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## Supporting Information

# Synthesis and biological evaluation of new fluconazole β-lactam conjugates linked via 1,2,3-triazole

Jaisingh M. Divse,[a] Santosh B. Mhaske,[a] Chaitanya R. Charolkar,[a] Duhita T. sant,[b] Mukund V. Deshpande,[b] Vijay M. Khedkar,[c,d] Laxman U. Nawale,[e] Dhiman Sarkar,[e] and Vandana S. Pore*[a]

[a] Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune 411008, India.
[b] Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune 411008, India.
[c] School of Health Sciences, University of KwaZulu Natal, Westville Campus, Durban 4000, SouthAfrica.
[d] Department of Pharmaceutical Chemistry, St. John Institute of Pharmacy & Research, Palghar (E) 401404, India.
[e] Combichem-Bioresource Center, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune 411008, India.

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# Supporting Information

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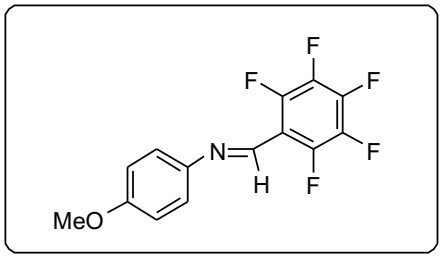
[d] School of Health Sciences, University of KwaZulu Natal, Westville Campus, Durban 4000, SouthAfrica.

**General procedure for synthesis of Imines (6a-l)**

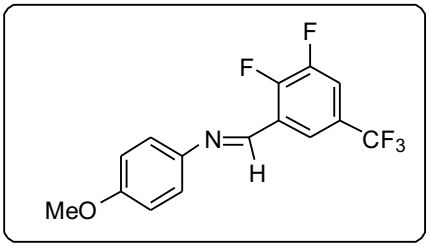
Amine (1mmol), and aldehyde (1-1.2mmol) was taken in a conical flask and kept it inside ordinary domestic microwave oven for 5-8 mins under 540 W. The conical flask was removed out and kept for 15-20 mins at room temperature. The reaction mixture in liquid form was slowly became solid. The solid obtained was then first washed with pet ether and then by 5:95 ethyl acetate: pet ether solution. The solid was obtained in high yeilds (80-98%) and used further without any purification.

***(E)-N-(4-methoxyphenyl)-1-(perfluorophenyl) methanimine (6a)***



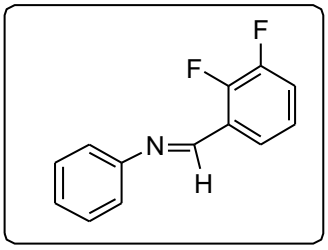

Yield: 92%; M.P 122-123 $^{0}$C; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^{1}$H NMR ($CDCl_3$, 200 MHz) δ 8.50 (S, 1H), 7.64-7.71 (m, 1H), 7.45-7.52 (m, 1H), 7.19-7.31 (m, 1H), 6.94-7.0 (m, 1H), 3.88 (s, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 165.5, 160.6, 158.5, 156.6, 144.2, 138.8, 130.2, 124.7, 122.2, 114.6, 55.45. ESI-MS (m/z): 302.14$[M+H]^+$.

***(E)-1-(2,3-difluoro-5-(trifluoromethyl)phenyl)-N-(4-methoxyphenyl)methanimine (6b)***



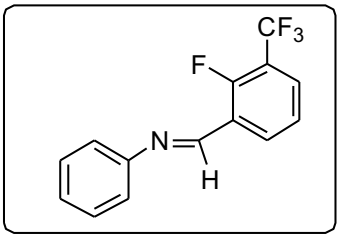

Yield: 89%;M.P 58-59 $^{0}$C;IR ($CHCl_3$, $cm^{-1}$) 1617, 3019;$^{1}$H NMR ($CDCl_3$, 200 MHz) δ 8.78 (s, 1H), 8.42 (dd, J = 5.56 Hz, 1H), 7.75 (dd, J = 6.44 1H), 7.30-7.35 (m, 2H), 6.94-6.99 (m, 2H), 3.86 (s, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 162.0, 159.3, 156.9, 147.9, 143.4, 129.2, 123.5, 122.7, 120.2, 114.5, 55.5; ESI-MS (m/z): 316.24$[M+H]^+$.

***(E)-1-(2,3-difluorophenyl)-N-phenylmethanimine (6c)***



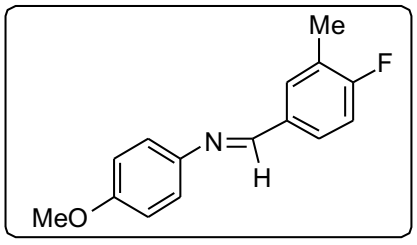

Yield: 80%; sticky solid; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019;$^{1}$H NMR ($CDCl_3$, 200 MHz) δ 8.65 (s, 1H), 7.80-7.87 (m, 1H), 7.28-7.35(m, 1H), 7.04-7.26 (m, 6H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 153.1, 152.1, 151.3, 148.4, 146.2, 129.2, 126.6, 122.5, 120.9, 118.5, 115.0; ESI-MS (m/z): 218.24$[M+H]^+$.

***(E)-1-(2-fluoro-3-(trifluoromethyl)phenyl)-N-phenylmethanimine (6d)***



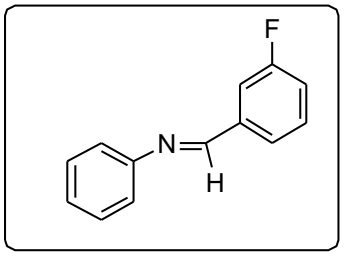

Yield: 75%; sticky solid; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^{1}$H NMR ($CDCl_3$, 400 MHz) δ 8.82 (s, 1H), 8.44 (t, *J*= 7.34 Hz, 1H), 7.75 (t, *J* = 7.34 Hz, 1H), 7.43-7.47 (m, 2H), 7.35-7.39 (m, 1H), 7.28-7.31 (m, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 151.6, 151.2, 131.8, 129.2, 126.8, 125.3, 124.2, 123.7, 120.9; ESI-MS (m/z): 268.08$[M+H]^+$.

***(E)-1-(4-fluoro-3-methylphenyl)-N-(4-methoxyphenyl)methanimine (6e)***



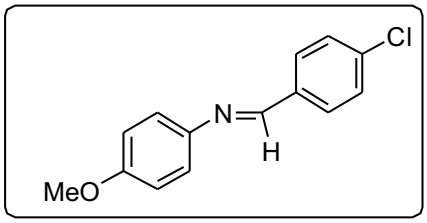

Yield: 93%; M.P 74-75 $^{0}$C; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^{1}$H NMR ($CDCl_3$, 200 MHz) δ 8.46 (s, 1H), 7.68-7.86 (m, 2H), 7.25-7.30 (m, 2H), 7.12-7.18 (m, 1H), 6.97-7.01 (m, 2H), 3.89 (s, 1H), 2.39 (s, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 190.7, 165.5, 160.6, 158.2, 157.2, 144.6, 132.3, 131.4, 131.3, 128.2, 125.3, 122.0, 115.5, 114.3, 55.4, 14.4; ESI-MS (m/z): 244.28$[M+H]^+$.

***(E)-1-(3-fluorophenyl)-N-phenylmethanimine (6f)***



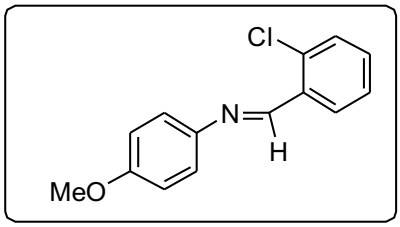

Yield: 88%; sticky solid; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.48 (s, 1H), 7.66-7.73 (m, 2H), 7.43-7.51 (m, 3H), 7.18-7.32 (m, 4H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 165.5, 160.6, 158.8, 151.4, 138.5, 138.4, 130.3, 129.1, 126.2, 120.8, 118.5, 115.1 ; ESI-MS (m/z): 200.08$[M+H]^+$.

***(E)-1-(4-chlorophenyl)-N-(4-methoxyphenyl)methanimine (6g)***



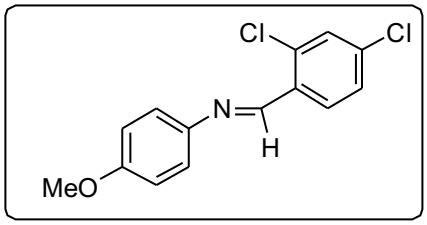

Yield: 98%; M.P 115-116 $^0C$; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 8.49 (s, 1H), 7.88 (d, *J* = 8.46 Hz, 2H), 7.49 (d, *J* = 8.46, 2H), 7.29 (d, *J* = 8.84, 2H), 6.99 (d, *J* = 6.69, 2H), 3.89 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 158.4, 156.5, 144.2, 136.8, 134.8, 129.6, 128.9, 122.1, 114.3, 55.3; ESI-MS (m/z): 246.08$[M+H]^+$.

***(E)-1-(2-chlorophenyl)-N-(4-methoxyphenyl)methanimine (6h)***



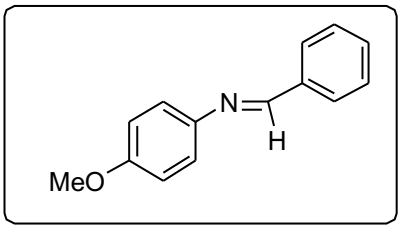

Yield: 98%; M.P 56-57$^0C$; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 8.95 (s, 1H), 8.22-8.26 (m, 1H), 7.27-7.41 (m, 5H), 6.93-6.98 (m, 2H), 3.85 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 158.5, 154.6, 144.4, 135.7, 131.7, 129.8, 128.2, 122.4, 114.3, 55.4; ESI-MS (m/z): 246.08$[M+H]^+$.

***(E)-1-(2,4-dichlorophenyl)-N-(4-methoxyphenyl)methanimine (6i)***



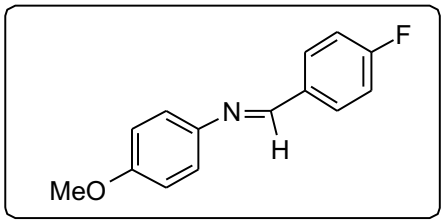

Yield: 96%; M.P 94-95$^0C$; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 8.88 (s, 1H), 8.20 (d, J = 8.46 Hz, 1H), 7.45 (d, J = 2.02Hz, 1H), 7.27-7.37 (m, 3H), 6.94-6.98 (m, 2H), 3.86(s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 158.8, 153.1, 144.1, 137.0, 136.1, 132.0, 129.5, 129.1, 122.5, 114.4, 55.4; ESI-MS (m/z): 280.04$[M+H]^+$.

***(E)-N-(4-methoxyphenyl)-1-phenylmethanimine (6j)***



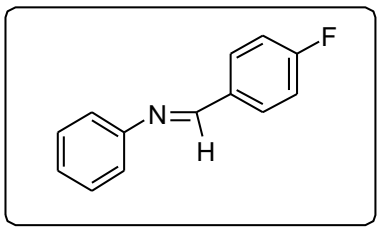

Yield: 98%; M.P 70-71 $^0$C; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1$H NMR ($CDCl_3$, 200 MHz) δ 8.48 (s, 1H), 7.86-7.91 (m, 2H), 7.44-7.49 (m, 3H), 7.20-7.26 (m, 2H), 6.89-6.97 (m, 2H), 3.83 (s, 3H) ; $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 158.2, 158.1, 144.7, 136.3, 130.9, 128.6, 122.1, 114.2, 55.3 ; ESI-MS (m/z): 212.08$[M+H]^+$.

***(E)-1-(4-fluorophenyl)-N-(4-methoxyphenyl)methanimine (6k)***




Yield: 97%; M.P 86-87 $^0$C; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1$H NMR ($CDCl_3$, 200 MHz) δ 8.45 (s, 1H), 7.86-7.93 (m, 2H), 7.16-7.27 (m, 4H), 6.92-6.97 (m, 2H), 3.85 (s, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 165.6, 158.2, 156.6, 144.5, 132.7, 130.3, 122.1, 115.8, 114.3, 55.3; ESI-MS (m/z): 230.08$[M+H]^+$.

***(E)-1-(4-fluorophenyl)-N-phenylmethanimine (6l)***

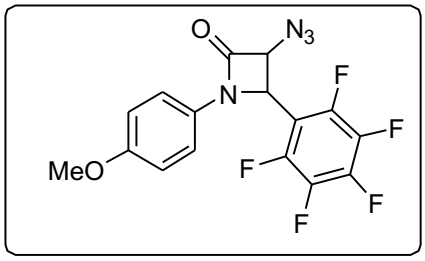



Yield: 80%; M.P 37-38 $^0$C; IR ($CHCl_3$, $cm^{-1}$) 1617, 3019; $^1$H NMR ($CDCl_3$, 200 MHz) δ 8.47 (s, 1H), 7.92-7.99 (m, 2H), 7.41-7.49 (m, 2H), 7.17-7.32 (m, 5H); $^{13}$C NMR ($CDCl_3$, 50 MHz) δ 167.1, 162.1, 158.7, 151.7, 135.5, 130.8, 129.1, 125.9, 120.8, 116.0, 115.0 ; ESI-MS (m/z): 200.08$[M+H]^+$.

**General procedure for synthesis of β-lactams (7+8 a-l)**

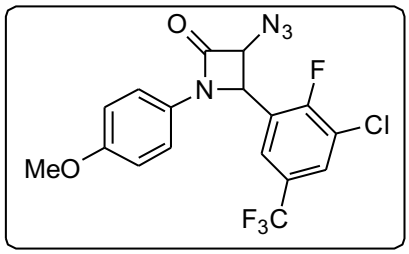
A solution of triphosgene (0.296 g, 1 mmol), in anhydrous $CH_2Cl_2$ (15 ml), was added slowly to a mixture of potassium salt of azidoacetic acid **3** (2 mmol), imine **6a-l** (2 mmol) and triethyl amine (0.84 ml, 6 mmol) in anhydrous $CH_2Cl_2$ (20 mL) at 0 $^0$C. After the addition, the reaction mixture was allowed to warm up to room temperature (28 $^0$C) and stirred for 15 h. The reaction mixture was then washed with water (20 ml), saturated sodium bicarbonate solution (2×15 ml) and brine (15 ml). The organic layer was dried over anhydrous sodium sulphate and concentrated to get crude product, which was purified by column chromatography to give pure β-lactams.

***3-azido-1-(4-methoxyphenyl)-4-(perfluorophenyl)azetidin-2-one (7+8a)***



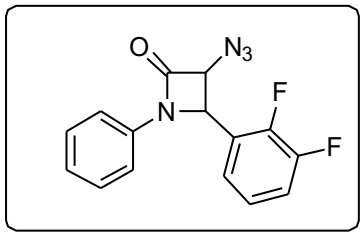

Yield: 81%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1763 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.20 (d, *J* = 9.09 Hz, 2H), 6.85 (d, *J* = 9.10 Hz, 2H), 5.61 (d, *J* = 5.43, 1H), 5.24 (d, *J* = 5.3, 1H), 3.78 (s, 3H, $OCH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 159.7, 157.0, 129.5, 118.1, 114.7, 66.6, 55.4, 51.7; ESI-MS (m/z): 349.06$[M+H]^+$.

***3-azido-4-(3-chloro-2-fluoro-5-(trifluoromethyl)phenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8b)***



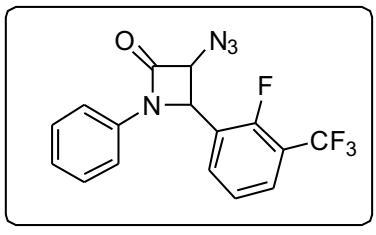

Yield: 80%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.72-7.76 (m, 1H), 7.33-7.36 (m, 1H), 7.22-7.26 (m, 2H), 6.84-6.89 (m, 2H), 5.58 (d, *J* = 5.43, 1H), 5.20 (d, *J* = 5.43, 1H), 3.79 (s, 3H, $OCH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 160.2, 157.0, 129.4, 128.6, 124.1, 118.4, 114.7, 67.2, 55.4, 54.6; ESI-MS (m/z): 415.06$[M+H]^+$.

***3-azido-4-(2,3-difluorophenyl)-1-phenylazetidin-2-one (7+8c)***



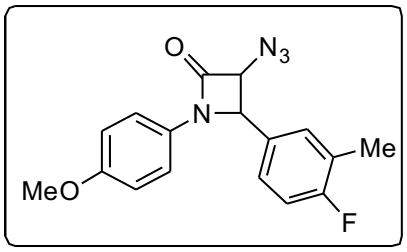

Yield: 77%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.12-7.25 (m, 5H), 6.91-7.10 (m, 3H), 5.56 (d, *J* = 5.44, 1H), 5.09 (d, *J* = 5.55, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 161.0, 136.3, 129.3, 129.2, 125.1, 117.3, 117.2, 67.1, 60.1, 54.6; ESI-MS (m/z): 301.06$[M+H]^+$.

***3-azido-4-(2-fluoro-3-(trifluoromethyl)phenyl)-1-phenylazetidin-2-one (7+8d)***



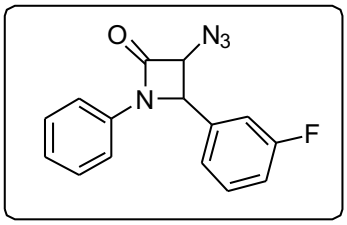

Yield: 75%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.63-7.70 (m, 1H), 7.36-7.42 (m, 1H), 7.31-7.35 (m, 3H), 7.27-7.30 (m, 1H), 7.14-7.26 (m, 2H), 5.69 (d, *J* = 5.43, 1H), 5.15 (d, *J* = 5.43 Hz, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 160.9, 136.2, 132.2, 129.4, 125.2, 124.4, 124.3, 117.1, 67.2, 54.2; ESI-MS (m/z): 351.08$[M+H]^+$.

***3-azido-4-(4-fluoro-3-methylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8e)***



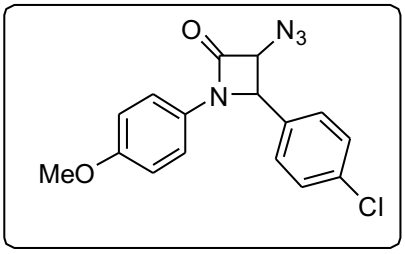

Yield: 52%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.26-7.30 (m, 2H), 7.07-7.20 (m, 3H), 6.83-6.87 (m, 2H), 5.26 (d, *J* = 5.31 Hz, 1H), 5.04 (d, *J* = 5.18 Hz, 1H), 3.80 (s, 3H), 2.31 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 164.0, 160.9, 159.1, 156.6, 130.6, 130.5, 130.0, 127.9, ,126.6, 126.4, 118.7, 115.7, 114.4, 67.3, 60.2, 55.4, 49.9, 14.6; ESI-MS (m/z): 326.08$[M+H]^+$.

***3-azido-4-(3-fluorophenyl)-1-phenylazetidin-2-one (7+8f)***



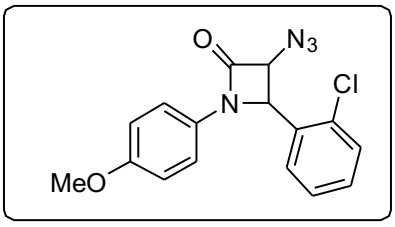

Yield: 81%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.37-7.43 (m, 1H), 7.28-7.32 (m, 4H), 7.12-7.16 (m, 1H), 7.03-7.11 (m, 3H), 5.32 (d, *J* = 5.43, 1H), 5.07 (d, *J* = 5.44 Hz, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 165.4, 161.1, 160.5, 136.4, 135.3, 130.7, 129.3, 125.0, 123.1, 117.3, 114.4, 67.3, 60.1; ESI-MS (m/z): 283.08$[M+H]^+$.

***3-azido-4-(4-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8g)***



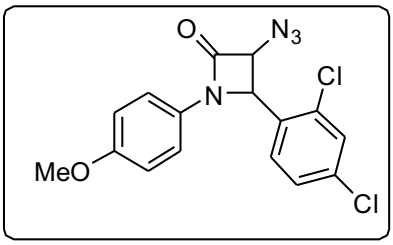

Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.41 (d, *J* = 7.82 Hz, 1H), 7.23-7.29 (m, 4H), 6.83 (d, *J*= 8.81 Hz, 1H), 5.27 (d, *J* = 5.38 Hz, 1H), 5.05 (d, *J* = 5.38 Hz, 1H), 3.77 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 160.6, 156.7, 135.1, 131.2, 129.2, 128.9, 118.7, 114.4, 67.3, 60.1, 55.4; ESI-MS (m/z): 329.08$[M+H]^+$.

***3-azido-4-(2-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8h)***



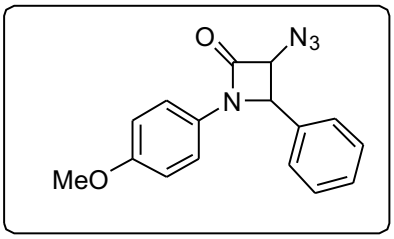

Yield: 91%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.48-7.53 (m, 1H), 7.28-7.36 (m, 2H), 7.21-7.26 (m, 3H), 6.84-6.88 (m, 2H), 5.67 (d, *J* = 5.31, 1H), 5.15 (d, *J* = 5.31 Hz, 1H), 3.79 (s, 3H); ESI-MS (m/z): 329.08$[M+H]^+$.

***3-azido-4-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8i)***



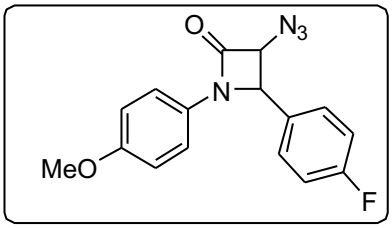

Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.51 (d, *J* = 1.89 Hz, 1H), 7.20-7.26 (m, 3H), 7.08-7.13 (m, 1H), 6.82-6.87 (m, 2H), 5.59 (d, *J* = 5.31, 1H), 5.15 (d, *J* = 5.30 Hz, 1H), 3.78 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 160.4, 156.8, 135.3, 129.7, 127.5, 118.5, 114.5, 67.5, 57.9, 55.4 ; ESI-MS (m/z): 363.04$[M+H]^+$.

***3-azido-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (7+8j)***



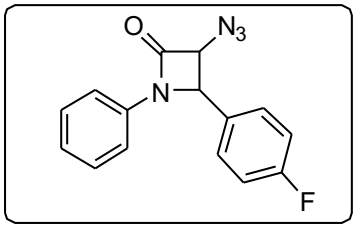

Yield: 94%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.25-7.41 (m, 6H), 6.68-6.84 (m, 3H), 5.30 (d, *J* = 5.30, 1H), 5.03 (d, *J* = 5.31 Hz, 1H), 3.76 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 160.8, 156.5, 132.6, 129.1, 128.8, 127.5, 118.7, 114.4, 67.3, 60.7, 55.4; ESI-MS (m/z): 295.14$[M+H]^+$.

***3-azido-4-(4-fluorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (7+8k)***




Yield: 92%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.29-7.36 (m, 2H), 7.07-7.22 (m, 3H), 6.68-6.84 (m, 3H), 5.28 (d, *J* = 5.31, 1H), 5.03 (d, *J* = 5.31 Hz, 1H), 3.77 (s, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 164.3, 161.8, 160.6, 156.6, 129.9, 129.4, 128.4, 118.7, 114.4, 67.3, 60.1, 55.4; ESI-MS (m/z): 313.14$[M+H]^+$.

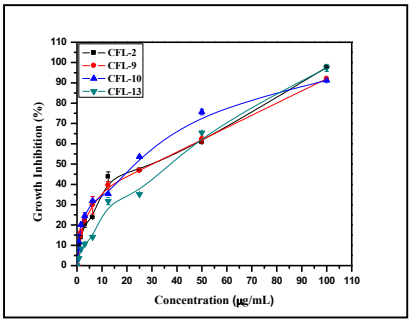
***3-azido-4-(4-fluorophenyl)-1-phenylazetidin-2-one (7+8l)***

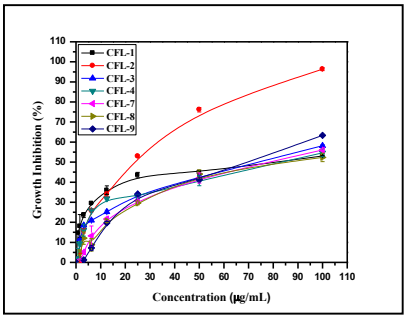


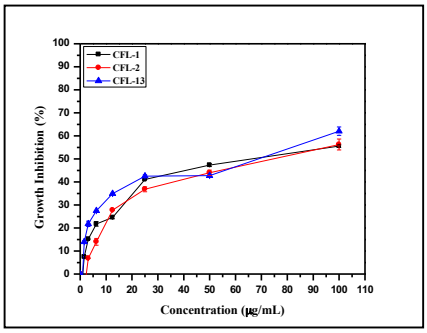

Yield: 72%; IR ($CHCl_3$, $cm^{-1}$) 2115 ($N_3$), 1751 (CO); $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.28-7.51 (m, 5H), 7.05-7.25 (m, 4H), 5.30 (d, *J* = 5.43, 1H), 5.03 (d, *J* = 5.30 Hz, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 165.4, 161.1, 160.5, 136.4, 135.3, 130.7, 129.3, 125.0, 123.1, 117.3, 114.4, 67.3, 60.1; ESI-MS (m/z): 283.10$[M+H]^+$.

**Table 1S. Cytotoxicity testing of the compounds 12 (a-l)**

| Sr. No. | Code | Cytotoxic Effect of inhibitors: MCF-7 | | Cytotoxic Effect of inhibitors: A431 | | Cytotoxic Effect of inhibitors: HUVEC | |
|---|---|---|---|---|---|---|---|
| | | IC50 | MIC | IC50 | MIC | IC50 | MIC |
| 1 | 12a | >100 | >100 | 80.43 | >100 | 63.17 | >100 |
| 2 | 12b | 31.71 | 89.67 | 23.21 | 83.56 | 73.83 | >100 |
| 3 | 12c | >100 | >100 | 73.74 | >100 | >100 | >100 |
| 4 | 12d | >100 | >100 | 85.76 | >100 | >100 | >100 |
| 5 | 12e | >100 | >100 | >100 | >100 | >100 | >100 |
| 6 | 12f | >100 | >100 | >100 | >100 | >100 | >100 |
| 7 | 12g | >100 | >100 | 77.99 | >100 | >100 | >100 |
| 8 | 12h | >100 | >100 | 86.25 | >100 | >100 | >100 |
| 9 | 12i | 29.85 | 97.44 | 67.77 | >100 | >100 | >100 |
| 10 | 12j | >100 | >100 | >100 | >100 | >100 | >100 |
| 11 | 12k | >100 | >100 | >100 | >100 | >100 | >100 |
| 12 | 12l | 37.09 | 88.2 | >100 | >100 | 74.14 | >100 |




## Experimental: Molecular Docking

Molecular docking study was carried out using the Glide (Grid-Based Ligand Docking with Energetics) module incorporated in the Schrödinger molecular modeling package (Schrödinger, LLC, New York, NY, 2016) Friesner, R.A et. al,[1]Halgren, T. A. et.al.,[2]Friesner, R. A. et. al.[3]to gauge the binding affinity of the new fluconazole β-lactam conjugates linked via 1,2,3-triazole towards sterol 14α-demethylase (CYP51) enzyme in an effort to augment the understanding of their action as antifungal agents. With this purpose, the X-ray crystal structure of sterol 14α-demethylase (CYP51) in complex with Fluconazole was retrieved from the Protein Data Bank

(PDB) (www.rcsb.org) (PDB code: 3KHM). The enzyme-inhibitor complex was preprocessed for docking simulation using the *Protein Preparation Wizard* integrated in the docking program which involved eliminating all the crystallographically observed water molecules (as no water molecule was found to be conserved in the interaction with the enzyme), addition of missing hydrogens/side chain atoms, assigning the correct bond orders and the appropriate charge and protonation state to the protein structure. The missing hydrogens/side chain atoms were corresponding to pH 7.0 considering the appropriate ionization states for the acidic as well as basic amino acid residues. Finally the prepared complex was subjected to energy minimization using the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field, in order to relieve the steric clashes among the residues caused due to addition of hydrogen atoms, until the average root mean square deviation (r.m.s.d.) reached 0.3Å. The 3D structures of title compounds were sketched with the *build* panel in Maestro and optimized with the *ligprep* module which performs addition of hydrogens, adjusting realistic bond lengths and angles, correct the chiralities, ionization states and ring conformations and generation of tautomers,. Partial charges were assigned OPLS-2005 force-field and the resulting structures were further optimized by energy minimization until a energy gradient of 0.001 kcal/mol/Å is reached. After ensuring that the enzyme and ligands were in the correct form, the the active site of the enzyme was defined for docking calculation using the *Receptor Grid Generation* panel in Glide which generates two cubical boxes having a common centroid to organize the calculations: a larger enclosing and a smaller binding box. With the non-covalently bound native ligand-fluconazole in place, the active site (receptor) grid was defined by a box that has dimensions of 10X10X10Å centered on the centroid of fluconazole in the crystal complex which was large enough to explore a large portion of the enzyme. The optimized enzyme and ligand structures were then used as input for the docking simulation utilizing the *extra precision* (XP) Glide scoring function to rank the docking poses and to estimate the binding affinities of the ligands to the CYP51 target. The *extra precision* (XP) scoring function in Glide is equipped with force field-based parameters accounting for contributions from steric interactions and electrostatic energies as well as solvation and repulsive interactions along with hydrophobic, hydrogen bonding and metal-ligand interactions all integrated in the empirical energy functions. The output files in terms of the docking poses of the ligands were visualized and analyzed key elements of interaction with the enzyme using the Maestro's Pose Viewer utility. Meanwhile, to validate the accuracy of the

docking protocol the co-crystallized ligand (fluconazole) was extracted from the crystal structure and re-docked into the active site defining the above mentioned parameters (**Figure 1S**). A very good agreement was observed between the localization of the fluconazole upon docking and from the X-ray bound conformation with an rmsd of less than 1.0Å indicating the reliability of the docking protocol in accurately predicting the binding mode for the title molecules.

**Table 2S:** Quantitative estimates of the per-residue interaction analysis of the fluconazole β-lactam conjugates (**12a-l**) with sterol 14α-demethylase (CYP51).

| Compound ID | MIC | Docking score | Binding energy | Per-residue interaction energy analysis | | |
|---|---|---|---|---|---|---|
| | | | | **van der Waals (kcal/mol)** | **Electrostatic (kcal/mol)** | **π- π stacking (Å)** |
| **12a** | **0.50** | -7.46 | -53.38 | Heme500(-5.67), Val461(-1.124), Thr459(-1.31), Leu356(-1.30), His294(-1.22), Phe290(-3.07), Ala287(-1.26), Leu208(-2.65), Glu205(-1.33), Leu127(-1.01), Phe110(-1.29), Met106(-1.60), Ile105(-1.19), Tyr103(-1.95) | Heme500(-3.80), Tyr116(-1.58) | His294(2.04), Tyr116(1.86) |
| **12b** | **0.25** | -7.61 | -55.12 | Heme500(-6.55), Val461(-2.07), Met460(-2.40), Thr459(-1.43), Thr295(-1.12), His294(-2.06), Ala291(-1.10), Ala287(-1.47), Leu208(-2.00), Glu205(-1.76), Leu127(-1.03), Tyr116(-2.83), Phe110(-1.73), Met106(-1.18), Ile105(-1.94), Tyr103(-2.28) | Heme500(-4.39), Glu205(-1.20), Tyr116(-1.81) | Tyr103(2.40), Tyr116(2.29), His294(2.56) |
| **12c** | **0.25** | -7.58 | -55.54 | Heme500(-6.80), Met460(-2.17), Thr459(-1.36), Leu356(-1.90), Thr295(-1.13), His294(-2.10), Ala291(-1.15), Phe290(-3.16), Leu208(-2.62), Glu205(-1.75), Tyr116(-2.61), Phe110(-1.39), Ile105(-1.20), | Heme500(-4.11), Phe290(-1.60), Glu205(-1.72), Tyr116(-1.52) | Tyr103(2.35), His294(2.30) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Tyr103(-2.18) | | |
| **12d** | **0.50** | -7.40 | -53.12 | Heme500(-5.50), Met460(-2.11), Thr459(-1.16), Thr295(-1.09), His294(-2.18), Ala291(-1.14), Phe290(-2.53), Ala287(-1.04), Leu208(-2.13), Glu205(-1.32), Leu127(-1.20), Tyr116(-2.16), Phe110(-1.42), Ile105(-1.08), Tyr103(-1.71) | Heme500(-3.80), Phe290(-1.14), Glu205(-1.17) | Phe110(2.84), Phe290(2.49), His294(2.24) |
| **12e** | **0.25** | -7.54 | -55.10 | Heme500(-6.33), Val461(-1.93), Met460(-2.37), Thr459(-1.56), Leu356(-1.80), Thr295(-1.26), His294(-2.15), Ala291(-1.19), Phe290(-3.58), Ala287(-1.56), Leu208(-3.11), Glu205(-1.80), Leu127(-1.13), Tyr116(-2.54), Phe110(-1.46), Met106(-1.77), Ile105(-1.24), Tyr103(-2.15) | Heme500(-4.14), Phe290(-1.07),Tyr116(-1.85) | Tyr103(2.40), Tyr116(2.30), His294(2.40) |
| **12f** | **0.125** | -7.95 | -56.27 | Heme500(-7.38), Met460(-3.18), Thr459(-1.91), Leu356(-2.16), Thr295(-1.13), His294(-2.09), Ala291(-1.12), Phe290(-3.81), Ala287(-1.78), Leu208(-2.96), Glu205(-1.67), Leu127(-1.04), Tyr116(-3.33), Phe110(-1.87), Ile105(-2.16), Tyr103(-2.194) | Heme500(-4.53), Phe290(-1.26), Glu205(-2.51) | Tyr103(2.33), Tyr116(2.28), His294(2.37) |
| **12g** | **0.125** | -7.91 | -56.79 | Heme500(-7.41), Met460(-3.15), Leu356(- | Heme500(-4.85), | Tyr103(2.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | 2.14), Thr295(-1.16), His294(-2.14), Ala291(-1.32), Phe290(-3.14), Ala287(-1.74), Leu208(-3.11), Glu205(-1.66), Leu127(-1.03), Tyr116(-3.23), Phe110(-1.87), Met106(-1.93), Ile105(-1.65), Tyr103(-2.20) | Phe290(-1.13) | 7), Tyr116(2.27), His294(2.91) |
| **12h** | **0.25** | -7.59 | -55.99 | Heme500(-6.66), Val461(-1.81), Met460(-2.49), Thr459(-1.63), Leu356(-1.86), Thr295(-1.16), His294(-1.95), Ala291(-1.62), Phe290(-2.92), Ala287(-1.40), Leu208(-3.12), Glu205(-1.71), Leu127(-1.16), Tyr116(-2.47), Phe110(-1.49), Ile105(-2.05), Tyr103(-2.35) | Heme500(-4.75), Glu205(-2.38) | Tyr103(2.19), Phe110(2.78), His294(2.81) |
| **12i** | **0.5** | -7.45 | -53.95 | Heme500(-5.86), Met460(-1.94), Thr459(-1.01), 422(-1.09), Leu356(-1.33), Thr295(-1.15), His294(-2.08), Ala291(-1.65), Phe290(-2.10), Leu208(-2.18), Glu205(-1.15), Phe110(-1.23), Tyr103(-1.75) | Heme500(-3.77), Tyr116(-1.29) | Tyr116(1.83) |
| **12j** | **0.031** | -8.50 | -58.96 | Heme500(-8.82), Val461(-2.59), Met460(-3.75), Thr459(-2.28), Val359(-1.47), Leu356(-2.74), His294(-2.84), Ala291(-1.79), Phe290(-4.15), Leu208(-3.48), | Heme500(-5.73), Ala291(-1.36), Phe290(-1.41), Glu205(-2.51), | His294(2.31) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Glu205(-2.54), Tyr116(-4.21), Phe110(-2.13), Met106(-2.03), Ile105(-2.40), Tyr103(-2.95) | Tyr116(-1.94), Tyr103(-1.23) | |
| **12k** | **0.0625** | -8.28 | -57.89 | Heme500(-8.29), Val461(-2.20), Met460(-3.37), Thr459(-2. 18), Leu356(-2.24), Thr295(-1.44), His294(-2.29), Ala291(-1.21), Phe290(-4.03), Ala287(-1.74), Leu208(-3.17), Glu205(-1.76), Leu127(-1.12), Tyr116(-3.85), Phe110(-1.82), Met106(-2.01), Ile105(-2.24), Tyr103(-2.24) | Heme500(-4.96), Ala291(-1.10), Phe290(-1.17), Tyr116(-1.88) | Tyr116(2.24), His294(2.52) |
| **12l** | **0.25** | -7.69 | -55.82 | Heme500(-6.45), Met460(-2.36), Thr459(-1.62), Leu356(-1.45), Thr295(-1.10), His294(-1.71), Phe290(-4.02), Ala287(-1.77), Leu208(-2.80), Glu205(-1.96), Leu127(-1.13), Tyr116(-2.28), Phe110(-1.55), Met106(-2.04), Ile105(-1.99), Tyr103(-2.18) | Heme500(-4.11), Phe290(-1.17) | Tyr103(2.34), Tyr116(2.27), His294(2.59) |

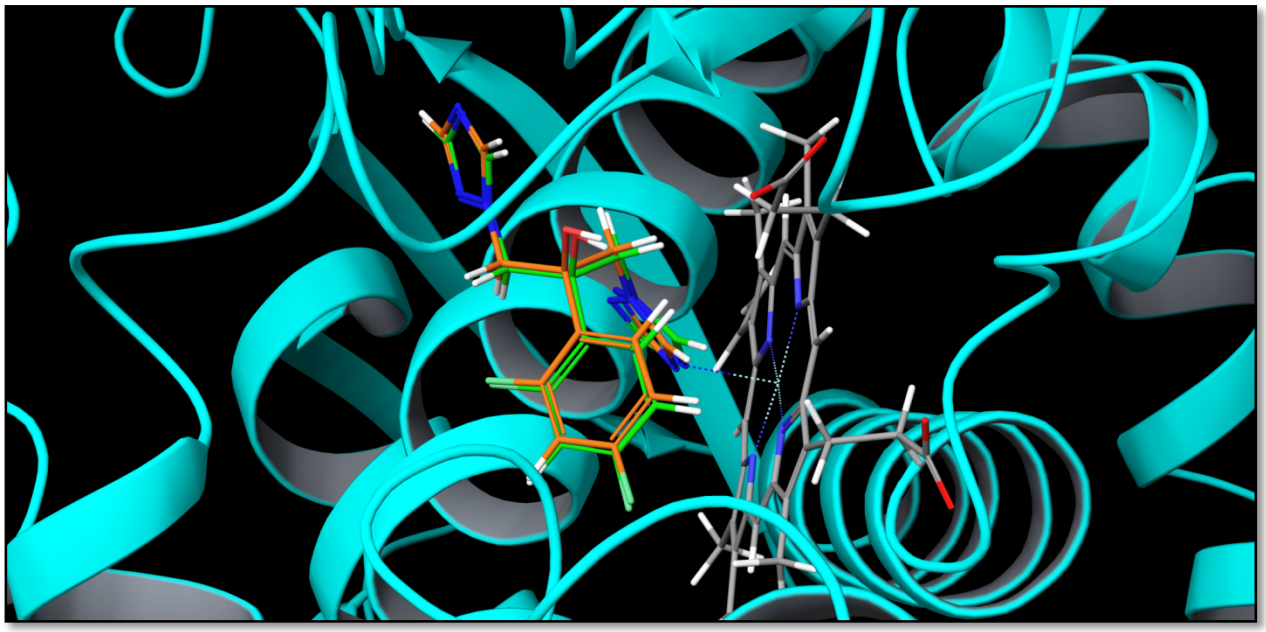


**Figure 1S:** Validation of docking protocol: Overlay of the best scoring pose for native ligand-Fluconazole obtained by docking (green carbon) against the X-ray bound conformation (orange carbon).

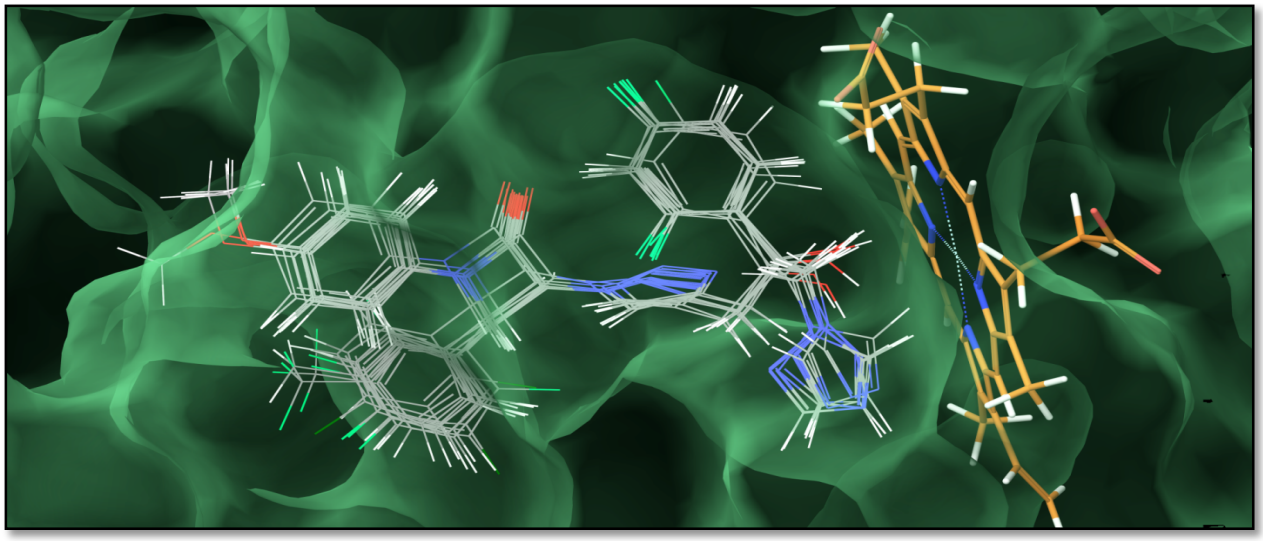


**Figure 2S.** Binding mode of fluconazole β-lactam conjugates linked via 1,2,3-triazole **12 (a-l)**into the active site of fungal sterol 14α-demethylase (CYP51).

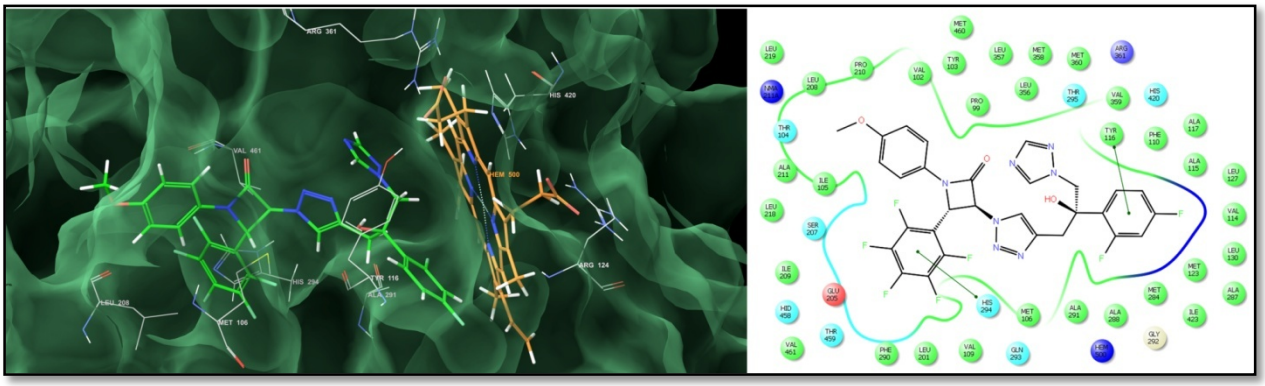


**Figure 3S.** Binding mode of **12a** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

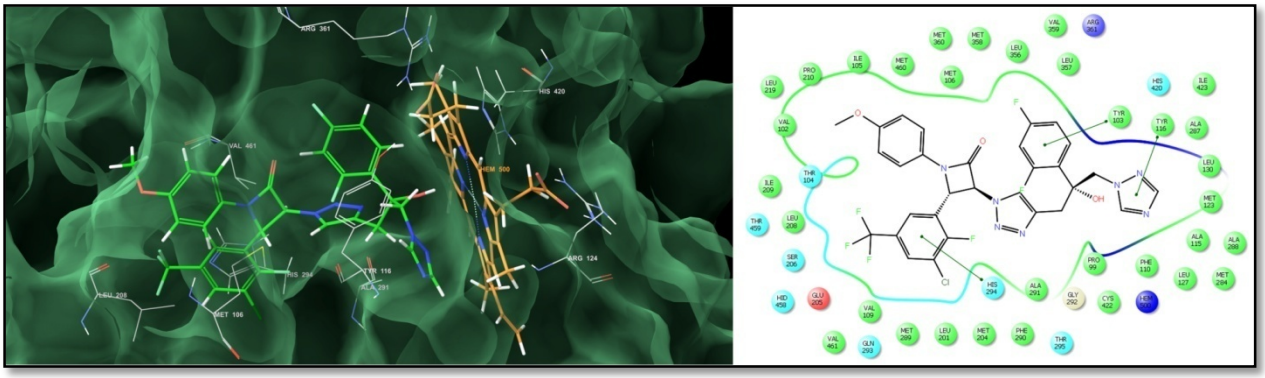


**Figure 4S.** Binding mode of **12b** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

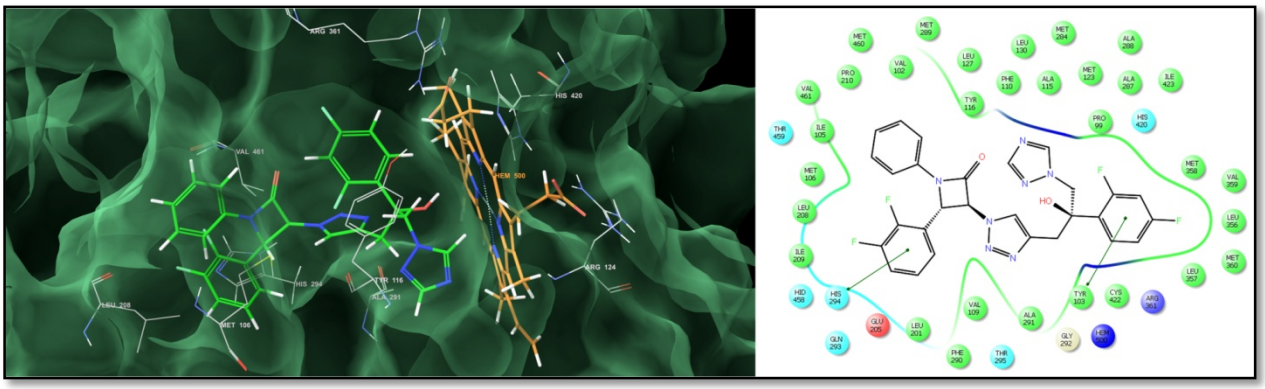


**Figure 5S.** Binding mode of **12c** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

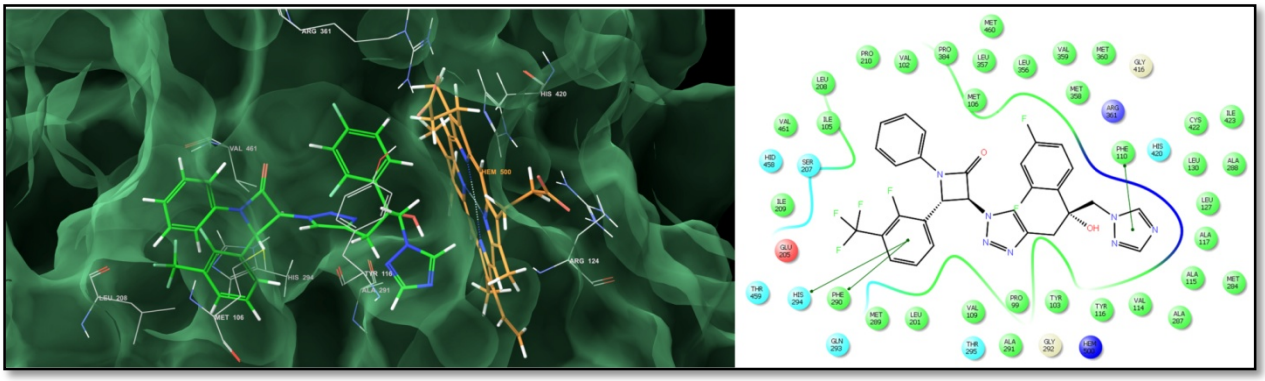


**Figure 6S.** Binding mode of **12d** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

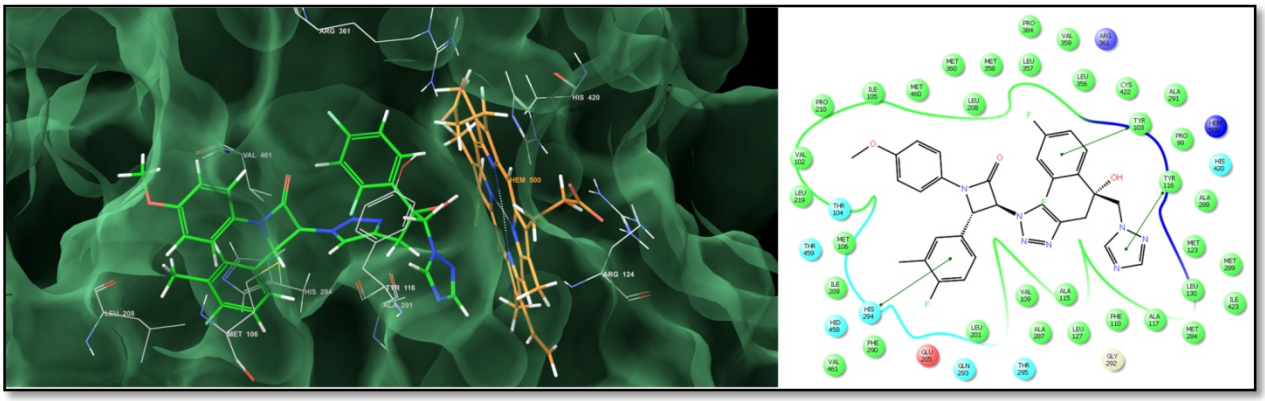


**Figure 7S.** Binding mode of **12e** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

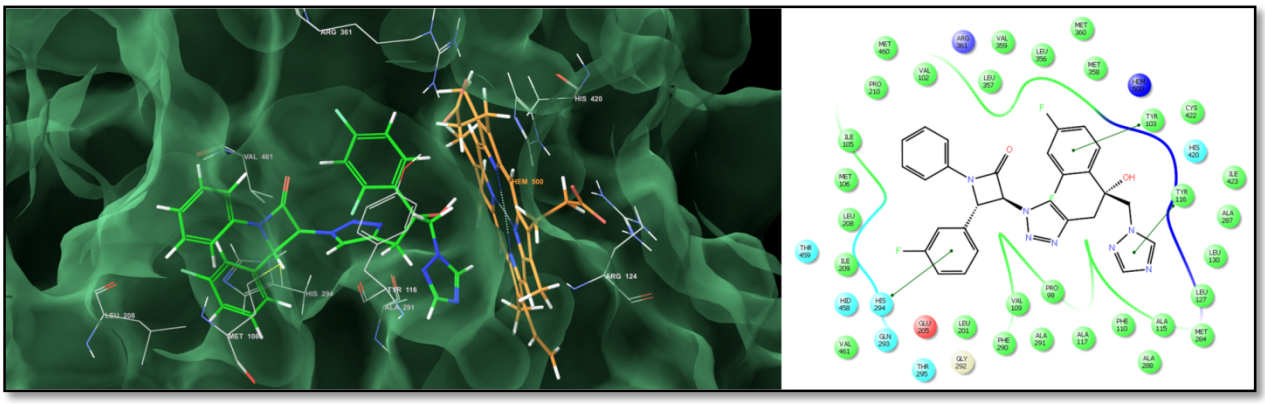


**Figure 8S.** Binding mode of **12f** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

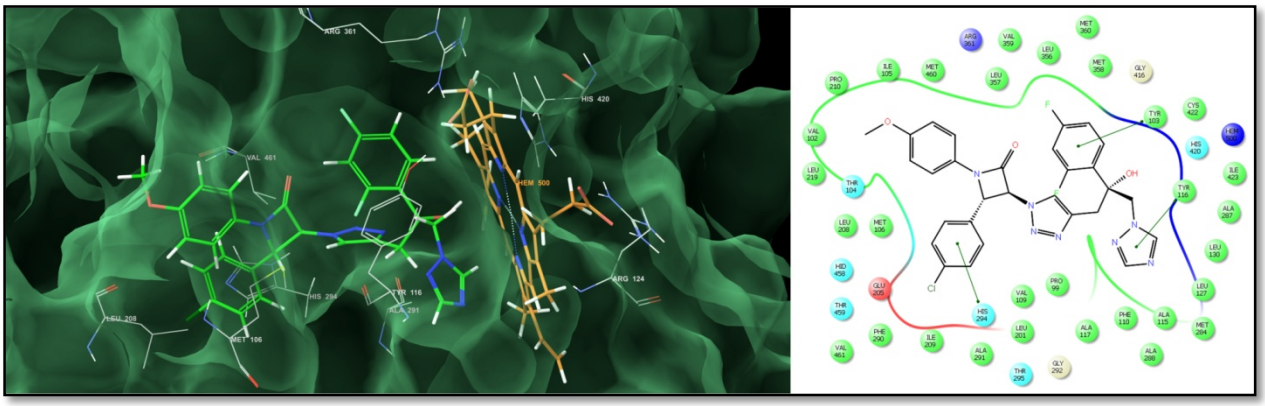


**Figure 9S.** Binding mode of **12g** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

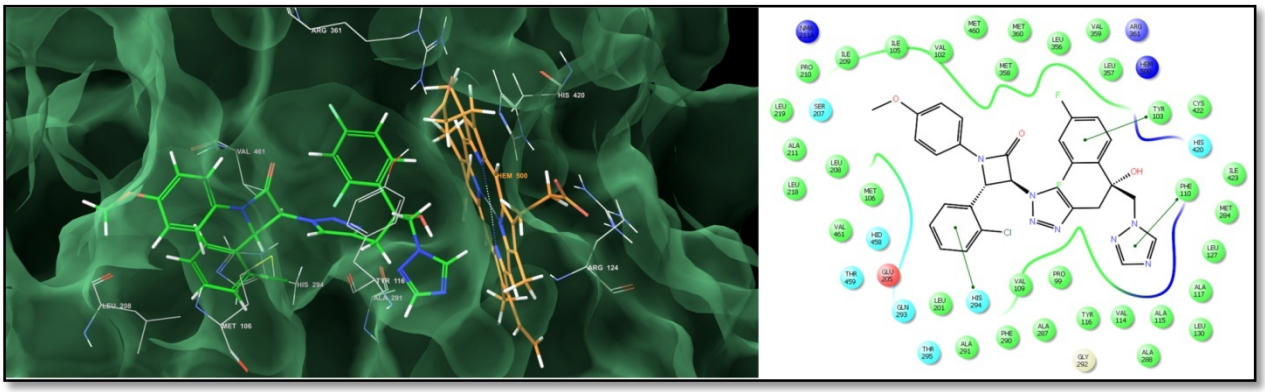


**Figure 10S.** Binding mode of **12h** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

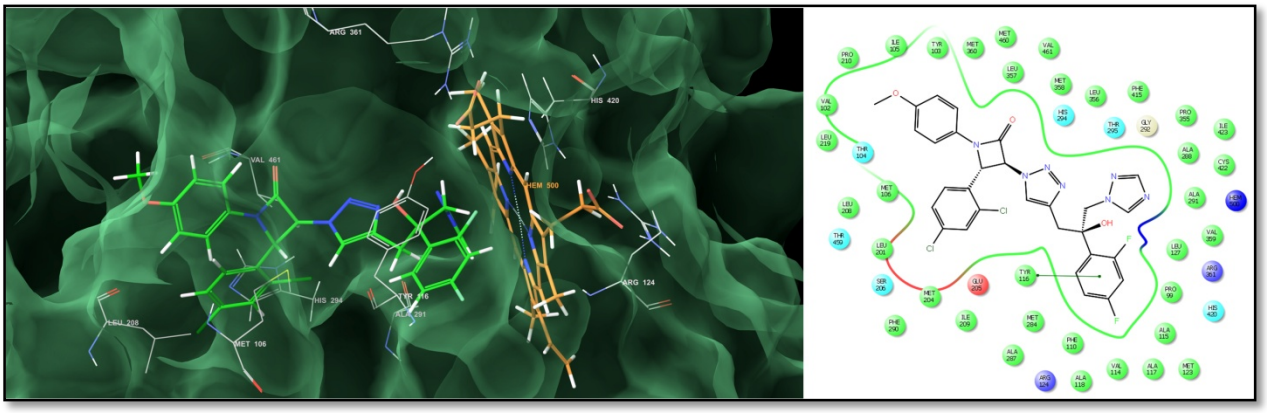


**Figure 11S.** Binding mode of **12i** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

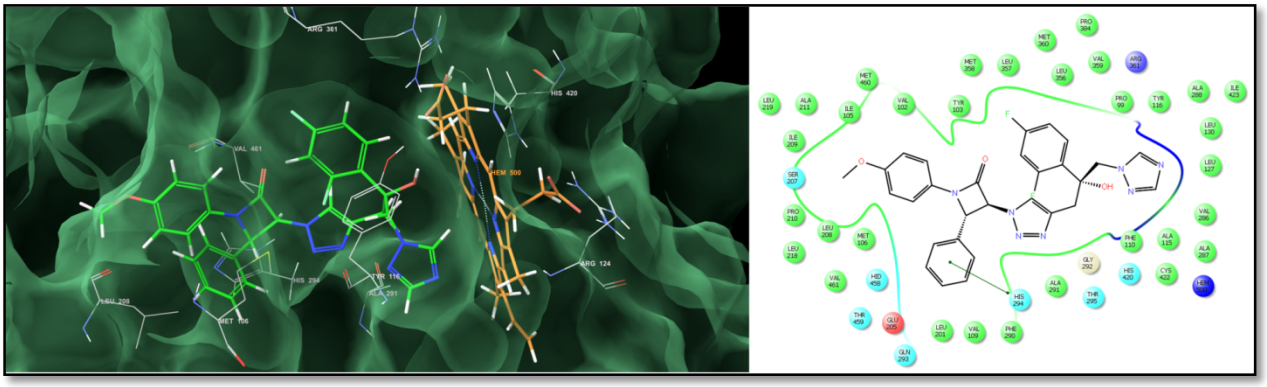


**Figure 12S.** Binding mode of **12j** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

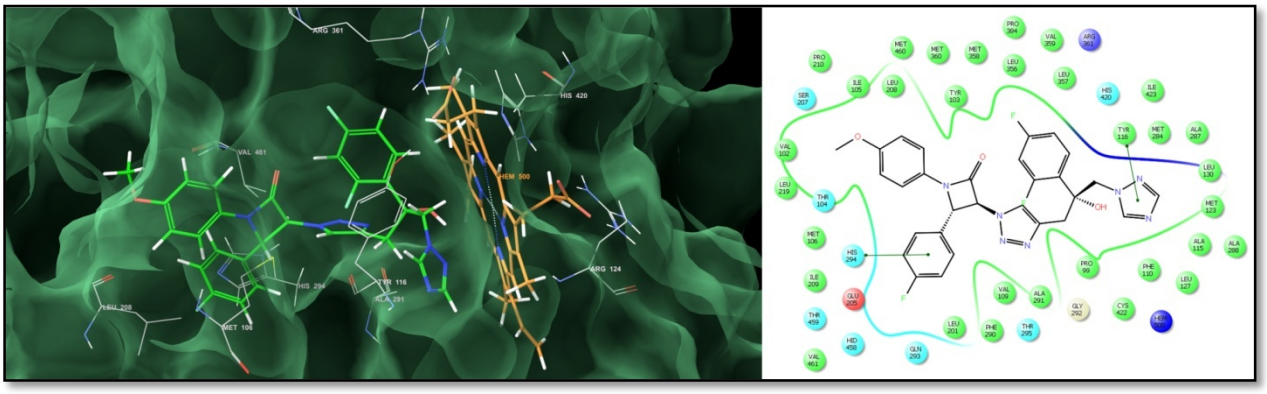


**Figure 13S.** Binding mode of **12k** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

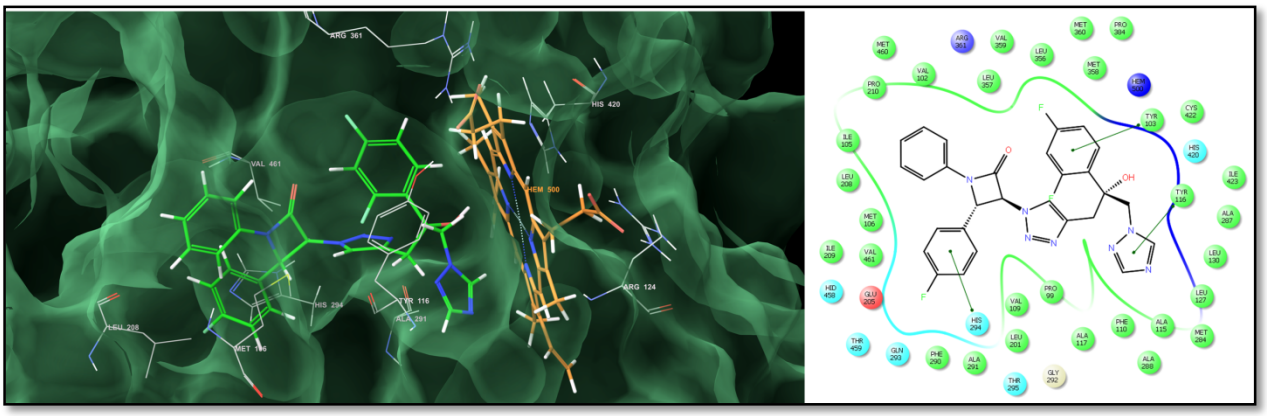


**Figure 14S.** Binding mode of **12l** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction has been represented using green lines).

## MTT assay (MTT: MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide))

In vitro cytotoxicity against above mentioned human cell lines was determined using MTT assay as described previously (Ciapetti et al, 1983).[4,5] Briefly, log phase cells were harvested using trypsin (0.05% trypsin, 0.02% EDTA, in PBS) from tissue culture flask and the suspension was diluted with appropriate culture medium to obtain cell density of $10^5$ cells/mL as determined by hemocytometry. An aliquot of 100 µl ($10^4$ cells/well) of each suspension was seeded in 96-wells cell culture plates and these were incubated at 37°C in an atmosphere of 5% $CO_2$ and 95% relative humidity in a $CO_2$ incubator. After 24 h, compounds (1 µl/well) at varying concentrations of 100 to 0.7825 µg/ml were added to the wells containing cells. Paclitaxel, Carboplatin, Doxorubicin were used as positive control. Suitable controls with equivalent concentration of DMSO were also included. The plates were further incubated for 48 h., at the end of the incubation period; the solution containing the unattached cells was discarded followed by addition of 10 µL MTT (5 mg/mL in PBS) to adhered cells in growth medium. After 3.5 h at 37°C for MTT cleavage, the formazan product was solubilized by the addition of 100 µL 0.04 N HCl in isopropanol. Absorbance was measured on a SPECTRAmax PLUS 384 plate reader at wavelength of 570 nm. Percentage cytotoxicity was calculated using the formula-

% Cytotoxicity = [(Control - CMP) / (Control - blank)] × 100

Where, Control = cell growth in medium without compounds

CMP = cell growth in presence of compounds

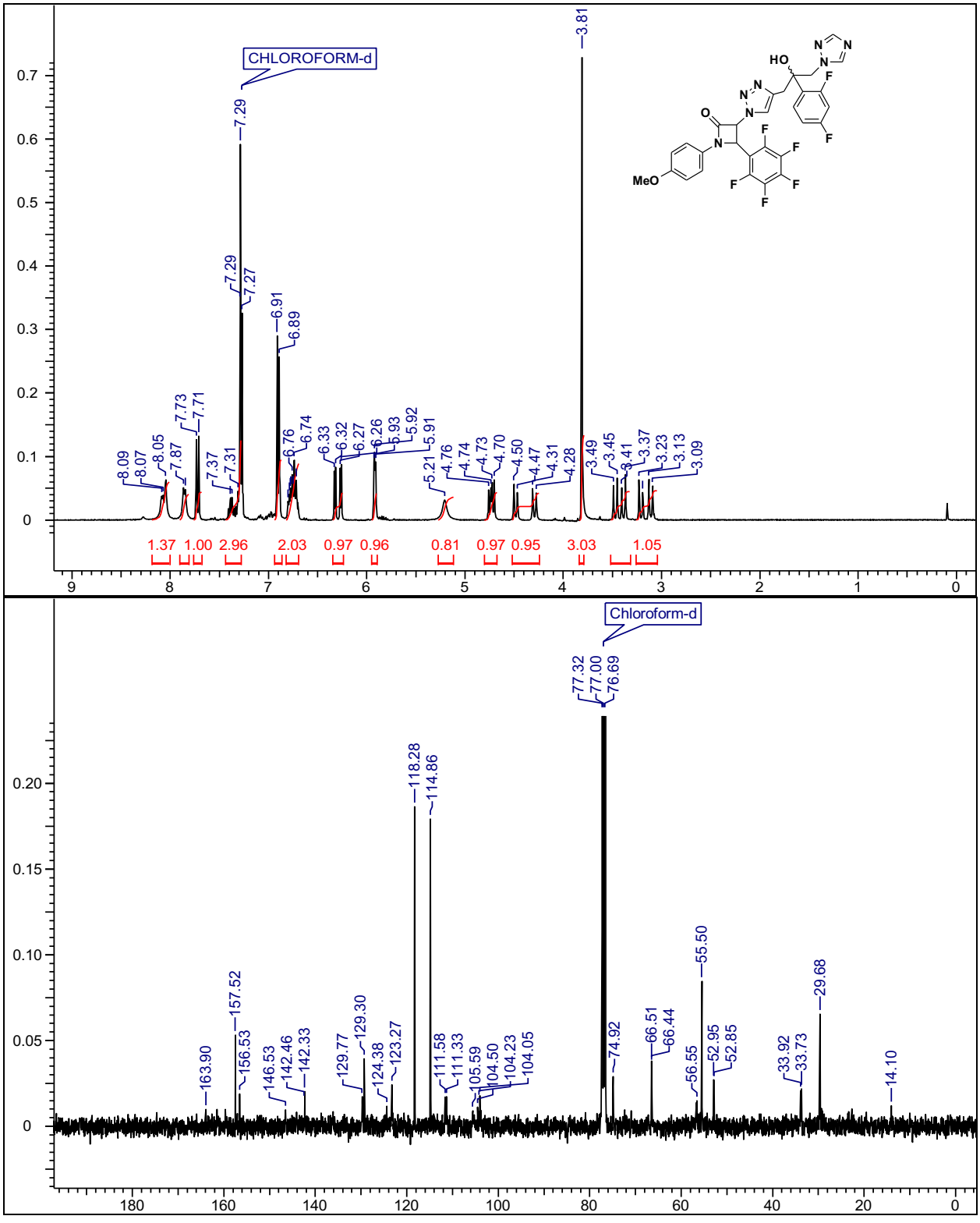
Blank = culture medium without cells

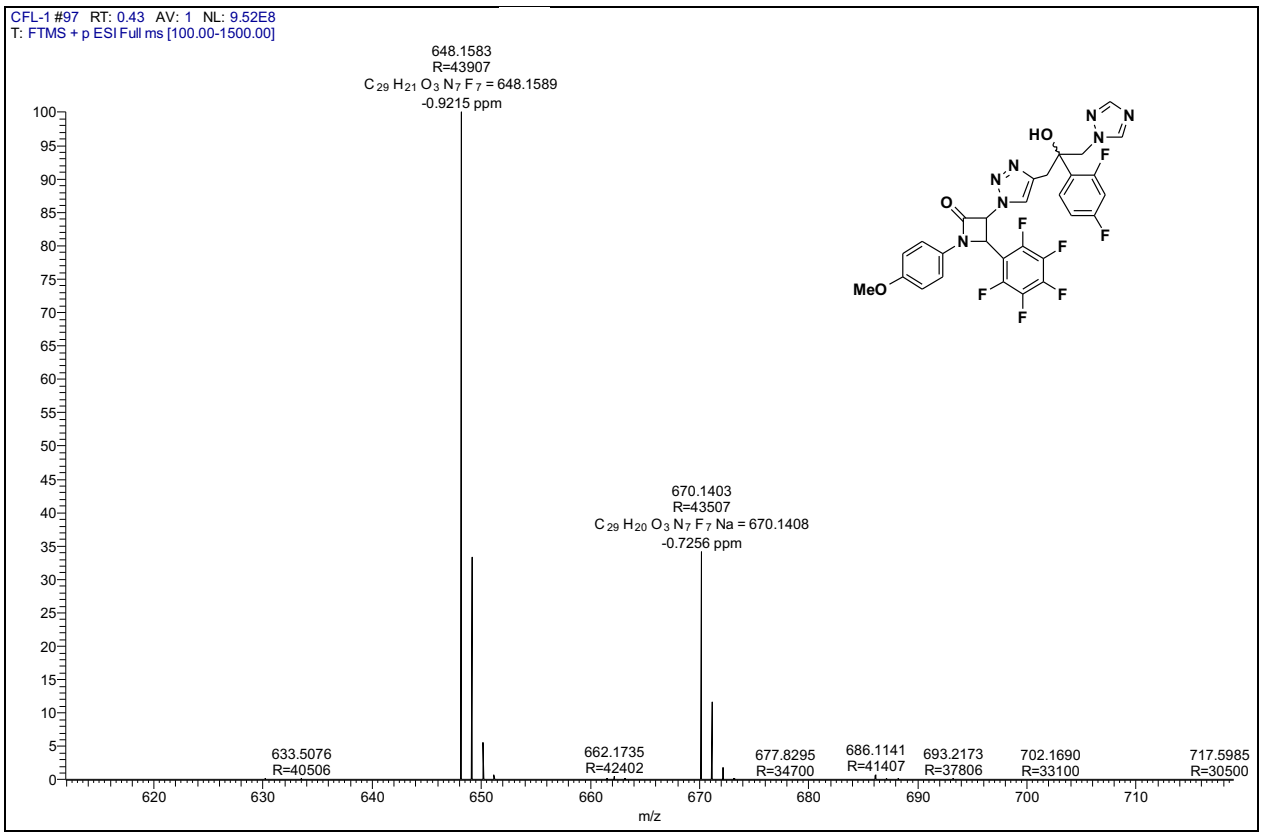
Experiment was performed in triplicates and the quantitative value was expressed as the average ± standard deviation.

**Experimental Data**

*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)-4-(perfluorophenyl)azetidin-2-one ;* **12a**. Yield: 90%; IR ($CHCl^3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.05-8.09 (m, 1H), 7.85 (d, *J*= 7.3 Hz, 1H), 7.71 (d, *J* = 9.5Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.31-7.40 (m, 1H, Ar-H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.70-6.80 (m, 2H), 6.26-6.33 (m, 1H), 5.91-5.93 (m, 1H), 5.21 (bs, 1H), 4.70-4.76 (m, 1H), 4.28-4.50 (m, 1H), 3.81 (s, 3H), 3.37-3.49 (m, 1H), 3.09-3.23 (m, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.9, 157.5, 156.5, 146.5, 142.4, 142.3, 129.7, 129.3, 124.4, 123.2, 118.3, 114.8, 111.6, 111.3, 105.6, 104.5, 104.2, 104.0, 74.9, 66.5, 56.5, 55.5, 52.9, 52.8, 33.9, 33.7, 29.7, 14.1; ESI-MS (m/z): 648.15 $[M+H]^+$; HRMS (ESI-qTOF): calcd for $C_{29}H_{21}F_7N_7O_3$ $[M+H]^+$,648.1589: found: 648.1583.

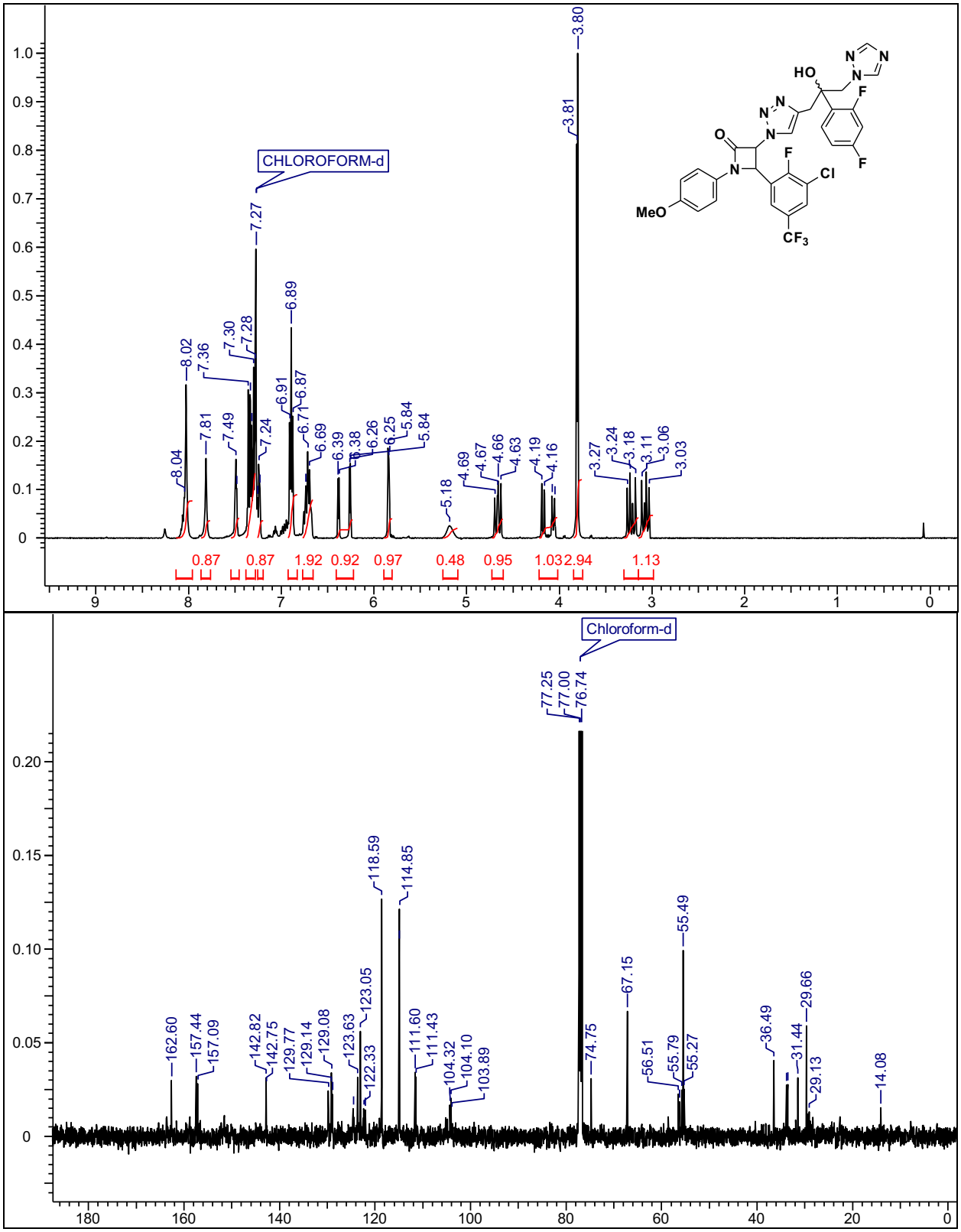
**12a**

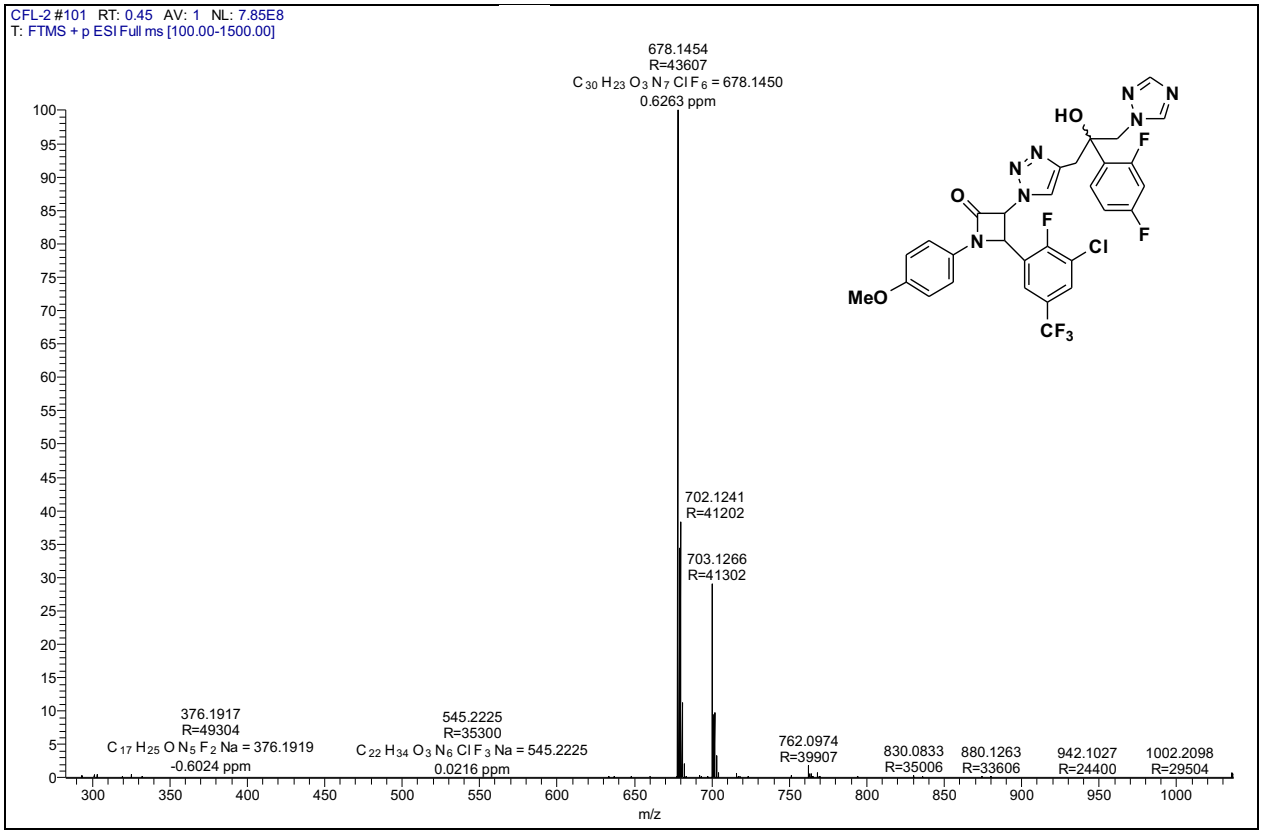





*4-(3-chloro-2-fluoro-5-(trifluoromethyl)phenyl)-3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)azetidin-2-one;* **12b.** Yield: 89%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 500 MHz) δ 8.02-8.06 (m, 2H), 7.81 (s, 1H), 7.47-7.51 (m, 1H, Ar-H), 7.28-7.36 (m, 3H, Ar-H), 7.23-7.26 (m, 1H, Ar-H), 6.89 (t, $J$ = 8.54 Hz, 2H), 6.67-6.78 (m, 2H), 6.25-6.39 (m, 1H), 5.83-5.84 (m,1H), 5.18 (bs, 1H), 4.63-4.69 (m, 1H), 4.05-4.19 (m, 1H), 3.81 (s, 3H), 3.18-3.27 (m, 1H), 3.03-3.11 (m, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 162.6, 157.4, 157.1, 142.8, 142.7, 129.8, 129.1, 124.5, 123.6, 123.0, 118.6, 114.8, 111.6, 111.4, 104.3, 104.1, 103.9, 74.7, 67.1, 56.5, 55.8, 55.5, 55.3, 36.5, 33.8, 33.6, 31.4, 29.7, 29.1, 14.1; ESI-MS (m/z): 678.14 $[M+H]^+$; HRMS (ESI-qTOF): calcd for $C_{30}H_{23}ClF_6N_7O_3$ $[M+H]^+$,678.1450: found: 678.1454.

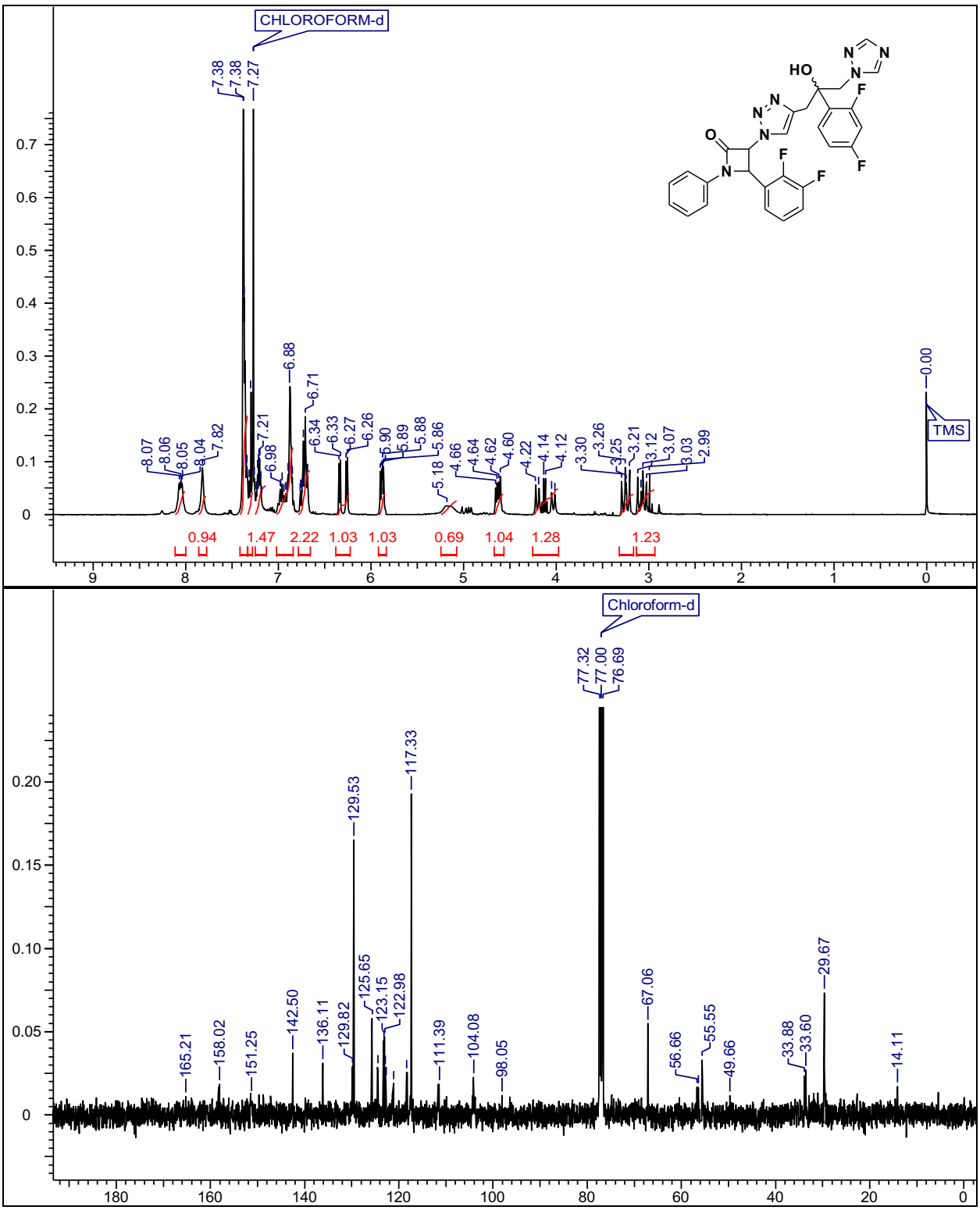
**12b**


CHLOROFORM-d
Chloroform-d
HO
F
F
F
Cl
MeO
CF3
O
N
N
N
N
N
3.80
3.81
7.27
6.89
8.02
7.36
7.30
7.28
6.91
6.87
6.71
7.81
7.49
7.24
6.69
6.39
6.38
6.26
6.25
5.84
5.84
8.04
5.18
4.69
4.67
4.66
4.63
4.19
4.16
3.27
3.24
3.18
3.11
3.06
3.03
0.87
0.87
1.92
0.92
0.97
0.48
0.95
1.03
2.94
1.13
77.25
77.00
76.74
118.59
114.85
123.05
111.60
111.43
67.15
55.49
162.60
157.44
157.09
142.82
142.75
129.77
129.14
129.08
123.63
122.33
104.32
104.10
103.89
74.75
56.51
55.79
55.27
36.49
31.44
29.66
29.13
14.08




*4-(2,3-difluorophenyl)-3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-phenylazetidin-2-one;* **12c**. Yield: 91%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.03-8.07 (m, 1H), 7.82 (s, 1H), 7.33-7.40 (m, 5H, Ar-H), 7.28-7.31 (m, 1H, Ar-H), 7.18-7.23 (m, 1H, Ar-H), 6.85-6.98 (m, 3H), 6.68-6.76 (m, 2H), 6.25-6.34 (m, 1H), 5.86-5.89 (m, 1H), 5.18 (bs, 1H), 4.60-4.65 (m, 1H), 4.01-4.22 (m, 1H), 3.20-3.29 (m, 1H), 2.99-3.12 (m,1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 165.2, 158.2, 158.0, 151.2, 142.5, 136.1, 129.8, 129.5, 125.6, 123.0, 117.3, 111.4, 104.1, 98.0, 67.0, 56.6, 56.3, 55.5, 49.6, 33.9, 33.6, 29.7, 14.1; ESI-MS(m/z): 564.17$[M+H]^+$; HRMS (ESI-qTOF): calcd for $C_{28}H_{22}F_4N_7O_2$ $[M+H]^+$,564.1766: found: 564.1769.

**12c**


CHLOROFORM-d
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7.38
7.27
6.88
6.71
6.34
6.33
6.27
6.26
6.90
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5.88
5.86
8.07
8.06
8.05
8.04
7.82
7.21
6.98
5.18
4.66
4.64
4.62
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4.22
4.14
4.12
3.30
3.26
3.25
3.21
3.12
3.07
3.03
2.99
0.00
TMS
0.94
1.47
2.22
1.03
1.03
0.69
1.04
1.28
1.23
HO
N
N
N
N
N
O
F
F
F
F
Chloroform-d
77.32
77.00
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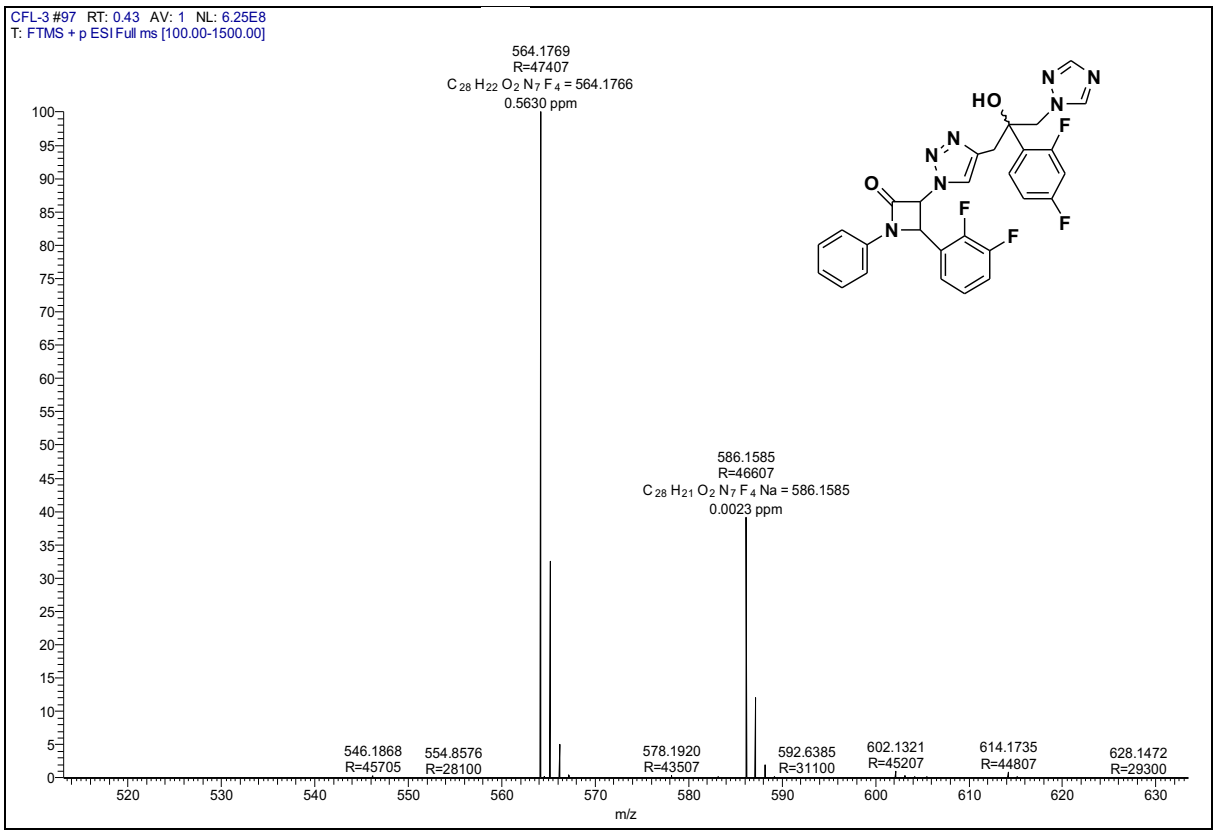
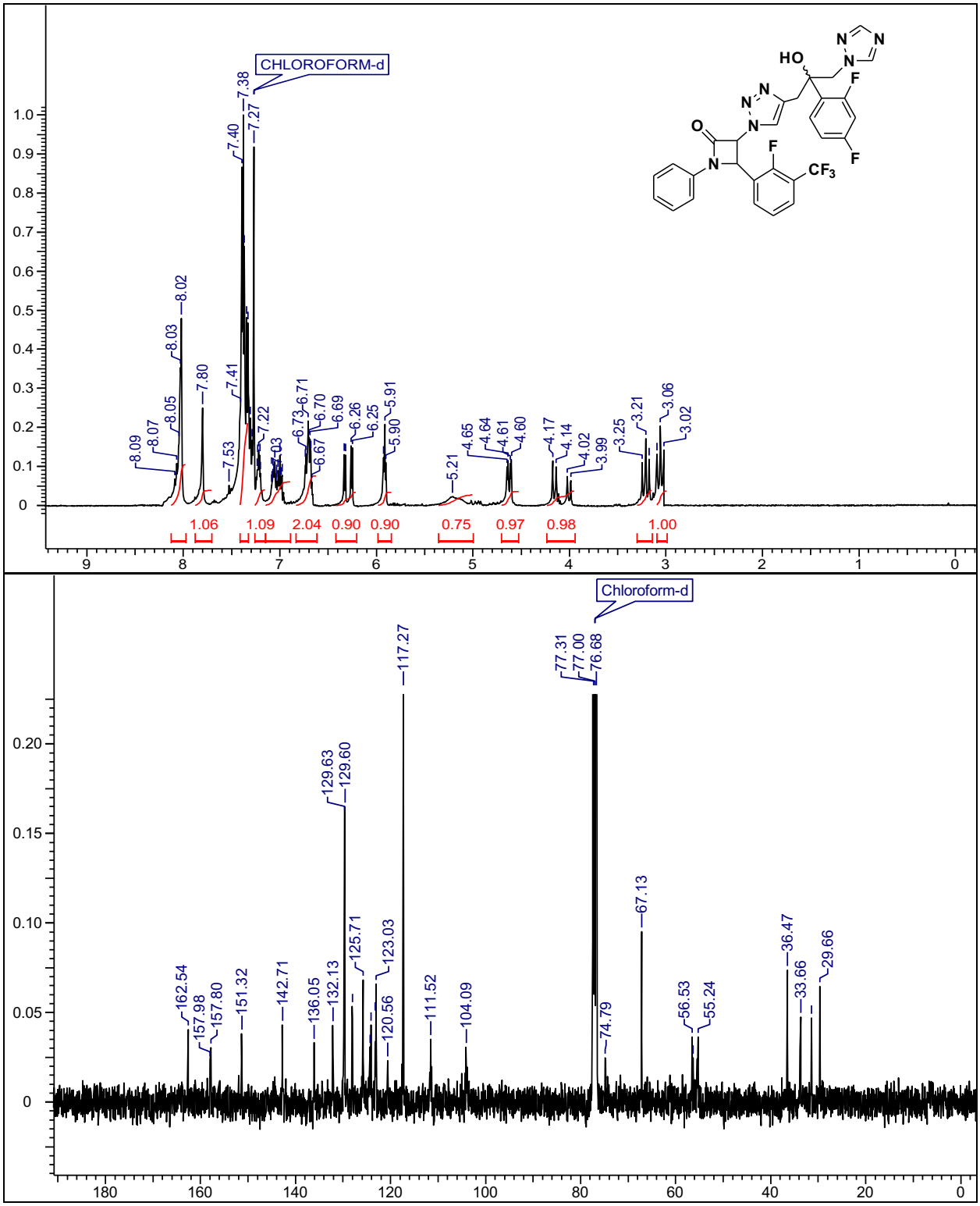
49.66
33.88
33.60
29.67
14.11




*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-4-(2-fluoro-3-(trifluoromethyl)phenyl)-1-phenylazetidin-2-one;***12d**. Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.02-8.07 (m, 3H), 7.80 (s, 1H), 7.33-7.41 (m, 6H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 7.00-7.21 (m, 1H, Ar-H), 6.67-6.74 (m, 2H), 6.25-6.33 (m,1H), 5.90-5.93 (m, 1H), 5.21 (bs, 1H), 4.60-4.65 (m, 1H), 3.99-4.17 (m, 1H), 3.17-3.25 (m, 1H), 3.02-3.10 (m, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 162.5, 158.0, 157.8, 151.3, 142.7, 136.0, 132.16, 129.6, 125.7, 123.0, 117.3, 111.5, 104.1, 74.8, 67.1, 56.5, 56.3, 55.2, 36.5, 33.7, 31.4, 29.7; ESI-MS (m/z): 614.17 $[M+H]^+$; HRMS (ESI-qTOF): calcd for $C_{29}H_{22}F_6N_7O_2$ $[M+H]^+$,614.1734: found: 614.1727.

**12d**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8.05
8.03
8.02
7.80
7.53
7.41
7.40
7.38
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7.22
7.03
6.73
6.71
6.70
6.69
6.67
6.26
6.25
5.91
5.90
5.21
4.65
4.64
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4.60
4.17
4.14
4.02
3.99
3.25
3.21
3.06
3.02
1.06
1.09
2.04
0.90
0.90
0.75
0.97
0.98
1.00
HO
N
N
N
N
N
O
N
F
F
F
CF3
Chloroform-d
162.54
157.98
157.80
151.32
142.71
136.05
132.13
129.63
129.60
125.71
123.03
120.56
117.27
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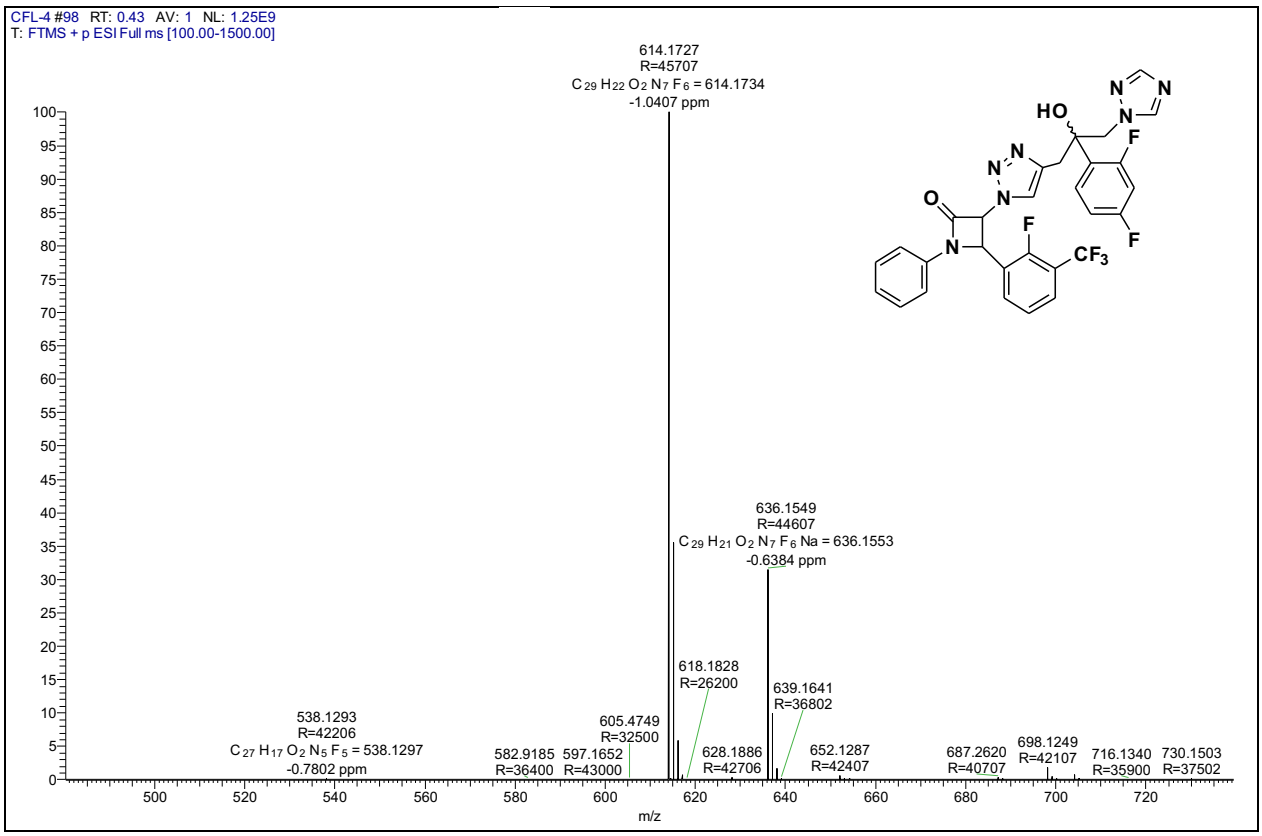
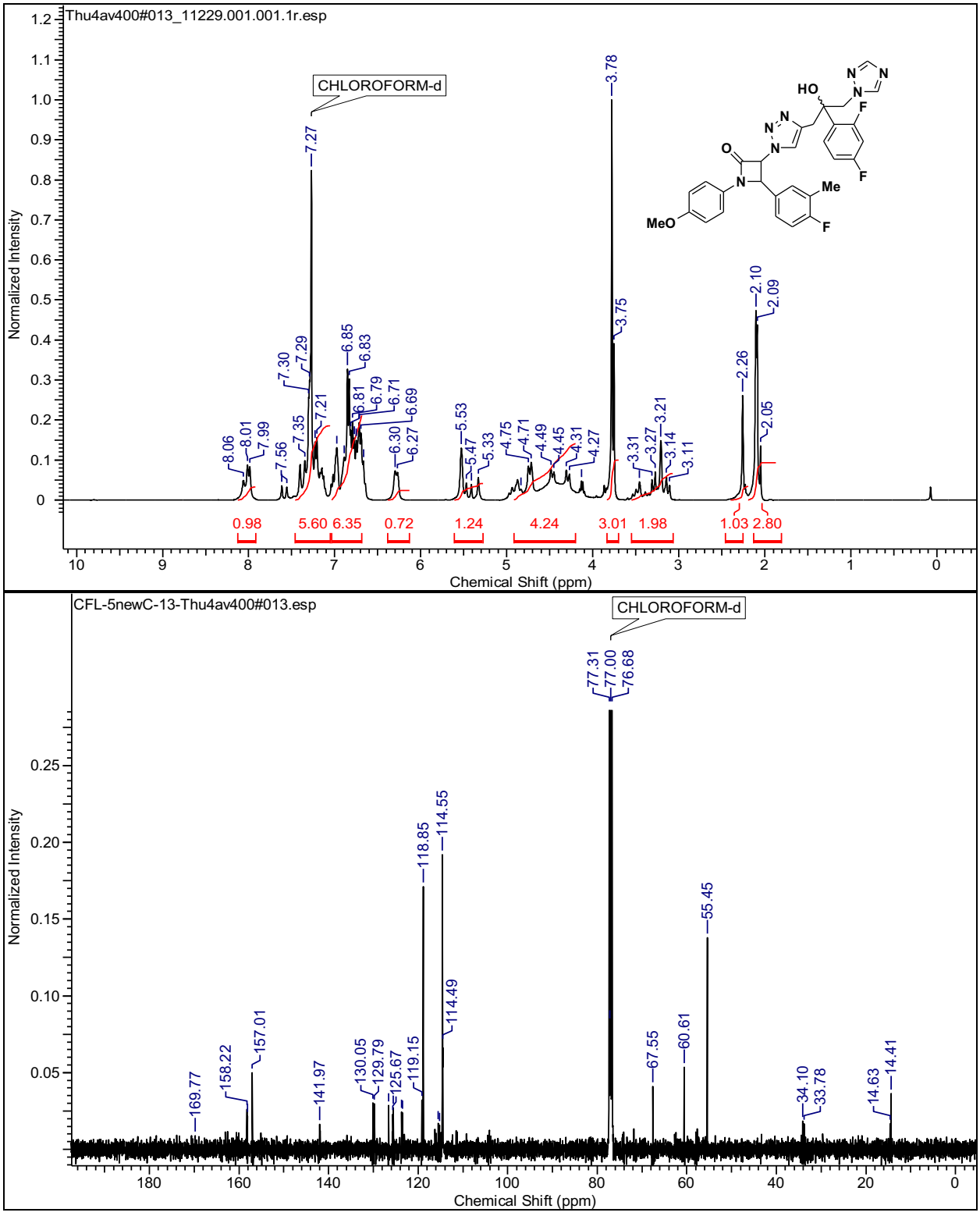
67.13
56.53
55.24
36.47
33.66
29.66




*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-4-(4-fluoro-3-methylphenyl)-1-(4-methoxyphenyl)azetidin-2-one;***12e***. Yield: 88%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.99-8.06 (m, 1H), 7.15-7.40 (m, 6H), 6.67-7.02 (m, 6H), 6.29 (d, *J* = 10.9 Hz, 1H), 5.33-5.53 (m, 1H), 4.13-4.84 (m, 4H), 3.76 (m, 3H), 3.11-3.46 (m, 2H), 2.26 (s, 1H), 2.05-2.10 (m, 3H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 169.7, 158.2, 157.0, 141.9, 130.0, 129.7, 126.5, 125.6, 119.1, 118.8, 115.4 114.5, 67.5, 60.6, 55.4, 33.7, 14.6, 14.4; HRMS (ESI-qTOF): calcd for $C_{30}H_{27}F_3N_7O_3$ $[M+H]^+$,590.2122: found: 590.2108.

**12e**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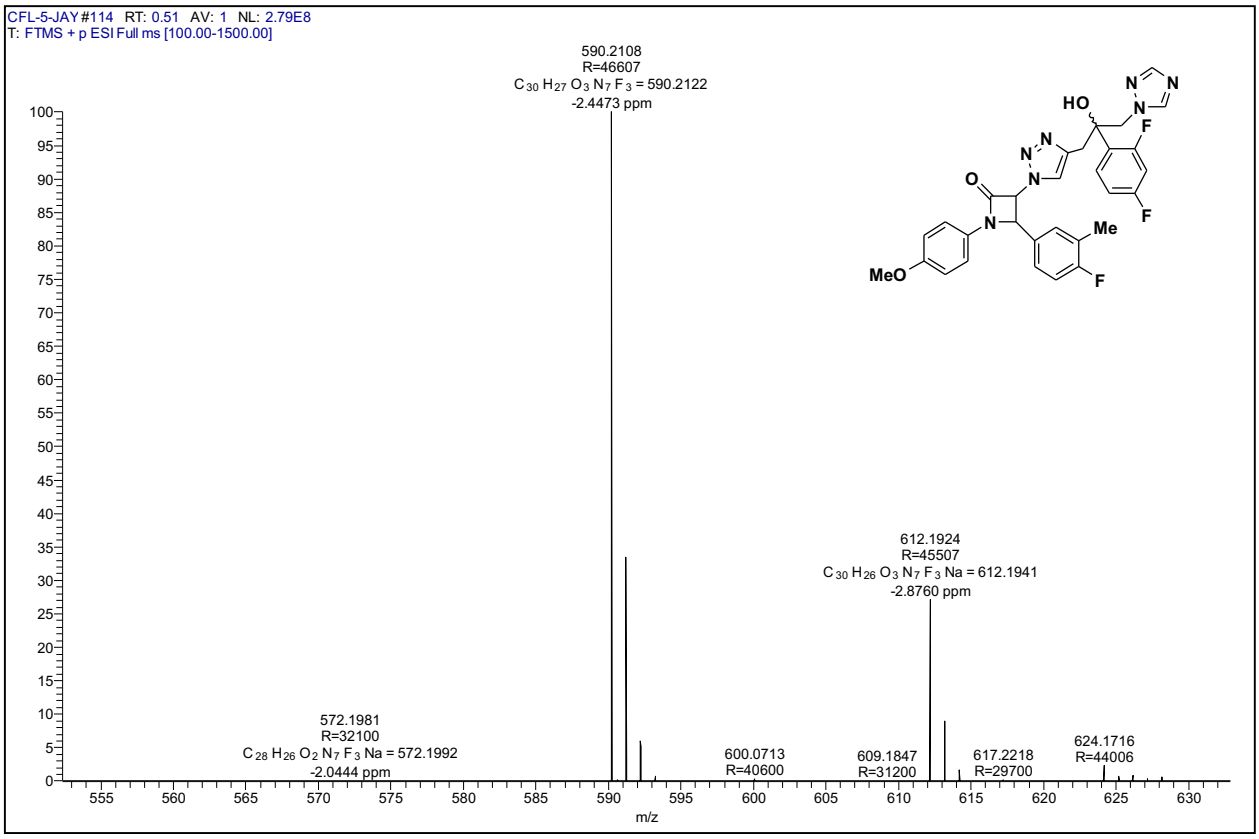
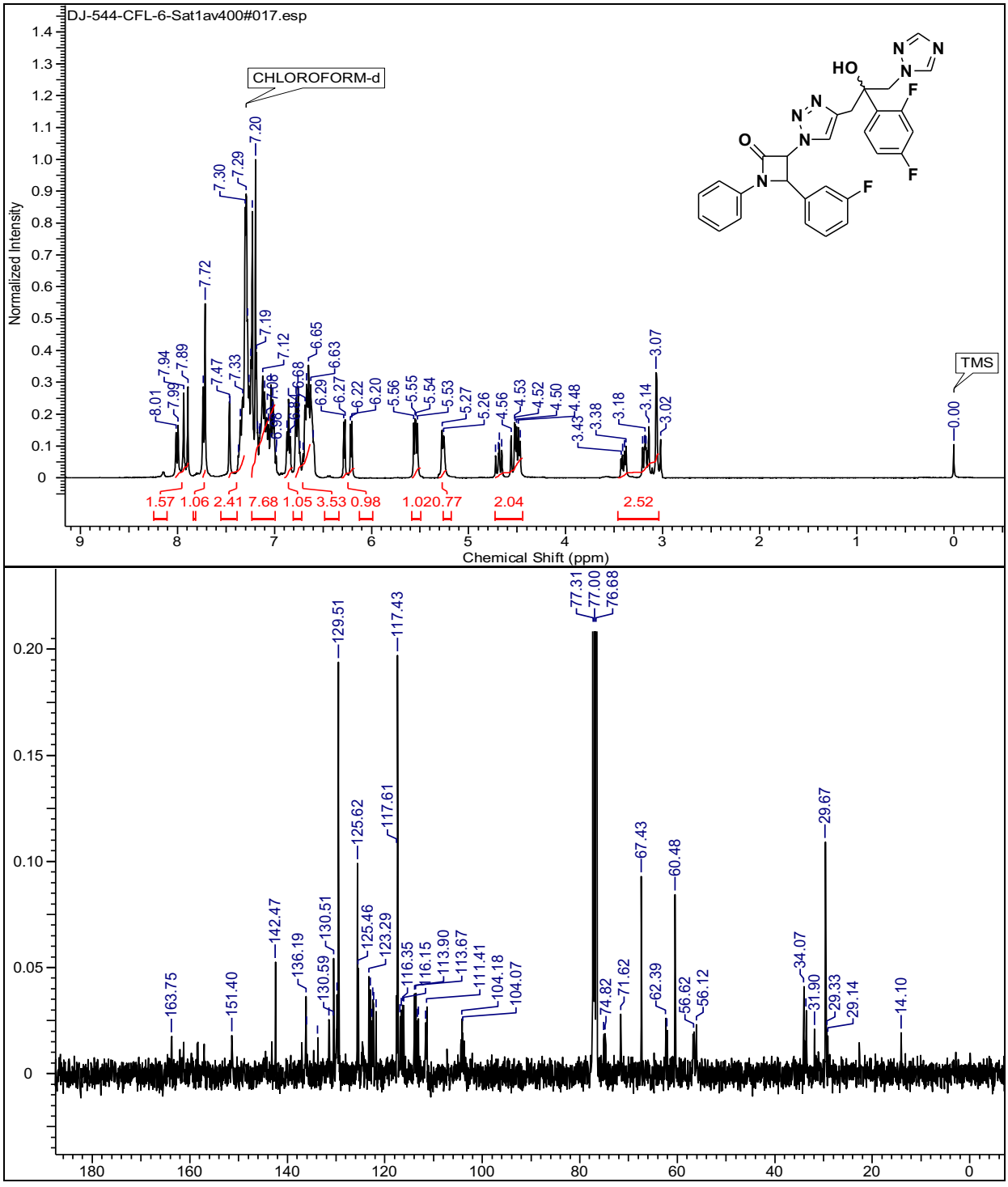
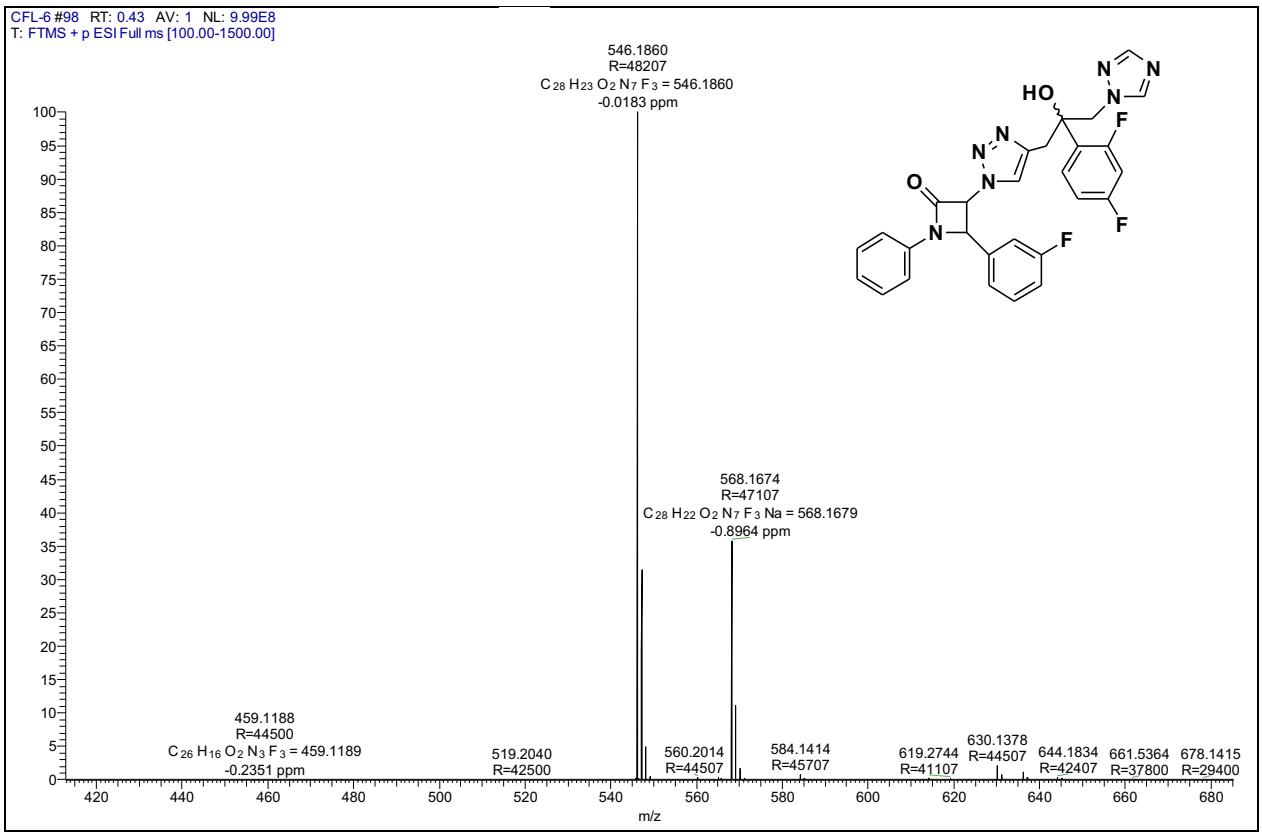
CHLOROFORM-d
Normalized Intensity
Chemical Shift (ppm)
CFL-5newC-13-Thu4av400#013.esp
CHLOROFORM-d
Normalized Intensity
Chemical Shift (ppm)




*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-4-(3-fluorophenyl)-1-phenylazetidin-2-one;* **12f**. Yield: 91%; semi-solid; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl^3$, 400 MHz) δ 7.89-8.01 (m, 1H), 7.73 (d, *J* = 7.82 Hz, 1H), 7.30-7.37 (m, 2H, Ar-H), 7.0-7.24 (m, 7H, Ar-H), 6.84-6.87 (m, 1H, Ar-H), 6.63-6.78(m, 3H), 6.20-6.29 (m, 1H), 5.53-5.56 (m, 1H), 5.26 (bs, 1H), 4.47-4.72 (m, 2H), 3.02-3.43 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.7, 161.2, 158.5, 158.4, 154.3, 151.4, 144.2, 142.4, 136.2, 130.4, 129.8, 129.5, 125.6, 123.2, 117.4, 116.3, 113.8, 111.5, 104.1, 75.1, 74.8, 67.4, 60.4, 56.5, 534.1, 33.9, 29.6, 21.0, 14.1.ESI-MS (m/z): 546.18 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{28}H_{23}F_3N_7O_3$ $[M+H]^+$,546.1860: found: 546.1860.

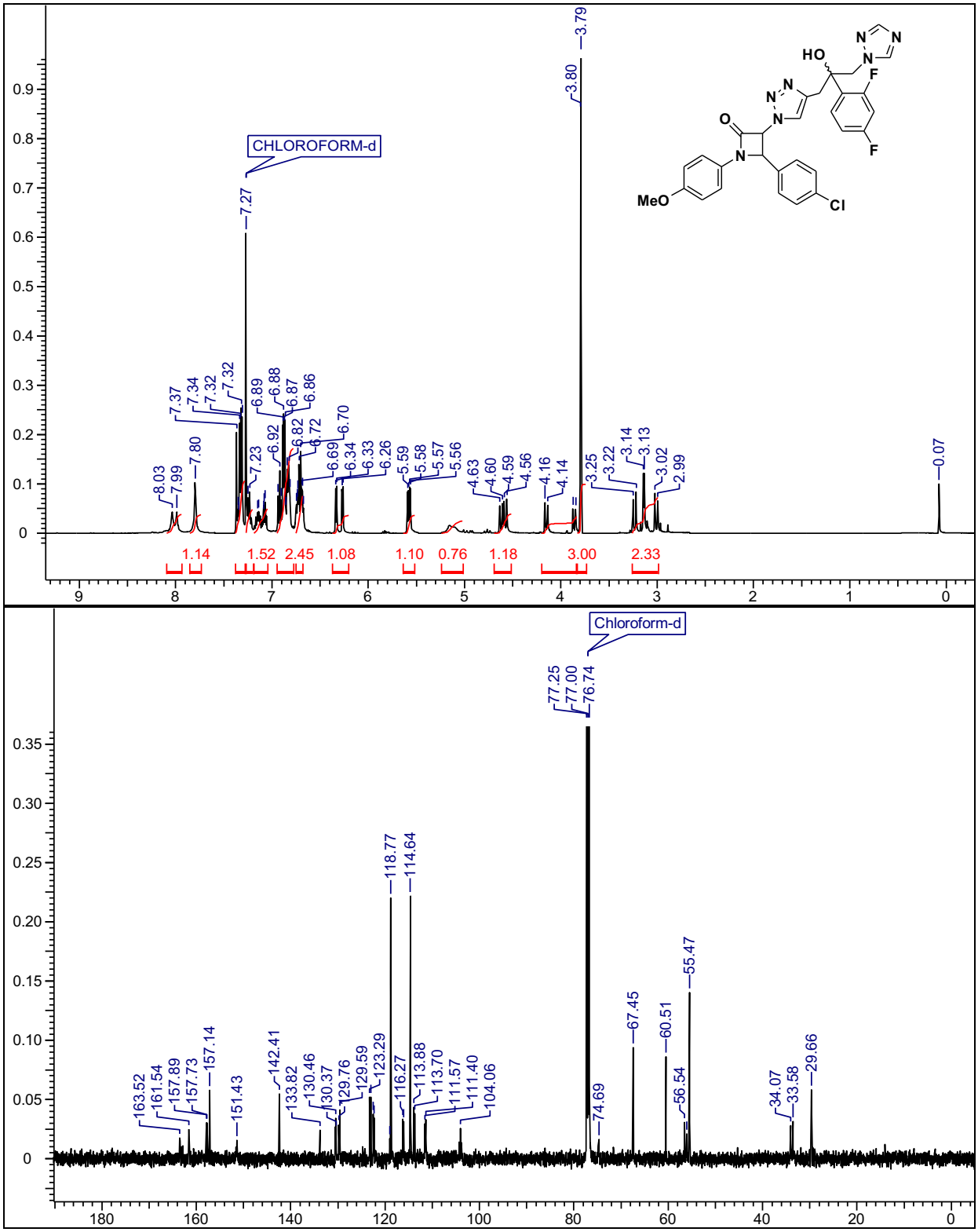
**12f**


DJ-544-CFL-6-Sat1av400#017.esp
CHLOROFORM-d
TMS
Normalized Intensity
Chemical Shift (ppm)
1.57 1.06 2.41 7.68 1.05 3.53 0.98 1.020.77 2.04 2.52
HO
N
F
O




*4-(4-chlorophenyl)-3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)azetidin-2-one;***12g**. Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 500 MHz) δ 7.99-8.03 (m, 1H), 7.80 (s, 1H), 7.31-7.37 (m, 3H, Ar-H), 7.23-7.26 (m, 1H, Ar-H), 7.07-7.15 (m, 1H), 6.81-6.93 (m, 5H), 6.68-6.74 (m, 2H), 6.26-6.34 (m, 1H), 5.56-5.59 (m, 1H), 5.15 (bs, 1H), 4.56-4.63 (m, 1H), 3.87-4.16 (m, 1H), 3.80 (s, 3H), 2.99-3.25 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.5, 161.5, 157.8, 157.1, 151.4, 142.4, 133.8, 130.4, 129.7, 129.5, 123.2, 122.4, 118.7, 114.6, 113.8, 113.7, 111.5, 111.4, 104.0, 74.6, 67.45, 60.5, 66.5, 55.4, 34.0, 33.5, 29.6; ESI-MS (m/z): 592.14 $[M+H]^+$; HRMS (ESI-qTOF): calcd for $C_{29}H_{25}ClF_2N_7O_3$ $[M+H]^+$,592.1670: found: 592.1663.

**12g**


CHLOROFORM-d
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3.79
3.80
HO
N
N
N
N
N
F
F
O
N
MeO
Cl
8.03
7.99
7.80
7.37
7.34
7.32
7.32
7.23
6.92
6.89
6.88
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6.86
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6.70
6.69
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6.33
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5.58
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5.56
4.63
4.60
4.59
4.56
4.16
4.14
3.25
3.22
3.14
3.13
3.02
2.99
0.07
1.14
1.52
2.45
1.08
1.10
0.76
1.18
3.00
2.33
Chloroform-d
77.25
77.00
76.74
74.69
163.52
161.54
157.89
157.73
157.14
151.43
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133.82
130.46
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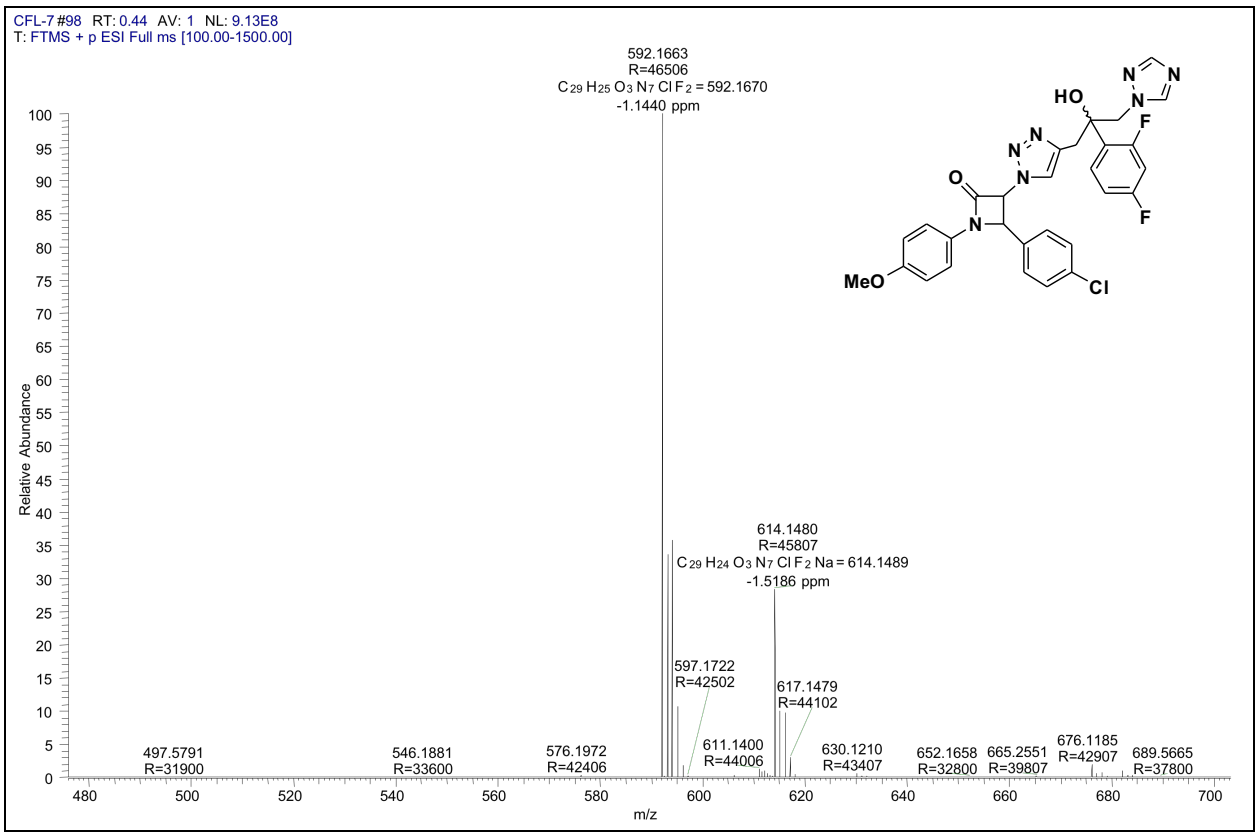
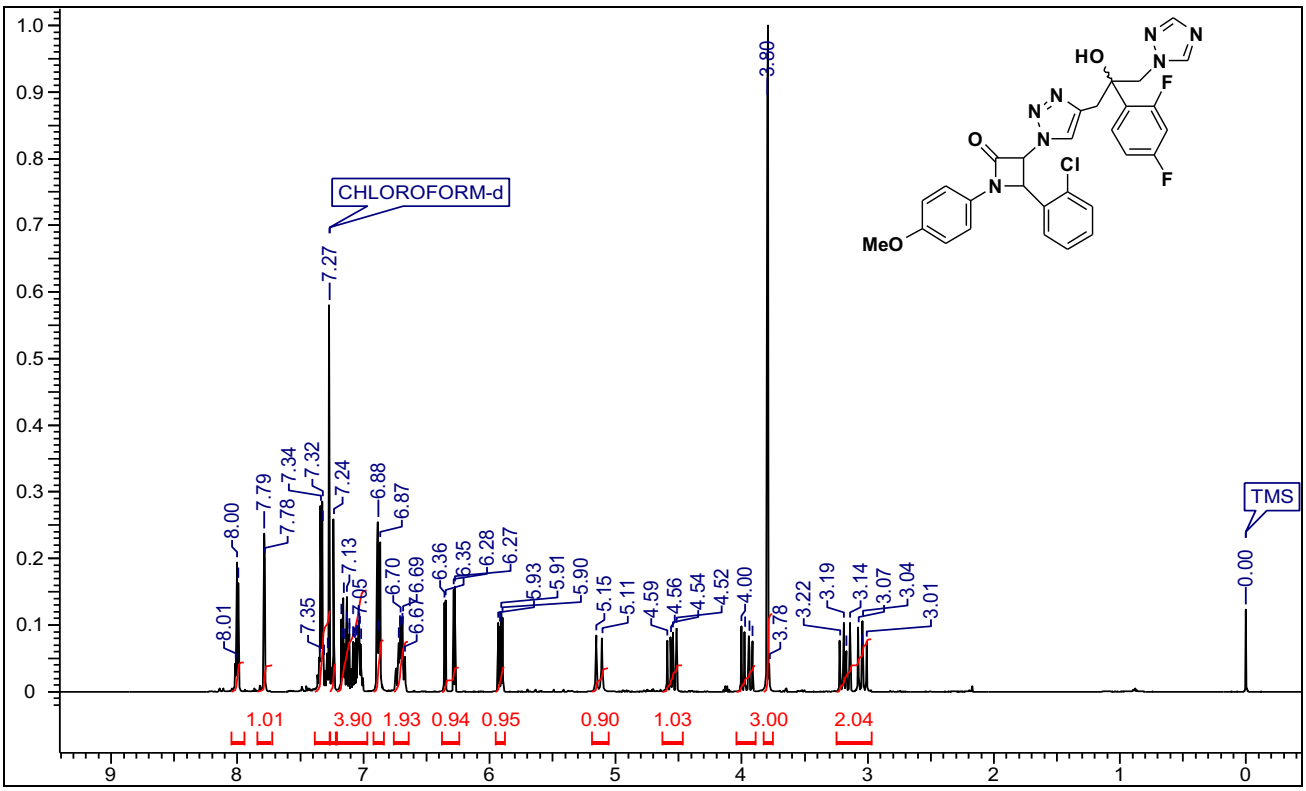
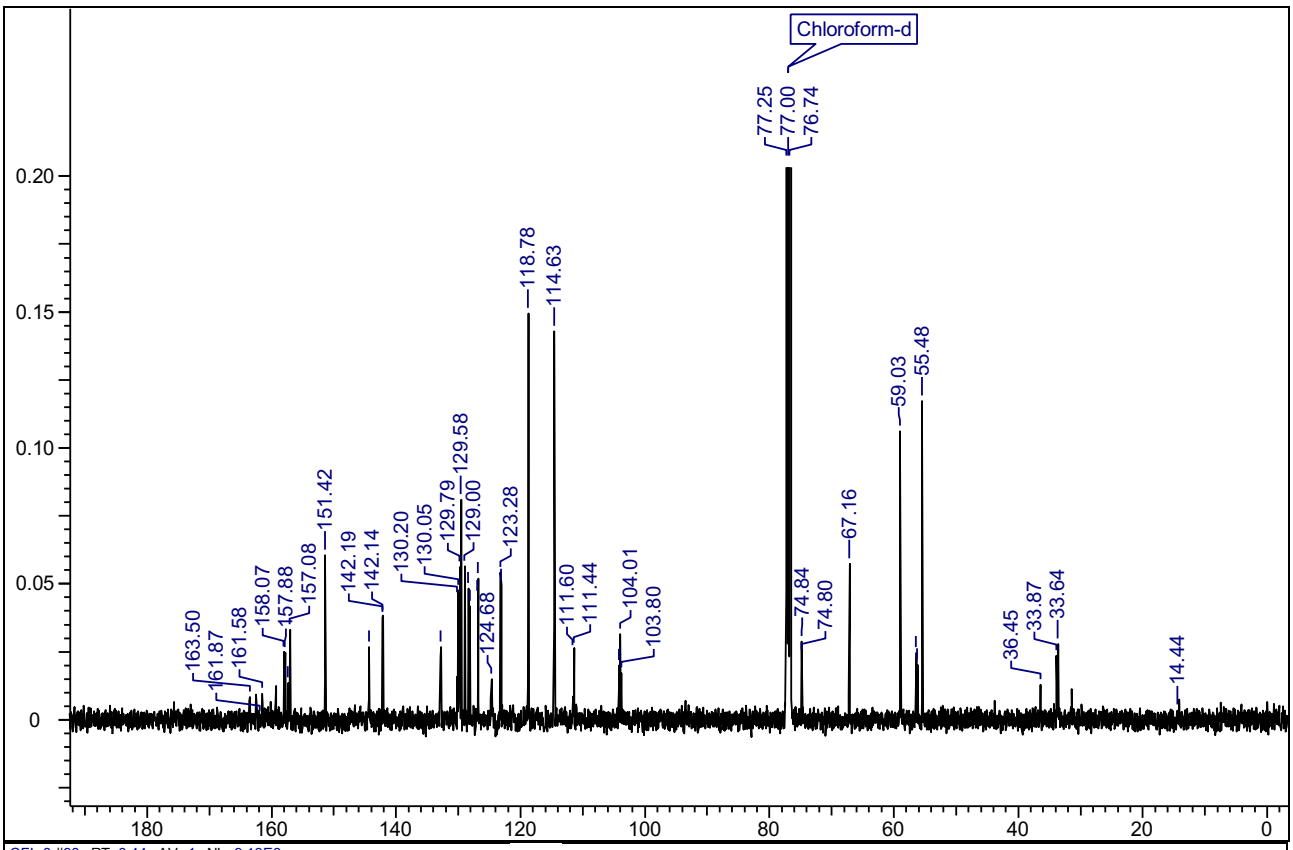
129.76
129.59
123.29
118.77
116.27
114.64
113.88
113.70
111.57
111.40
104.06
67.45
60.51
56.54
55.47
34.07
33.58
29.66

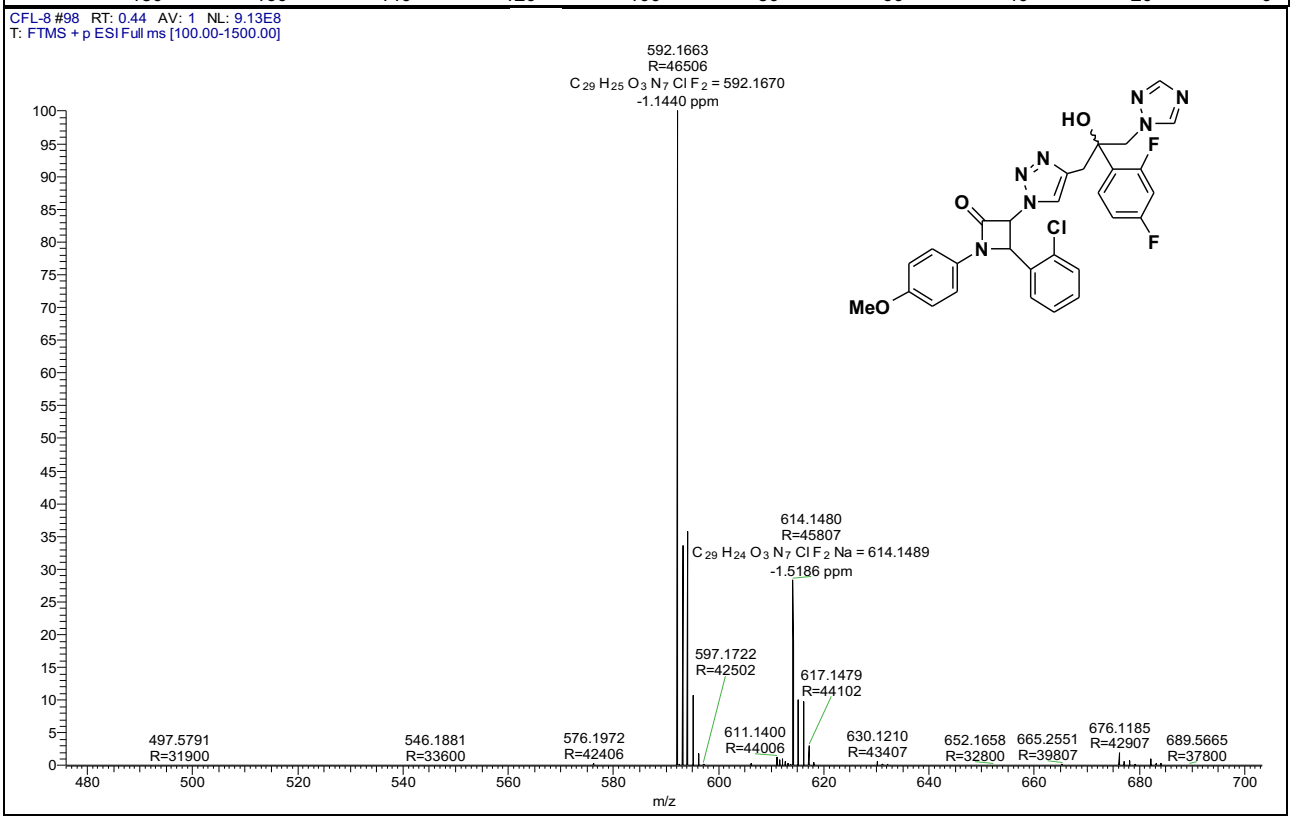



*4-(2-chlorophenyl)-3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)azetidin-2-one;***12h**. Yield: 92%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 500 MHz) δ 7.99-8.01 (m, 1H), 7.79 (s, 1H), 7.28-7.35 (m, 3H, Ar-H), 7.23-7.24 (m, 1H, Ar-H), 7.02-7.17 (m, 4H), 6.87-6.89 (m, 2H), 6.67-6.72 (m, 2H), 6.27-6.36 (m, 1H), 5.90-5.93 (m, 1H), 5.13 (bs, 1H), 4.52-4.59 (m, 1H), 3.91-4.0 (m, 1H), 3.80 (s, 3H), 3.01-3.22 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.5, 161.8, 161.5, 158.0, 157.8, 157.1, 151.4, 142.4, 132.8, 130.0, 129.7, 129.5, 128.4, 123.2, 122.4, 118.7, 114.6, 113.8, 113.7, 111.5, 111.4, 104.0, 74.6, 74.8, 67.45, 59.0, 56.4, 56.2, 36.4, 33.8, 14.4; ESI-MS (m/z): 592.14 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{29}H_{25}ClF_2N_7O_3$ $[M+H]^+$,592.1670: found: 592.1663.

**12h**

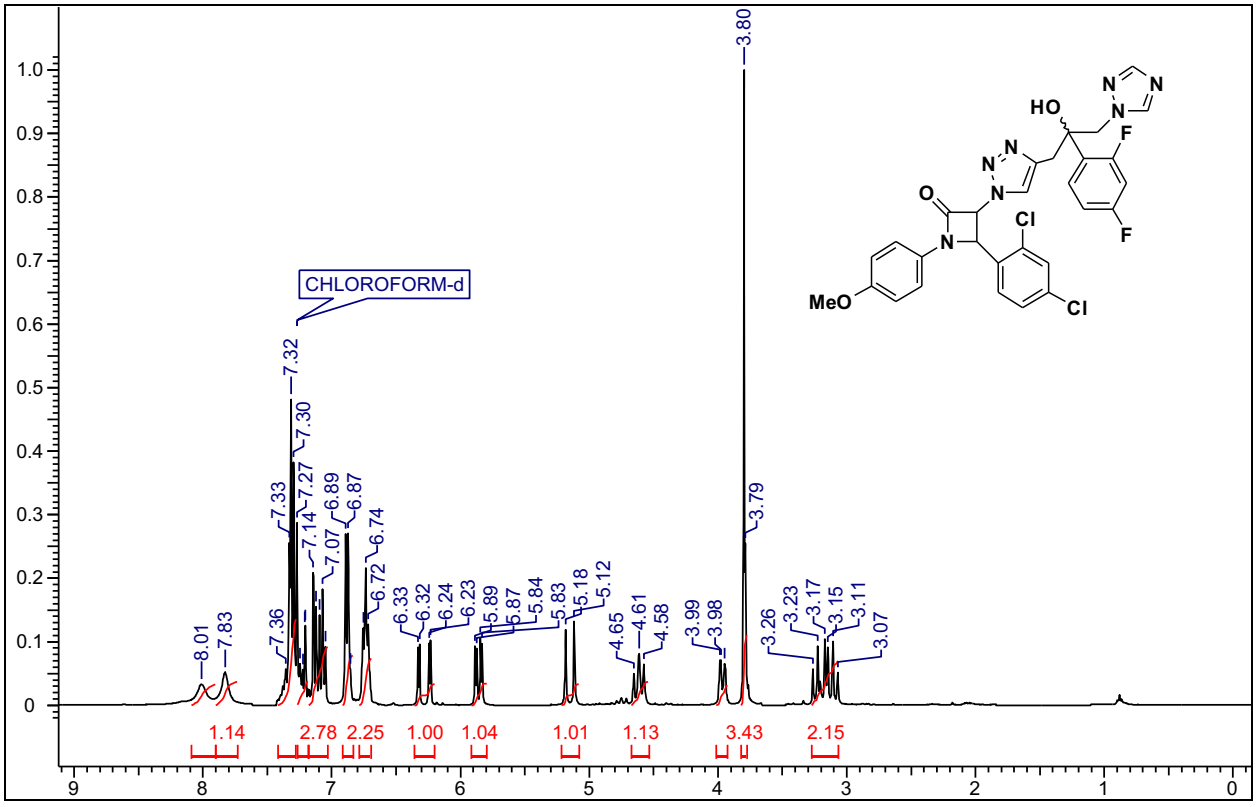

CHLOROFORM-d
TMS
HO
N
N
N
N
N
O
N
Cl
F
F
MeO
8.01
8.00
7.79
7.78
7.35
7.34
7.32
7.27
7.24
7.13
7.05
6.88
6.87
6.70
6.69
6.67
6.36
6.35
6.28
6.27
5.93
5.91
5.90
5.15
5.11
4.59
4.56
4.54
4.52
4.00
3.80
3.78
3.22
3.19
3.14
3.07
3.04
3.01
0.00
1.01
3.90
1.93
0.94
0.95
0.90
1.03
3.00
2.04


Chloroform-d
77.25
77.00
76.74
118.78
114.63
59.03
55.48
129.58
151.42
67.16
129.79
129.00
123.28
142.19
142.14
130.20
130.05
158.07
157.88
157.08
111.60
111.44
104.01
103.80
74.84
74.80
124.68
163.50
161.87
161.58
36.45
33.87
33.64
14.44
0.20
0.15
0.10
0.05
0
180
160
140
120
100
80
60
40
20
0

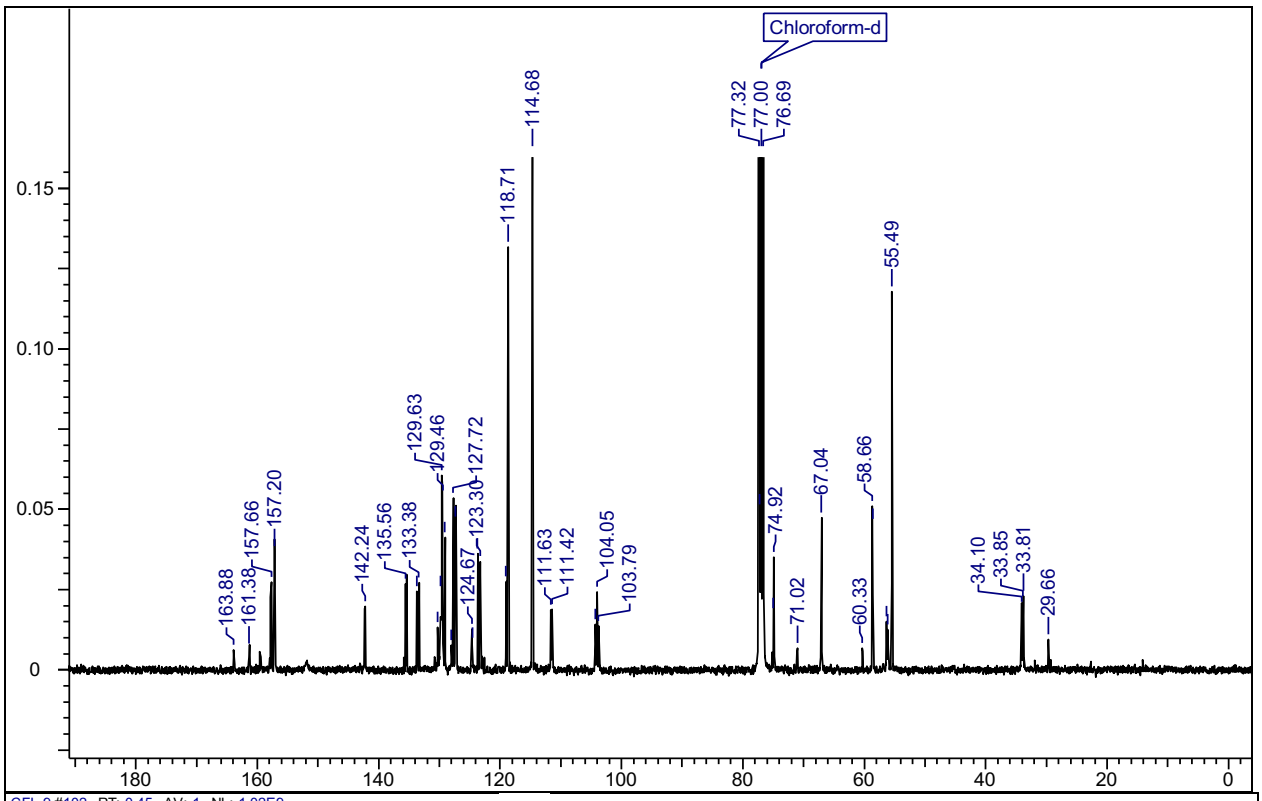

CFL-8 #98 RT: 0.44 AV: 1 NL: 9.13E8
T: FTMS + p ESI Full ms [100.00-1500.00]
592.1663
R=46506
C29 H25 O3 N7 Cl F2 = 592.1670
-1.1440 ppm
614.1480
R=45807
C29 H24 O3 N7 Cl F2 Na = 614.1489
-1.5186 ppm
597.1722
R=42502
617.1479
R=44102
497.5791
R=31900
546.1881
R=33600
576.1972
R=42406
611.1400
R=44006
630.1210
R=43407
652.1658
R=32800
665.2551
R=39807
676.1185
R=42907
689.5665
R=37800
m/z
HO
N
F
O
Cl
MeO

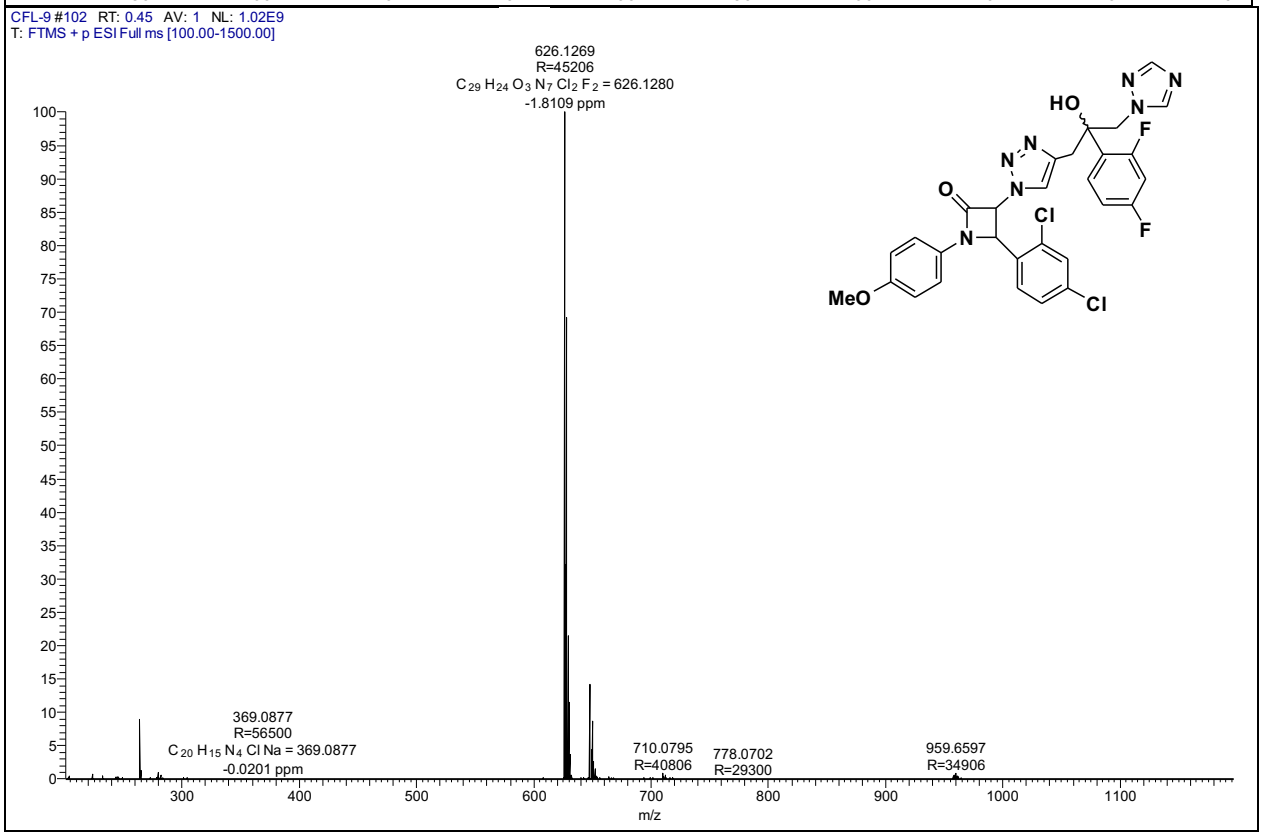
*4-(2,4-dichlorophenyl)-3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)azetidin-2-one;***12i**. Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.01 (s, 1H), 7.83 (s, 1H), 7.30-7.36 (m, 4H, Ar-H), 7.21-7.25 (m, 1H, Ar-H), 7.05-7.14 (m, 2H), 6.86-6.90 (m, 2H), 6.72-6.76 (m, 2H), 6.23-6.33 (m, 1H), 5.83-5.89 (m, 1H), 5.14 (bs, 1H), 4.58-4.65 (m, 1H), 3.95-3.99 (m, 1H), 3.80 (s, 3H), 3.07-3.26 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.8, 161.3, 161.2, 157.8, 157.6, 157.1, 142.2, 165.5, 135.4, 129.7, 129.6, 127.7, 127.2, 118.7, 114.6, 111.6, 111.4, 104.3, 103.7, 74.9, 71.0, 67.0, 60.3, 58.6, 58.5, 55.4, 34.1, 33.8, 29.6; ESI-MS (m/z): 626.14 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{29}H_{24}Cl_2F_2N_7O_3$ $[M+H]^+$,626.1280: found: 626.1269.

**12i**



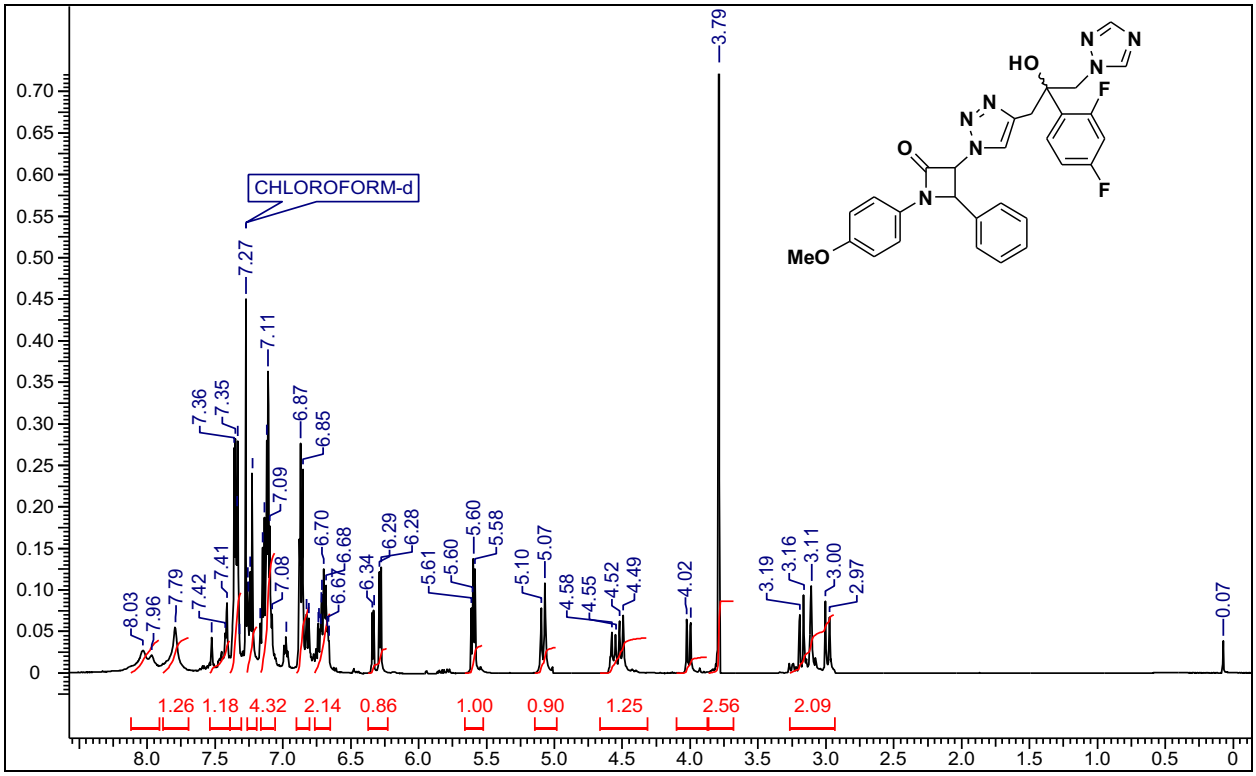

**12i**


Chloroform-d
77.32
77.00
76.69
114.68
118.71
55.49
129.63
129.46
127.72
123.30
67.04
58.66
74.92
142.24
135.56
133.38
111.63
111.42
104.05
103.79
163.88
161.38
157.66
157.20
124.67
71.02
60.33
34.10
33.85
33.81
29.66
0.15
0.10
0.05
0
180
160
140
120
100
80
60
40
20
0

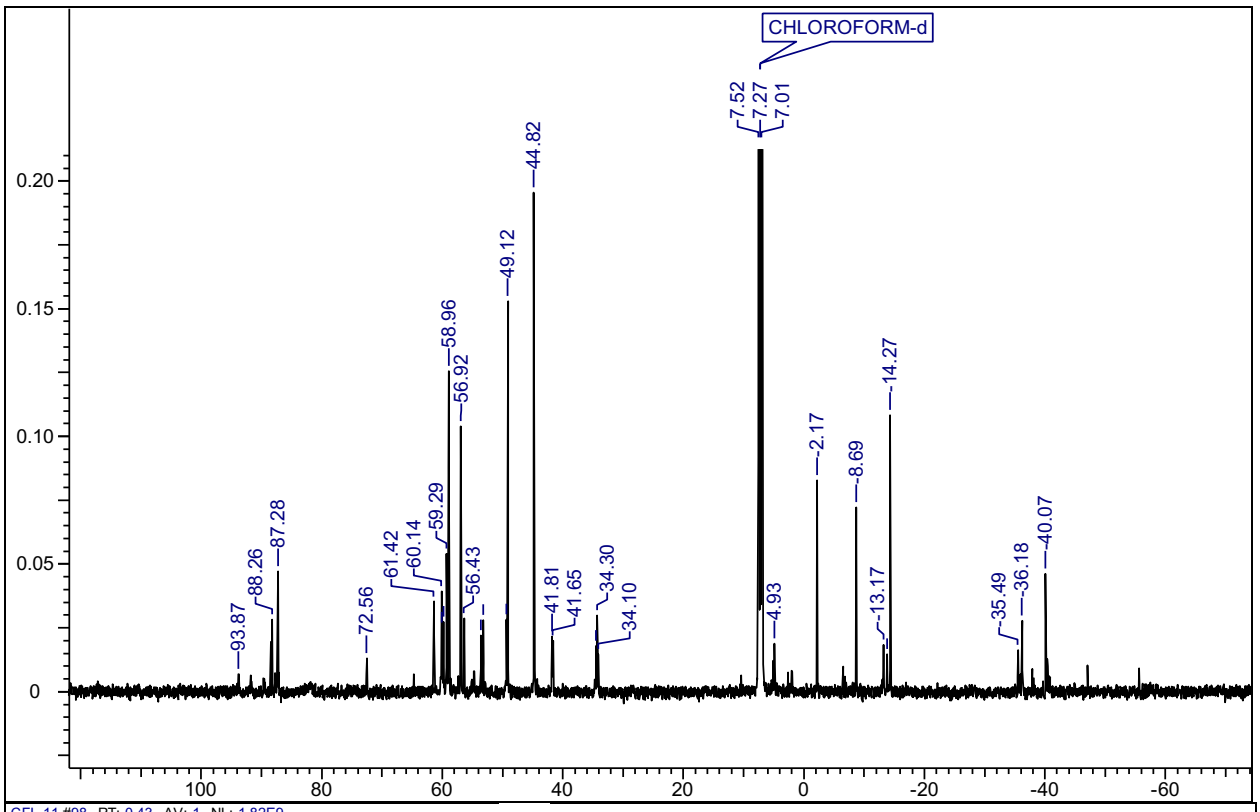

CFL-9 #102 RT: 0.45 AV: 1 NL: 1.02E9
T: FTMS + p ESI Full ms [100.00-1500.00]
626.1269
R=45206
C29 H24 O3 N7 Cl2 F2 = 626.1280
-1.8109 ppm
369.0877
R=56500
C20 H15 N4 Cl Na = 369.0877
-0.0201 ppm
710.0795
R=40806
778.0702
R=29300
959.6597
R=34906
m/z
HO
N
N
N
N
N
F
F
O
N
Cl
Cl
MeO

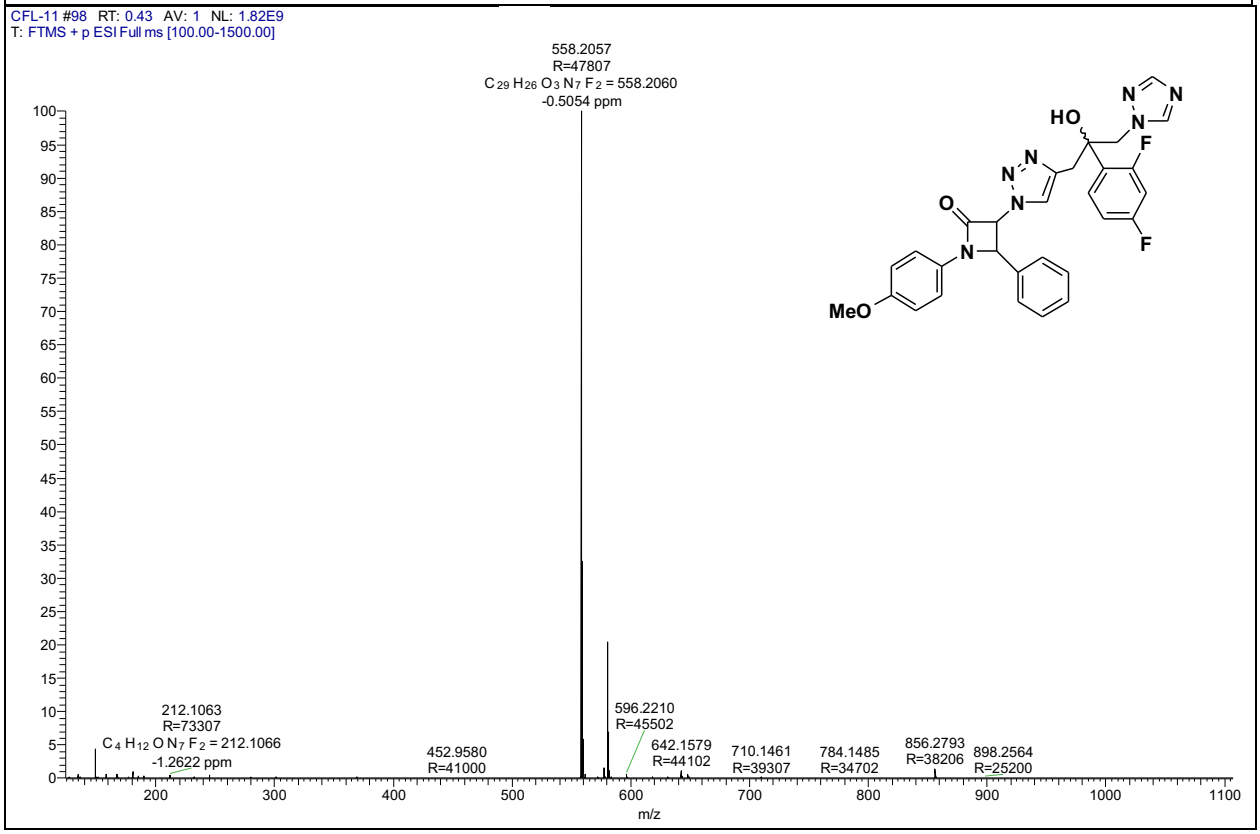
*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one;***12j**. Yield: 90%; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 500 MHz) δ 7.96-8.03 (m, 1H), 7.79 (s, 1H), 7.41-7.52 (m, 1H), 7.33-7.35 (m, 2H, Ar-H), 7.22-7.26 (m, 1H, Ar-H), 7.07-7.16 (m, 4H), 6.80-6.87 (m, 2H), 6.66-6.74 (m, 2H), 6.28-6.34 (m, 1H), 5.58-5.61 (m, 1H), 5.07 (bs, 1H), 4.49-4.58 (m, 1H), 4.0-4.02 (m, 1H), 3.78 (s, 3H), 2.97-3.19 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.6, 158.1, 157.9, 157.0, 142.2, 131.1, 129.8, 129.0, 128.6, 126.6, 126.5, 126.1, 119.0, 118.8, 114.5, 114.4, 111.5, 111.3, 104.0, 103.8, 74.6, 67.5, 61.04, 56.5, 55.9, 55.4, 34.2, 29.6; ESI-MS (m/z): 558.18 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{29}H_{26}F_2N_7O_3$ $[M+H]^+$,558.2060: found: 558.2057.

**12j**



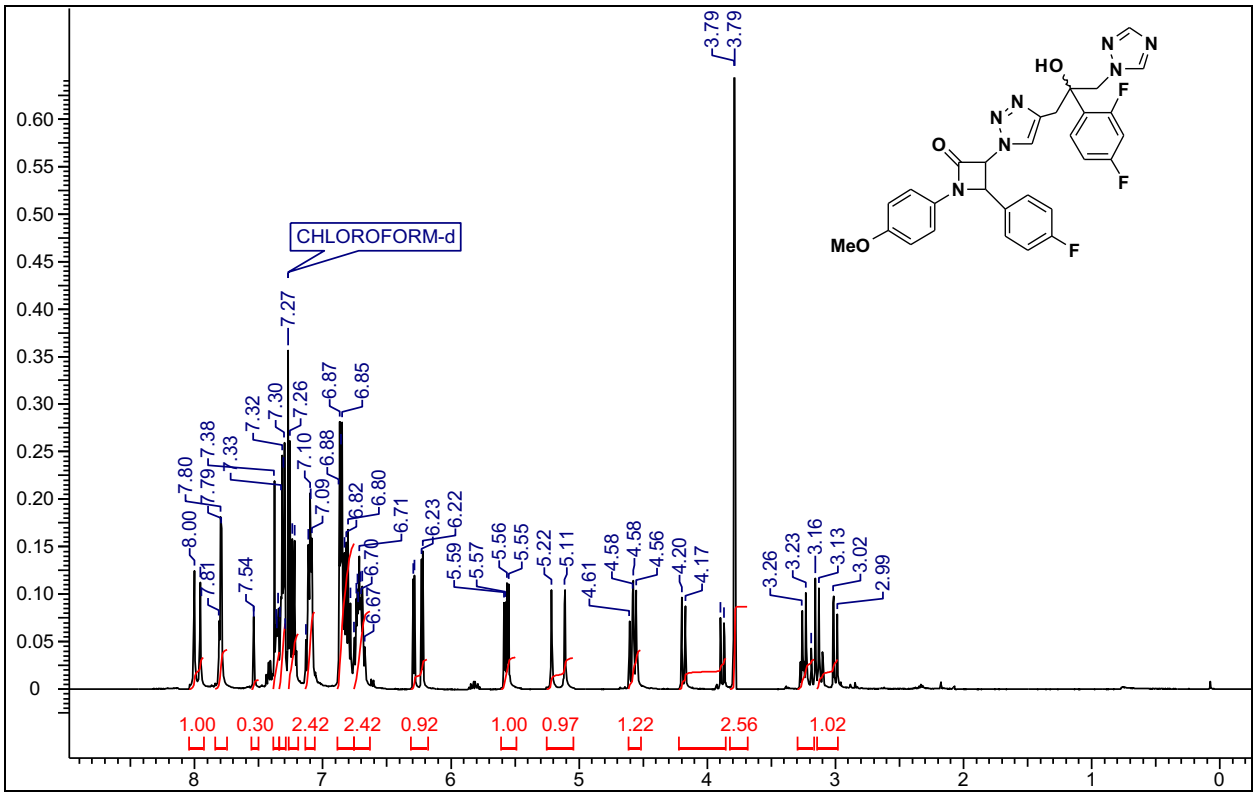

**12j**


CHLOROFORM-d
7.52
7.27
7.01
44.82
49.12
58.96
56.92
59.29
61.42
60.14
56.43
87.28
88.26
93.87
72.56
41.81
41.65
34.30
34.10
4.93
2.17
8.69
14.27
13.17
35.49
36.18
40.07
0.20
0.15
0.10
0.05
0
100
80
60
40
20
0
-20
-40
-60

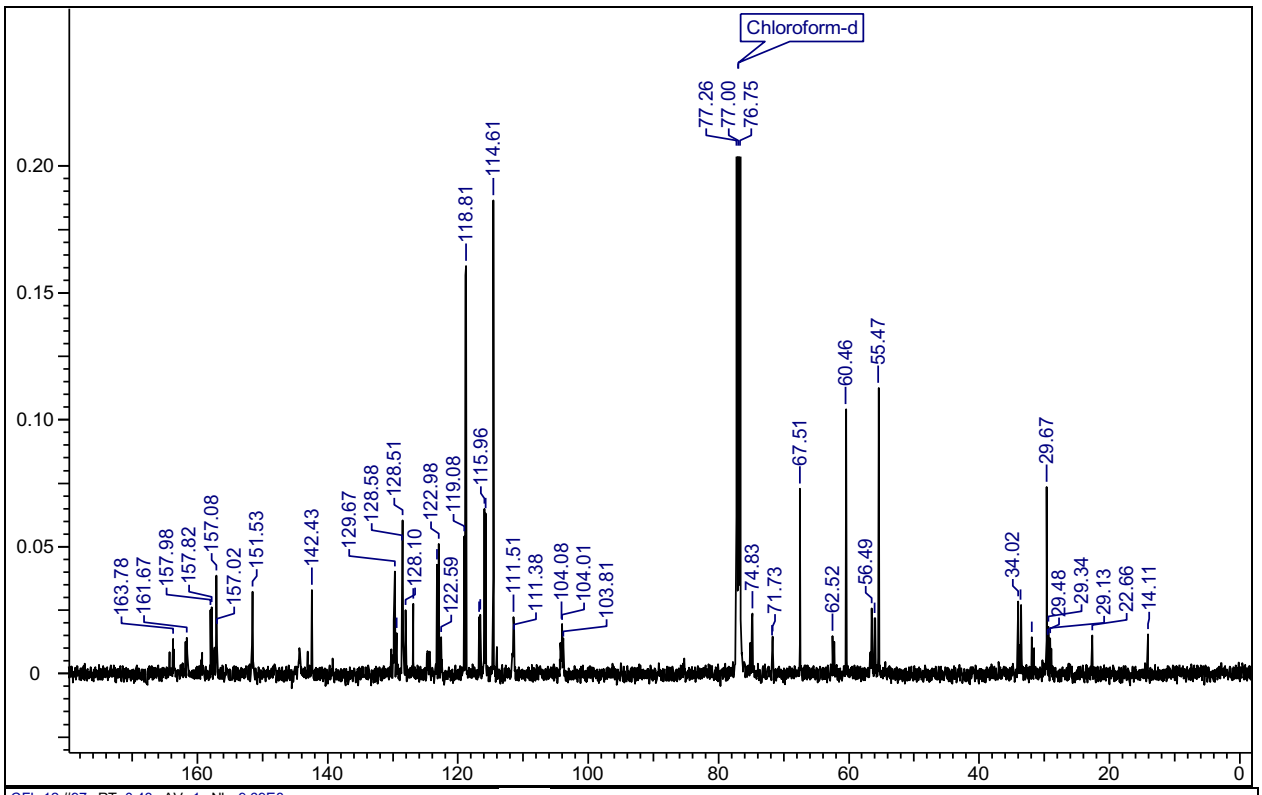

CFL-11 #98 RT: 0.43 AV: 1 NL: 1.82E9
T: FTMS + p ESI Full ms [100.00-1500.00]
558.2057
R=47807
C29 H26 O3 N7 F2 = 558.2060
-0.5054 ppm
212.1063
R=73307
C4 H12 O N7 F2 = 212.1066
-1.2622 ppm
452.9580
R=41000
596.2210
R=45502
642.1579
R=44102
710.1461
R=39307
784.1485
R=34702
856.2793
R=38206
898.2564
R=25200
m/z
HO
N
F
O
MeO

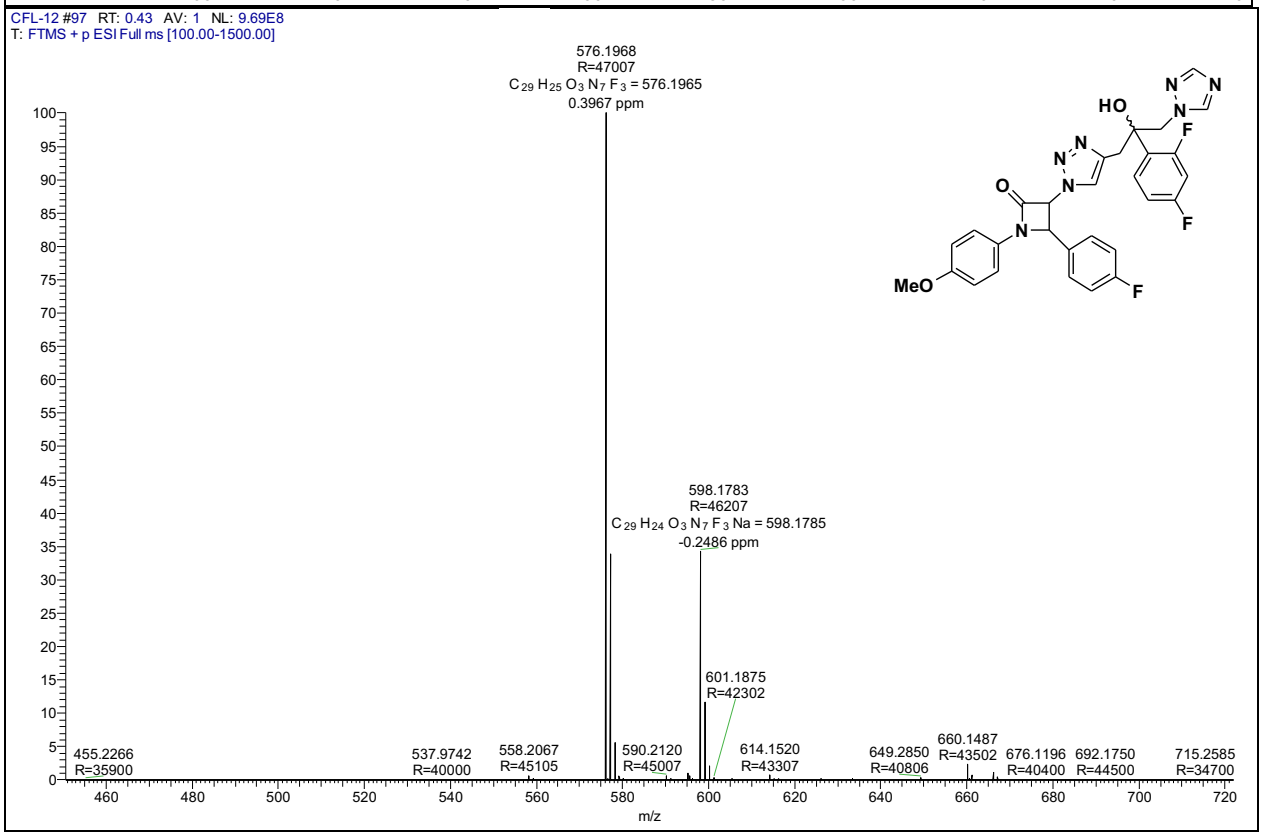
*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-4-(4-fluorophenyl)-1-(4-methoxyphenyl)azetidin-2-one;***12k**. Yield: 93%; semi-solid; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 500 MHz) δ 7.96-8.0 (m, 1H), 7.79-7.81 (m, 1H), 7.35-7.38 (m, 1H), 7.29-7.33 (m, 2H, Ar-H), 7.22-7.26 (m, 2H, Ar-H), 7.09-7.13 (m, 2H), 6.80-6.88 (m, 4H), 6.67-6.78 (m, 2H), 6.22-6.29 (m, 1H), 5.55-5.59 (m, 1H), 5.11 (bs, 1H), 4.56-4.61 (m, 1H), 3.87-4.20 (m, 1H), 3.79 (s, 3H), 3.19-3.26 (m, 1H), 2.99-3.13 (m, 1H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 163.7, 163.6, 161.6, 157.8, 151.5, 142.4, 129.6, 128.5, 126.9, 123.2, 122.9, 119.0, 118.8, 115.9, 115.7, 114.6, 111.5, 104.0, 74.9, 74.8, 67.5, 62.5, 60.4, 56.4, 34.0, 29.6, 14.1; ESI-MS (m/z): 576.19 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{29}H_{25}F_3N_7O_3$ $[M+H]^+$,576.1965: found: 576.1968.

**12k**



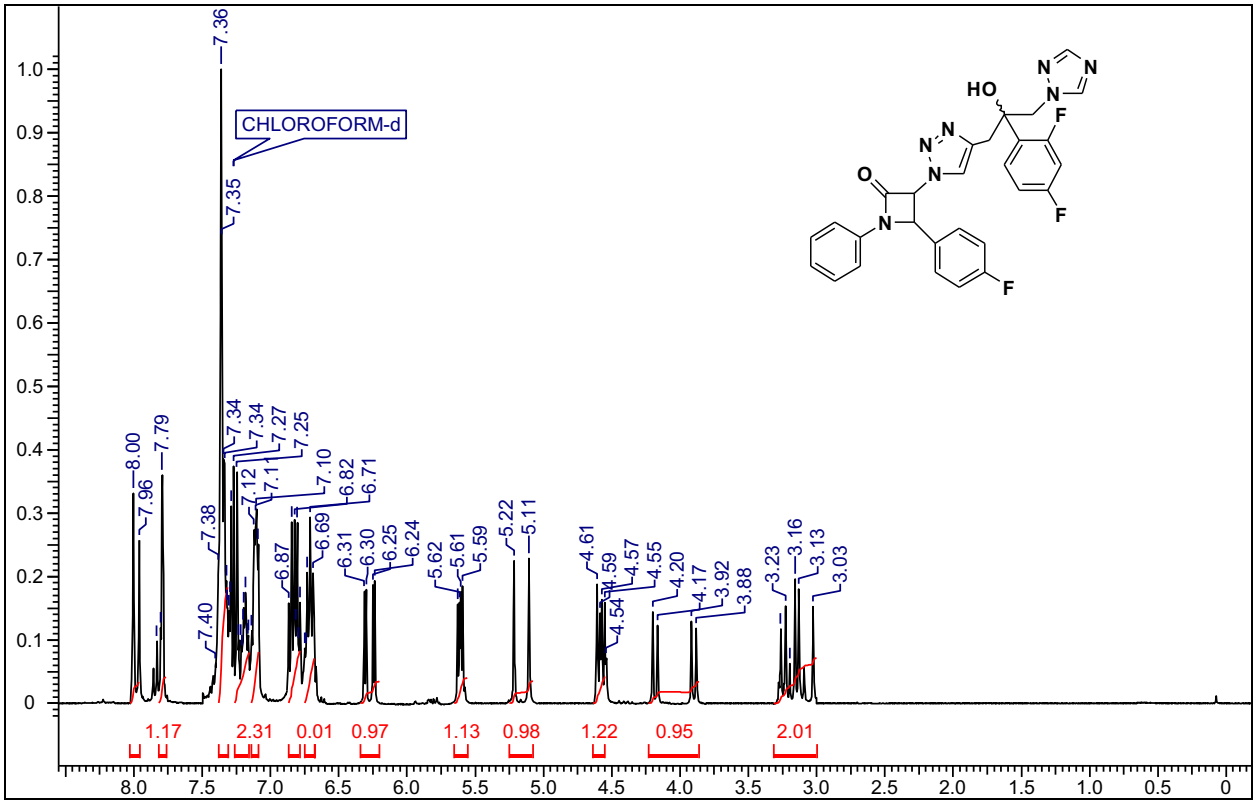

**12k**


Chloroform-d
77.26
77.00
76.75
114.61
118.81
115.96
119.08
122.98
128.51
128.58
129.67
142.43
151.53
157.02
157.08
157.82
157.98
161.67
163.78
122.59
128.10
111.38
111.51
103.81
104.01
104.08
74.83
71.73
67.51
62.52
60.46
56.49
55.47
34.02
29.67
29.48
29.34
29.13
22.66
14.11
0.20
0.15
0.10
0.05
0
160
140
120
100
80
60
40
20
0

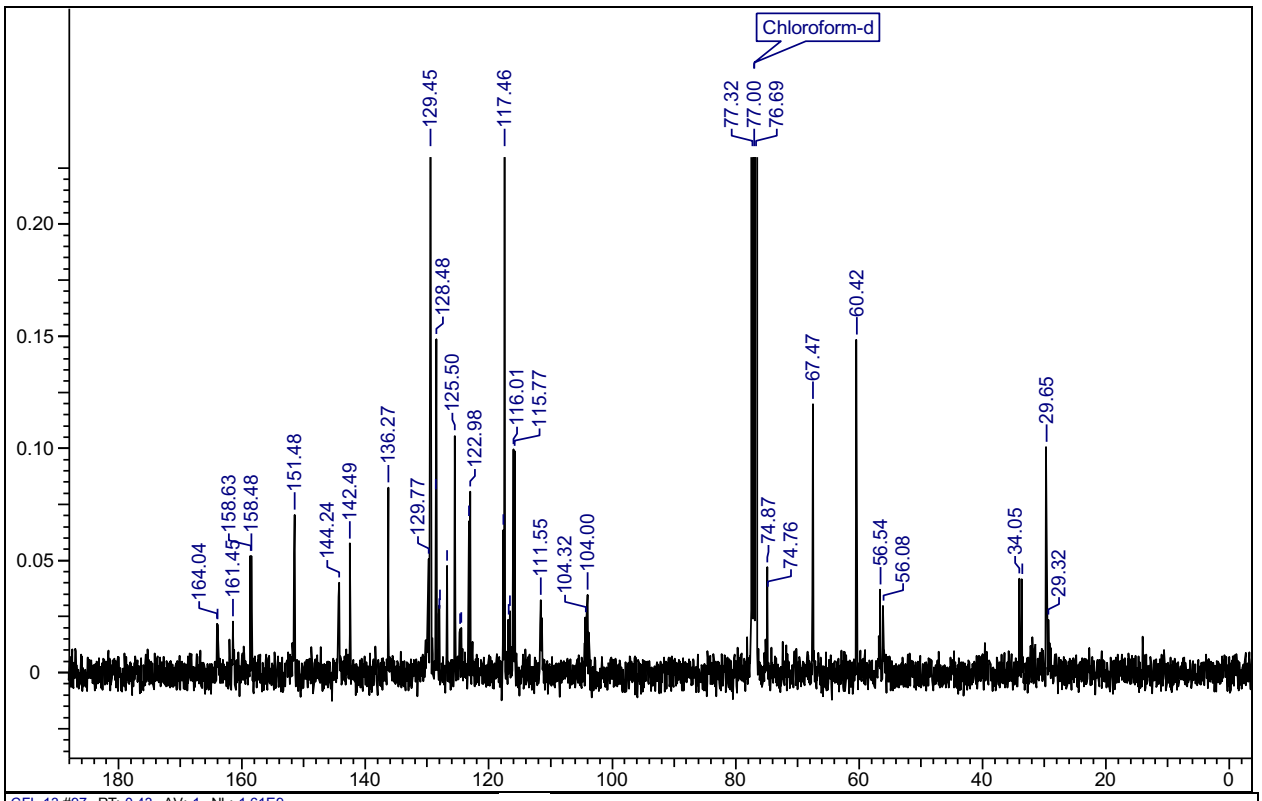

CFL-12 #97 RT: 0.43 AV: 1 NL: 9.69E8
T: FTMS + p ESI Full ms [100.00-1500.00]
576.1968
R=47007
C29 H25 O3 N7 F3 = 576.1965
0.3967 ppm
598.1783
R=46207
C29 H24 O3 N7 F3 Na = 598.1785
-0.2486 ppm
601.1875
R=42302
455.2266
R=35900
537.9742
R=40000
558.2067
R=45105
590.2120
R=45007
614.1520
R=43307
649.2850
R=40806
660.1487
R=43502
676.1196
R=40400
692.1750
R=44500
715.2585
R=34700
m/z
HO
N
F
O
MeO

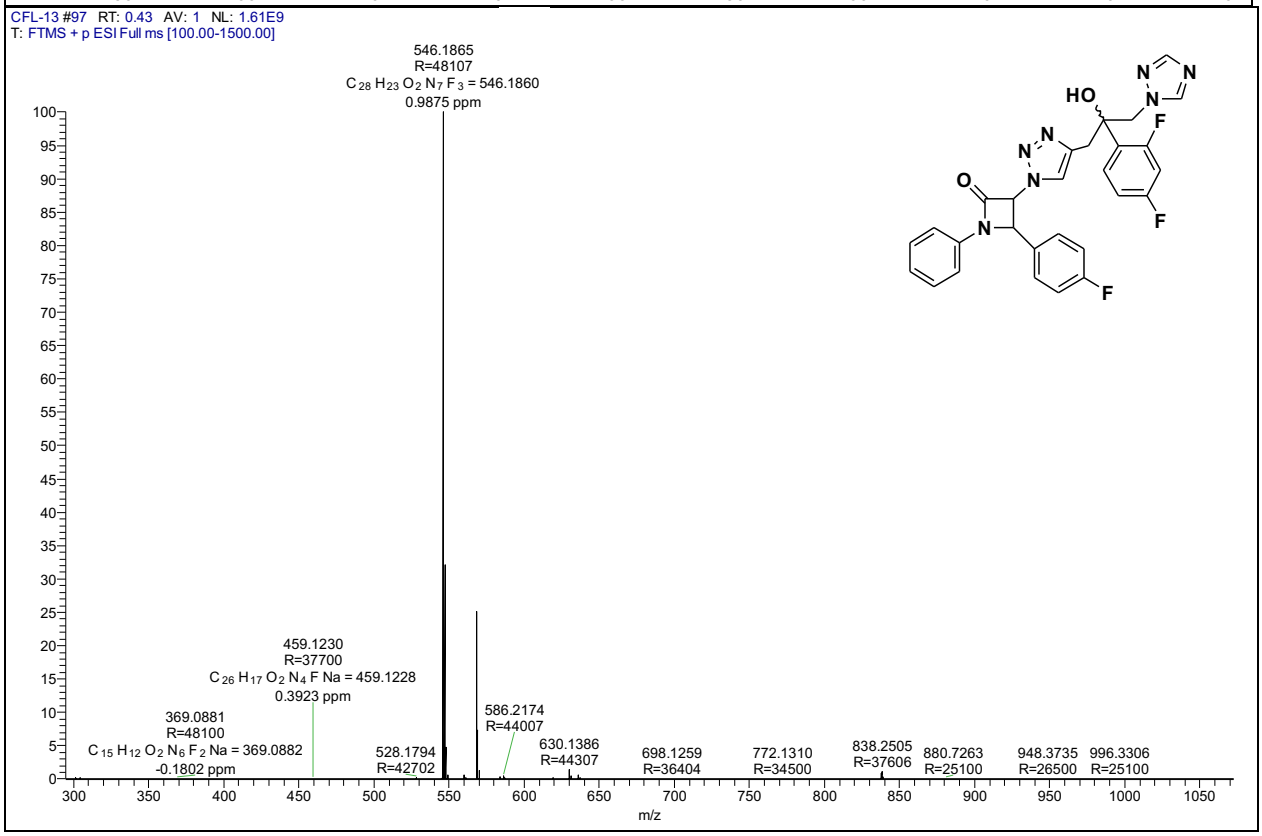
*3-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)-4-(4-fluorophenyl)-1-phenylazetidin-2-one;* **12l**. Yield: 90%; semi-solid; IR ($CHCl_3$, $cm^{-1}$) 3415, 1761; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.96-8.0 (m, 1H), 7.79-7.83 (m, 1H), 7.32-7.40 (m, 5H, Ar-H), 7.20-7.25 (m, 2H, Ar-H), 7.09-7.18 (m, 2H, Ar-H), 6.78-6.87 (m, 2H), 6.69-6.73 (m, 2H), 6.24-6.31 (m, 1H), 5.59-5.63 (m, 1H), 5.11-5.22 (m, 1H), 4.54-4.61 (m, 1H), 3.88-4.20 (m, 1H), 3.03-3.26 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 164.0, 163.9, 161.4, 158.6, 158.4, 151.4, 144.2, 142.4, 136.2, 129.7, 129.4, 128.4, 125.5, 122.9, 117.4, 116.0, 115.7, 111.5, 104.3, 104.0, 74.92, 74.8, 74.7, 67.4, 60.4, 56.5, 34.0, 33.6, 29.3. ESI-MS (m/z): 546.18 $[M+H]^+$ ; HRMS (ESI-qTOF): calcd for $C_{28}H_{23}F_3N_7O_2$ $[M+H]^+$,546.1860: found: 546.1865.

**12l**




**12l**


Chloroform-d
129.45
117.46
77.32
77.00
76.69
128.48
125.50
122.98
116.01
115.77
67.47
60.42
136.27
151.48
142.49
129.77
144.24
158.63
158.48
164.04
161.45
111.55
104.32
104.00
74.87
74.76
56.54
56.08
34.05
29.65
29.32
0.20
0.15
0.10
0.05
0
180
160
140
120
100
80
60
40
20
0


CFL-13 #97 RT: 0.43 AV: 1 NL: 1.61E9
T: FTMS + p ESI Full ms [100.00-1500.00]
546.1865
R=48107
C 28 H 23 O 2 N 7 F 3 = 546.1860
0.9875 ppm
459.1230
R=37700
C 26 H 17 O 2 N 4 F Na = 459.1228
0.3923 ppm
369.0881
R=48100
C 15 H 12 O 2 N 6 F 2 Na = 369.0882
-0.1802 ppm
528.1794
R=42702
586.2174
R=44007
630.1386
R=44307
698.1259
R=36404
772.1310
R=34500
838.2505
R=37606
880.7263
R=25100
948.3735
R=26500
996.3306
R=25100
m/z
HO
N
F
O

## HPLC Data of compound 12j

D-7000 HPLC System Manager Report

Analyzed: 10/27/15 04:16 AM

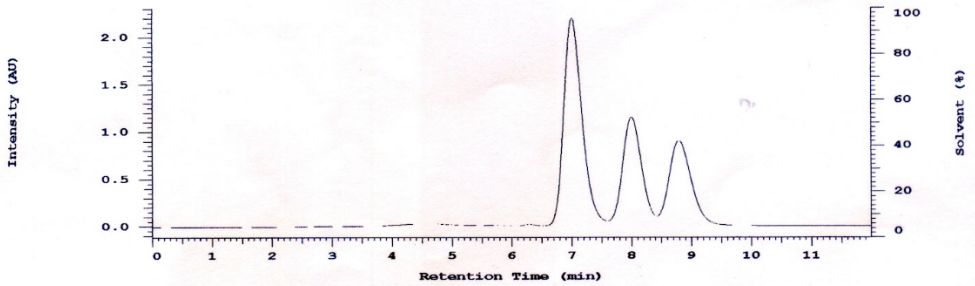
Reported: 10/30/15 12:51 PM
Processed: 10/30/15 12:50 PM

Data Path: C:\WIN32APP\HSM\HPLC\DATA\8560\
Processing Method: SANTOSHH

System(acquisition): Sys 1
Application: HPLC
Sample Name: DJ=1
Injection from this vial: 1 of 1
Sample Description: MEOH:H2O (80:20)

Series:8560
Volume: 10.0 ul

Chrom Type: HPLC Channel : 1




| No. | RT | Height | Area | Area % |
|---|---|---|---|---|
| 1 | 6.29 | 8593 | 101643 | 0.211 |
| 2 | 7.01 | 1094569 | 23573347 | 48.831 |
| 3 | 8.00 | 573295 | 12845485 | 26.609 |
| 4 | 8.79 | 449741 | 11754985 | 24.350 |
| | | 2126198 | 48275460 | 100.000 |

Peak rejection level: 0

```
Group Leader   :-Dr.V.S.Pore
COLUMN         :-Chiralcel OD-R(150 X 4.6mm)
MOBILE PHASE   :-MEOH:H2O:(90:10)
WAVELENGTH     :- 254nm
FLOW RATE      :- 0.5 ml/min (1160 psi)
SAMPLE CONC    :-X mg/1ml Injection vol:20ul
```

**Reference**


1 R.A. Friesner et. al, *J. Med. Chem*., 2006,**49**, 6177.

2 T. A. Halgrenet.al.,*J. Med. Chem.,* 2004, **47**, 1750.

3 R.A. Friesner et. al, *J. Med. Chem*., 2004, **47**, 1739.

4 T. Mosmann, *J. Immunol. Methods,* 1983, **65**, 55.

5 G. Ciapetti, E. Cenni, L. Pratelli, A. Pizzoferrato, *Biomaterials,*1993, **14**, 359.