.8, 30.6.

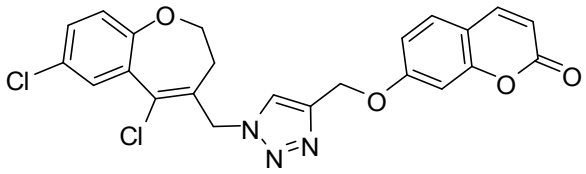
*8-((1-((5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)quinoline (3**m**)*



Pale yellow solid; yield 89%; mp 98-100 ºC; R_f 0.5 ($CHCl_3$); MS *m/z* 453 (M+1 100%); IR: 3381, 3106, 3056, 2953, 2885, 1621, 1501, 1381, 1314, 1259, 1105, 824, 790; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.94 (dd, 1H, *J* = 4.1, 1.5 Hz), 8.14 (dd, 1H, *J* = 8.3, 1.5 Hz), 7.92 (s, 1H), 7.58 (d, 1H, *J* = 2.5 Hz), 7.46 - 7.41 (m, 3H), 7.31 (dd, 1H, *J* = 7.0, 1.6 Hz), 7.24 (d, 1H, *J* = 2.5 Hz), 6.96 (d, 1H, *J* = 8.6 Hz), 5.59 (s, 2H), 5.41 (s, 2H), 4.25 (t, 2H, *J* = 6.1 Hz), 2.31 (t, 2H, *J* = 6.2 Hz);

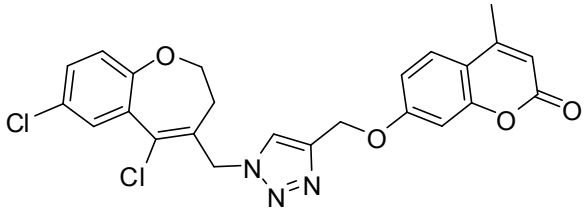
^{13}CNMR ($CDCl_3$, 100 MHz) δ 155.2, 153.7, 149.3, 144.6, 140.2, 136.1, 130.8, 130.6, 130.0, 129.7, 129.5, 126.7, 124.0, 123.6, 122.3, 121.7, 120.3, 117.2, 110.0, 78.7, 62.9, 52.9, 30.0.

*7-((1-((5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (3**n**)*



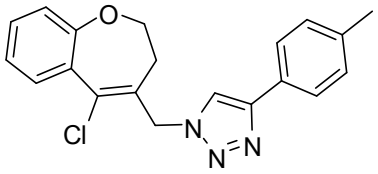
Pale yellow solid; yield 85%; mp 162-164 ºC; R_f 0.4 ($CHCl_3$: EtOAc 9:1); MS *m/z* 470.1 (M+1 100%); IR: 2924, 2876, 1722, 1622, 1555, 1465, 1291, 1135, 1025, 826, 806; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82 (s, 1H), 7.64 (d, 1H, *J* = 9.1 Hz), 7.61 (d, 1H, *J* = 2.5 Hz), 7.40 (d, 1H, *J* = 9.3 Hz), 7.30 -7.28 (m, 1H), 6.99 (d, 1H, *J* = 8.5 Hz), 6.96 – 6.94 (m, 2H), 6.28 (d, 1H, *J* = 9.4 Hz), 5.46 (s, 2H), 5.28 (s, 2H), 4.26 (t, 2H, *J* = 6.1 Hz), 2.31 (t, 2H, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$,100 MHz): δ 161.3, 161.1, 155.7, 152.9, 143.6, 143.4, 133.7, 131.5, 130.6, 130.2, 130.0, 129.9, 123.3, 122.0, 117.2, 113.4, 113.0, 112.9, 102.0, 78.8, 62.2, 53.0, 30.8.

*7-((1-((5,7-Dichloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one (3**o**)*



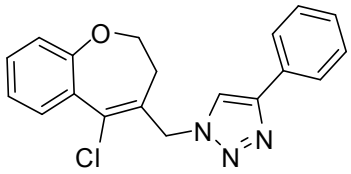
Pale yellow solid; yield 90%; mp 162-164 ºC; R_f 0.4 ($CHCl_3$: EtOAc 9:1); MS *m/z* 484 (M+1 100%); IR: 3067, 2923, 2882, 1726, 1617, 1388, 1279, 1149, 1023; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82 (s, 1H), 7.61 (d, 1H, *J* = 2.5 Hz), 7.52 (d, 1H, *J* = 8.8 Hz), 7.28 (d, 1H, *J* = 2.5 Hz), 6.99 – 6.93 (m, 3H), 6.16 (s, 1H), 5.40 (s, 2H), 5.28 (s, 2H), 4.22 (t, 2H, *J* = 6.2 Hz), 2.40 (s, 3H), 2.38 (t, 2H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$,100 MHz) δ 161.3, 161.1, 155.7, 152.9, 143.6, 143.4, 133.7, 131.5, 130.6, 130.2, 130.0, 129.9, 123.3, 122.0, 117.2, 113.4, 113.0, 112.9, 102.0, 78.8, 62.2, 53.0, 30.8, 20.9.

*1-((5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-(p-tolyl)-1H-1,2,3-triazole (3**p**)*



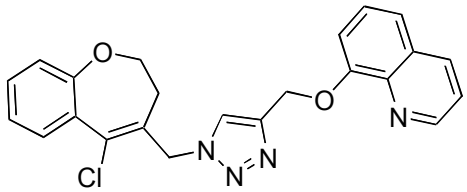
Pale yellow solid; yield 92%; mp 110-112 ºC; R_f 0.4 ($CHCl_3$); MS *m/z* 352 (M+1 100%); IR: 3105, 3027, 2926, 2871, 1634, 1600, 1481, 1441, 1222, 1041, 822, 789; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91 (s, 1H), 7.74 (d, 2H, *J* = 8.1 Hz), 7.65 (dd, 1H, *J* = 7.9, 1.6 Hz), 7.31 (td, 1H, *J* = 7.9, 1.6 Hz), 7.24 (d, 2H, *J* = 8.1 Hz), 7.12 (td, 1H, *J* = 7.8, 1.2 Hz), 7.05 (dd, 1H, *J* = 8.0, 1.2 Hz), 5.49 (s, 2H), 4.31 (t, 2H, *J* = 6.0 Hz), 2.41 (s, 3H), 2.37 (t, 2H, *J* = 6.0 Hz); ^{13}CNMR ($CDCl_3$, 100 MHz) δ: 153.8, 148.6, 132.5, 132.1, 130.6, 130.2, 129.5, 129.3, 128.9, 128.4, 128.3, 125.8, 123.7, 119.6, 78.9, 52.8, 30.6, 21.3.

*1-((5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (3**q**)*



Pale yellow solid; yield 85%; mp 124-126 ºC; R_f 0.4 ($CHCl_3$); MS *m/z* 338.1 (M+1 100%); IR: 3089, 2937, 2879, 1602, 1479, 1442, 1226, 1038, 767; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.94 (s, 1H), 7.86 (d, 2H, *J* = 7.3 Hz), 7.65 (dd, 1H, *J* = 7.9, 1.6 Hz), 7.43 (t, 2H, *J* = 7.3 Hz), 7.36 – 7.31 (m, 2H), 7.12 (td, 1H, *J* = 7.8, 1.2 Hz), 7.05 (dd, 1H, *J* = 8.0, 1.2 Hz), 5.50 (s, 2H), 4.31 (t, 2H, *J* = 6.2 Hz), 2.39 (t, 2H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.8, 148.6, 132.5, 132.1, 130.6, 130.2, 129.5, 129.3, 128.9, 128.4, 128.3, 125.8, 123.7, 119.6, 78.9, 52.8, 30.6.

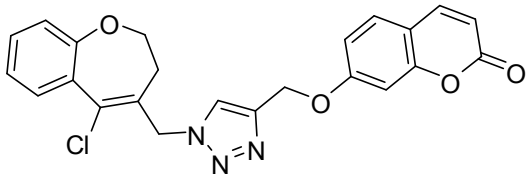
*8-((1-((5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) quinoline (3**r**)*



Pale yellow solid; yield 86%; mp 96-98 ºC; R_f 0.3 ($CHCl_3$: EtOAc 9:1); MS *m/z* 419.1 (M+1 100%); IR: 3083, 2932, 2862, 1601, 1467, 1434, 1221, 1035, 772; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.19 (d, 1H, *J* = 4.3 Hz), 7.95 (d, 1H, *J* = 8.0 Hz), 7.87 (s, 1H), 7.62 (d, 1H, *J* = 7.7 Hz), 7.32 – 7.02 (m, 5H), 6.73 (d, 1H, *J* = 8.2 Hz), 6.63 (t, 1H, *J* = 7.6 Hz), 5.48 (s, 2H), 5.46 (s, 2H), 4.29 (t,

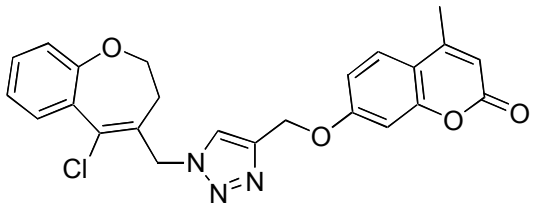
2H, *J* = 6.2 Hz), 2.35 (t, 2H, *J* = 6.2 Hz).[13]C NMR ($CDCl_3$, 100 MHz) δ 155.2, 153.7, 149.3, 144.6, 140.2, 136.1, 130.8, 130.6, 129.9, 129.7, 129.5, 126.7, 124.0, 123.6, 122.3, 121.7, 120.3, 110.0, 78.7, 62.9, 52.9, 30.2.

*7-((1-((5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (3**s**)*



Pale yellow solid; yield 90%; mp 134-136 ºC; R_f 0.5 ($CHCl_3$: EtOAc 9:1); MS *m/z* 436.1 (M+1 100%); IR: 3096, 2926, 2879, 1730, 1620, 1235, 1131, 832, 757; [1]H NMR ($CDCl_3$, 400 MHz): δ 7.87 (s, 1H), 7.65 (d, 1H, *J* = 9.4 Hz), 7.37 (td, 2H, *J* = 7.9, 1.6 Hz), 7.12 (dd, 1H, *J* = 8.2, 1.9 Hz), 6.95- 6.92 (m, 3H), 6.96 (d, 1H, *J* = 2.4 Hz), 6.16 (d, 1H, *J* = 8.6 Hz), 5.46 (s, 2H), 5.27 (s, 2H), 4.24 (t, 2H, *J* = 6.2 Hz), 2.34 (t, 2H, *J* = 6.2 Hz). [13]C NMR ($CDCl_3$, 100 MHz) δ 161.3, 157.1, 155.7, 153.9, 143.6, 133.7, 133.1, 131.5, 130.6, 130.2, 130.0, 129.8, 123.3, 122.0, 117.2, 113.4, 113.0, 112.9, 102.2, 78.8, 62.3, 53.1, 30.5.

*7-((1-((5-Chloro-2,3-dihydrobenzo[b]oxepin-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one (3**t**)*



Off white solid; yield 90%; mp 156-158 ºC; R_f 0.5 ($CHCl_3$: EtOAc 9:1); MS *m/z* 450.1 (M+1 100%); IR: 2923, 2853, 1729, 1616, 1449, 1385, 1106, 801; [1]H NMR ($CDCl_3$, 400 MHz): δ 7.85 (s, 1H), 7.63 (dd, 1H, *J* = 7.8, 1.5 Hz), 7.52 (d, 1H, *J* = 8.6 Hz), 7.33 (td, 1H, *J* = 7.9, 1.5 Hz), 7.21 (t, 1H, *J* = 7.0 Hz), 7.05 (d, 1H, *J* = 7.9 Hz), 6.98 (d, 1H, *J* = 2.2 Hz), 6.95 - 6.93 (m, 1H), 6.16 (s, 1H), 5.49 (s, 2H), 5.28 (s, 2H) 4.28 (t, 2H, *J* = 6.2 Hz), 2.40 (s, 3H), 2.37 (t, 2H, *J* = 6.2 Hz).[13]C NMR ($CDCl_3$, 100 MHz) δ 161.3, 155.7, 154.2, 152.9, 143.6, 133.7, 131.5, 130.6, 130.2, 129.9, 128.9, 126.3, 124.4, 123.0, 117.2, 113.4, 113.0, 102.0, 78.8, 62.2, 53.0, 30.8, 20.9.

Pharmacology

Antibacterial activity

Test organisms and culture condition

A collection of four organisms including two Gram-positive and two Gram-negative organisms were used for this study. *Escherichia coli* (MTCC 1563) and *Pseudomonas aeruginosa* (MTCC 6642) were obtained from Microbial Type Culture Collection, IMTECH, and Chandigarh, India. Clinical isolates such as *Staphylococcus aureus, Klebsiella pneumonia* were obtained from Microbiology laboratory of Global Hospital, Hyderabad. All strains were tested for purity by standard microbiological methods. The bacterial stock cultures were maintained on Mueller Hinton Agar (MHA) slants and stored at 4 °C.

Determination of antibacterial activity

An agar-well diffusion method was employed for evaluation of antibacterial activity . The bacterial strains were reactivated from stock cultures by transferring into Mueller Hinton Broth (MHB) and incubating at 37 °C for 18 h. A final inoculum containing 10^6 colony forming units (1 x 10^6 CFU/ml) was added aseptically to MHA medium and poured into sterile Petri dishes. Different test compounds at a concentration of 0.4 mg/50 µL were added to wells (8 mm in diameter) punched on agar surface. Plates were incubated overnight at 37 °C and diameter of inhibition zone (DIZ) around each well was measured in mm. Experiments were performed in triplicates. Antibiotic such as pefloxacin at a concentration of 0.04 mg/50 µL were used as positive reference to determine sensitivity of microorganisms tested. DMSO was used as a negative control.

In vitro cytotoxicity assay

The human tumor cell lines are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of test compounds.

After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of addition of test compound (Tz). Test compounds are dissolved in DMSO at 400-fold the desired final maximum test concentration and

stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/mL gentamicin. Additional four, 10-fold or ½log serial dilutions are made to provide a total of five drug (test compound) concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage control growth is calculated at each of the drug concentrations levels.

Three dose response parameters are calculated for each test compound.

GI_{50}: Growth inhibition of 50% (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

TGI: The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz.

LC_{50}: The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.