Supporting information

Synthesis and biological evaluation of 4′-[(benzimidazol-1-yl)methyl] biphenyl-2-amides as dual angiotensin II and endothelin A receptor antagonists

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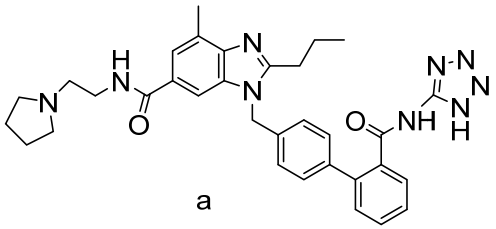
1. General method for preparation and data of compounds (1a-1w)

All reagents were supplied by Acros Organics, Aldrich, Avocado Scientific, BDH, Fischer, Lancaster, Merck, and VWR, and were used with no further purification. TLC was performed on silica gel 60 F254 plates (Merck). Melting points were uncorrected and were determined on a Stuart Scientific apparatus or hot stage microscope (Reichert-Austria). The proton magnetic resonance ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 400 MHz and Bruker APX400 spectrometer at 400 MHz in the specified solvent. Chemical shifts were reported on the d (delta) scale and were related to that of the solvent and J values are given in Hz. Mass spectra were recorded on Finnigan MAT, SSQ 7000, Mass spectrometer, at Q-Tof Micro mass spectrometer (ESI). All mass spectra were recorded in ESI mode unless otherwise stated.

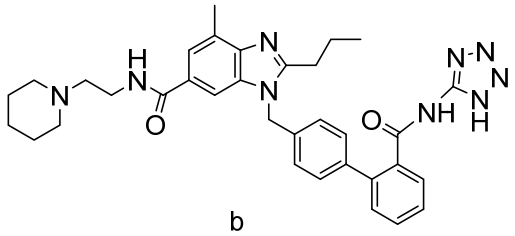


a

4'-[[6-(N-2-pyrrolidin-1-ylethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1, 1'-biphenyl]-2-N-(1H-tetrazol-5-yl) amide **(1a)**.

4'-[[6-(-N-2-pyrrolidin-1-yl)ethylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]biphenyl -2-carboxylic acid (7a). (178 mg, 0.3 mmol) and 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (152 mg, 0.4 mmol) were stirred in dry DMF (5 mL) for 1 h. Anhydrous 5-aminotetrazole (31 mg, 0.4 mmol) was then added and the mixture stirred for 2h. After cooling and filtration, the DMF was removed in vacuo and the solid residue dissolved in 2 N ammonium solution with gentle heating. Undissolved solid was removed by filtration, and the cooled filtrate was acidified to pH= 1 with concentrated HCl. The solid product was collected, boiled with 90% HCOOH (5 min), and finally recrystallized from DMF-H_2O to give 68.4 mg (56.1 %). m.p. 105 ~ 107℃。^{1}H NMR (400MHz, DMSO) δ: 0.96 (t, J = 7.23 Hz, 3H), 1.58 (t, J = 7.19 Hz, 4H), 1.74 (m, 2H), 2.26 (t, J = 7.17 Hz, 4H), 2.55 (s, 3H), 2.86 (t, J = 7.16 Hz, 4H), 3.51 (m, 2H), 5.56 (s, 2H), 7.09 ~ 7.91 (m, 10H), 8.43 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.1, 16.7, 20.9, 25.8, 29.1, 38.6, 46.7, 47.2, 53.4, 109.8, 123.7, 124.9, 127.9, 128.9, 129.1, 129.2, 130.2, 133.04, 133.3, 135.1, 135.7, 138.3, 138.8, 141.8, 143.1, 156.5, 165.1, 170.1; MS (ESI), m/z: 591.3 (M+H); Anal.

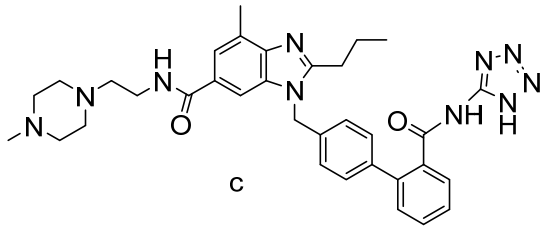
Calcd. for ($C_{33}H_{37}N_9O_2$) : C, 67.01; H, 6.29; N, 21.32; Found: C, 67.00; H, 6.29; N, 21.31.



b

4'-[[6-(N-2-piperidin-1-ylethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1b)**.

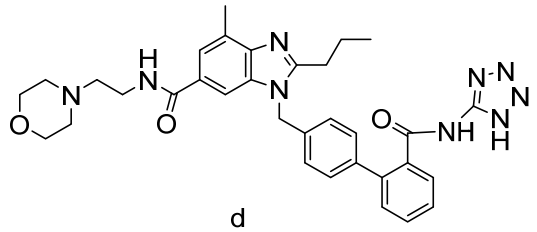
Light yellow solid (65.4 mg, 53.6 %), m.p. 107-108℃, ^{1}H NMR (400MHz, DMSO) δ: 0.94 (t, J = 7.24 Hz, 3H), 1.58 (m, 6H), 1.75 (m, 2H), 2.40 (t, J = 7.19 Hz, 4H), 2.55 (s, 3H), 2.78 (t, J = 7.22 Hz, 4H), 3.49 (m, 2H), 5.55 (s, 2H), 7.10 ~ 7.94 (m, 10H), 8.45 (s, 1H), 11.03 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 13.4, 16.0, 20.2, 25.5, 27.3, 28.3, 38.0, 45.9, 47.6, 53.4, 109.1, 123.0, 124.2, 127.2, 128.2, 128.4, 128.4, 129.5, 132.3, 132.7, 134.3, 135.0, 137.6, 138.0, 141.1, 142.5, 155.8, 164.4, 169.4; MS (ESI), m/z: 605.3 (M+H); Anal. Calcd. for ($C_{34}H_{39}N_9O_2$) : C, 67.45; H, 6.49; N, 20.79; Found: C, 67.44; H, 6.48; N, 20.79.



c

4'-[[6-(N-2-(4-methylpiperazin-1-yl)ethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1c)**.

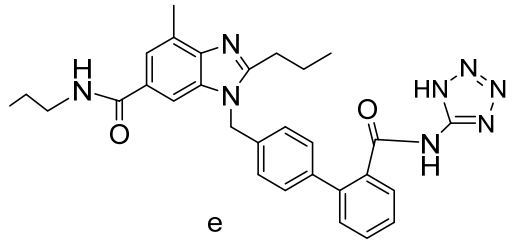
Light yellow solid (58.3 mg,47.8 %), m.p. 101-103℃, ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.20 Hz, 3H), 1.74 (m, 2H), 2.30 (s, 3H), 2.46 (t, J = 7.25 Hz, 4H), 2.56 (s, 3H), 2.67 (t, J = 7.22 Hz, 4H), 2.79 (t, J = 7.16 Hz, 4H), 3.46 (m, 2H), 5.56 (s, 2H), 7.06 ~ 7.91 (m, 10H), 8.44 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 13.4, 16.1, 20.3, 28.4, 38.2, 43.2, 46.0, 49.5, 52.4, 53.1, 109.2, 123.0, 124.3, 127.2, 128.3, 128.5, 128.5, 129.5, 132.4, 132.7, 134.4, 135.1, 137.7, 138.1, 141.2, 142.5, 155.9, 164.5, 169.5; MS (ESI), m/z: 620.3 (M+H); Anal. Calcd. for $C_{34}H_{40}N_{10}O_2$: C, 65.74; H, 6.50; N, 22.54; Found: C, 65.73; H, 6.50; N, 22.53.



d

4'-[[6-(N-2-morpholinoethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1d)**.

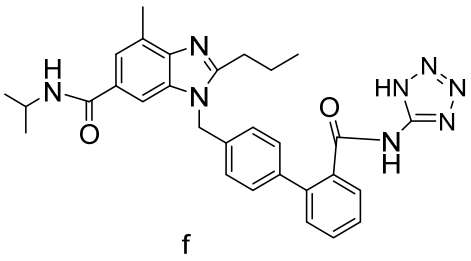
Light yellow solid (48.8 mg, 40%), m.p. 103-105℃, ^{1}H NMR (400MHz, DMSO) δ: 0.94 (t, J = 7.18 Hz, 3H), 1.73 (m, 2H), 2.36 (t, J = 7.21 Hz, 4H), 2.55 (s, 3H), 2.79 (m, 4H), 3.45 (m, 2H), 3.67 (m, 4H), 5.55 (s, 2H), 7.09 ~ 7.93 (m, 10H), 8.45 (s, 1H), 11.01 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 13.8, 16.5, 20.6, 28.8, 38.2, 46.4, 49.5, 53.5, 68.6, 109.5, 123.4, 124.7, 127.6, 128.6, 128.9, 128.9, 129.9, 132.8, 133.1, 134.8, 135,5, 138.1, 138.5, 141.6, 142.9, 156.3, 164.8, 169.9; MS (ESI), m/z: 607.3 (M+H); Anal. Calcd. for $C_{33}H_{37}N_9O_3$: C, 65.18; H, 6.14; N, 20.74; Found: C, 65.16; H, 6.14; N, 20.73.



e

4'-[(6-N-n-propylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1e)**.

Light yellow solid (70.2 mg, 57.5%), m.p. 113 ~ 115℃, ^{1}H NMR (400MHz, DMSO) δ: 0.91 (t, J = 7.23 Hz, 3H), 0.96 (t, J = 7.23 Hz, 3H), 1.58 (m, 2H), 1.75 (m, 2H), 2.55 (s, 3H), 2.77 (t, J = 7.16 Hz, 2H), 3.20 (t, J = 7.24 Hz, 2H), 5.55 (s, 2H), 7.07 ~ 7.89 (m, 10H), 8.45 (s, 1H), 11.08 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 12.0, 14.3, 17.0, 21.0, 29.2, 41.5, 46.2, 107.6, 121.6, 126.6, 127.6, 127.8, 128.6, 128.7, 129.3, 130.5, 135.1, 136.5, 136.7, 138.9, 144.1, 157.0, 167.0; MS (ESI), m/z: 536.3 (M+H); Anal. Calcd. for $C_{30}H_{32}N_8O_2$: C, 67.19; H, 6.01; N, 20.88; Found: C, 67.18; H, 6.01; N, 22.87.

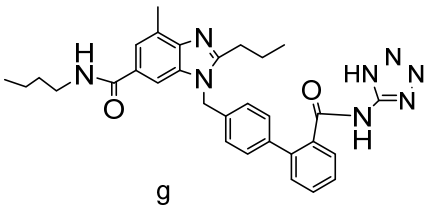


f

4'-[(6-N-iso-propylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]

-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1f)**.

Light yellow solid (66.8 mg, 54.8%), m.p. 111 ~ 113℃. ^{1}H NMR (400MHz, DMSO) δ: 0.94 (t, J = 7.20 Hz, 3H), 1.25 (d, J = 7.23 Hz, 6H), 1.76 (m, 2H), 2.55 (s, 3H), 2.79 (t, J = 7.16 Hz, 2H), 3.95 (m, 1H), 5.56 (s, 2H), 7.08 ~ 7.89 (m, 10H), 8.44 (s, 1H), 11.03 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.2, 16.9, 21.0, 22.9, 29.2, 46.2, 49.1, 107.7, 121.6, 126.6, 127.6, 127.7, 128.6, 128.8, 129.4, 130.3, 130.5, 135.1, 136.5, 138.9, 139.6, 144.1, 157.0, 166.2, 168.4; MS (ESI), m/z: 536.3 (M+H); Anal. Calcd. for $C_{30}H_{32}N_8O_2$: C, 67.13; H, 6.01; N, 20.88; Found: C, 67.11; H, 6.01; N, 20.87.

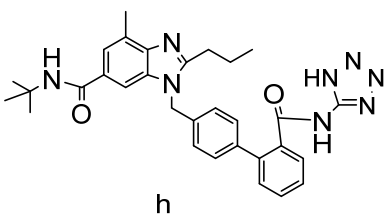


g

4'-[(6-N-n-butylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]

-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1g)**.

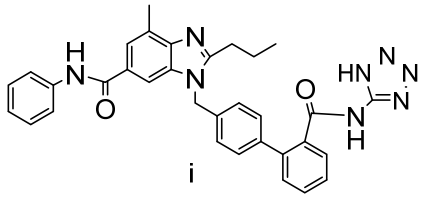
Light yellow solid (72.2 mg, 59.2%), m.p. 116 ~ 118℃. ^{1}H NMR (400MHz, DMSO) δ: 0.91 (t, J = 7.24 Hz, 3H), 0.95 (t, J = 7.25 Hz, 3H), 1.32 (m, 2H), 1.59 (m, 2H), 1.75 (m, 2H), 2.56 (s, 3H), 2.81 (t, J = 7.16 Hz, 2H), 3.53 (m, 2H), 5.55 (s, 2H), 7.11 ~ 7.92 (m, 10H), 8.45 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.2, 14.3, 17.0, 21.0, 31.9, 46.2, 49.1, 107.6, 121.6, 126.7, 127.6, 127.8, 128.7, 129.3, 130.3, 130.5, 135.1, 136.5, 139.0, 139.6, 144.1, 157.0, 166.9, 168.3; MS (ESI), m/z: 550.3 (M+H); Anal. Calcd. for $C_{31}H_{34}N_8O_2$: C, 67.66; H, 6.22; N, 20.35; Found: C, 67.65; H, 6.22; N, 20.34.



h

4'-[(6-N-tert-butylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1h)**.

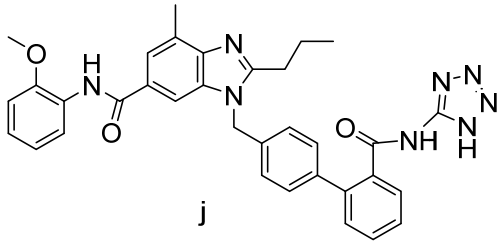
Light yellow solid (70.6 mg, 57.9%), m.p. 114~ 116℃. ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.23 Hz, 3H), 1.39 (s, 9H), 1.85 (m, 2H), 2.58 (s, 3H), 2.97 (t, J = 7.18 Hz, 2H), 5.56 (s, 2H), 7.09 ~ 7.94 (m, 10H), 8.43 (s, 1H), 11.02 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 16.9, 21.0, 29.2, 46.1, 49.1, 51.2, 107.7, 121.8, 126.6, 127.6, 128.7, 129.3, 130.5, 135.1, 136.3, 136.7, 139.0, 139.5, 144.0, 156.9, 167.1; MS (ESI), m/z: 550.3 (M+H); Anal. Calcd. for $C_{31}H_{34}N_8O_2$): C, 67.60; H, 6.22; N, 20.37; Found: C, 67.58; H, 6.21; N, 20.35.



i

4'-[(6-N-phenylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]-[1,1'-biphenyl]-2-N-(1H-tetrazol-5-yl)amide **(1i)**.

Light yellow solid (67.8 mg, 55.6 %), m.p. 109 ~ 111℃, ^{1}H NMR (400MHz, DMSO) δ: 0.96 (t, J = 7.24 Hz, 3H), 1.74 (m, 2H), 2.55 (s, 3H), 2.83 (t, J = 7.16 Hz, 2H), 5.55 (s, 2H), 6.82 ~ 7.95 (m, 15H), 8.85 (s, 1H), 10.98 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 29.2, 46.4, 108.4, 120.9, 122.0, 122.9, 124.1, 124.5, 127.1, 128.1, 128.3, 128.3, 129.0, 129.4, 131.3, 132.6, 135.2, 136.4, 141.0, 142.3, 155.7, 164.3, 167.0; MS (ESI), m/z: 570.2 (M+H); Anal. Calcd. for $C_{33}H_{30}N_8O_2$: C, 69.47; H, 5.30; N, 19.64; Found:C, C, 69.46; H, 5.30; N, 19.63.

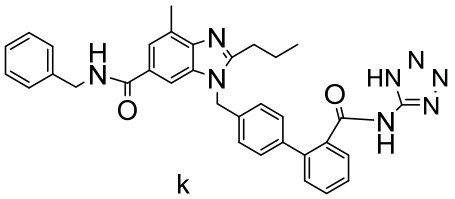


j

4'-[[6-(N-2-methoxyphenyl) aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl] methyl] - [1, 1'-biphenyl]-2- N-(1H-tetrazol-5-yl) amide **(1j)**.

Light yellow solid (69.3 mg, 56.8 %), m.p. 107 ~ 108 ℃ ^{1}H NMR (400MHz, DMSO) δ: 0.93 (t, J = 7.22 Hz, 3H), 1.75 (m, 2H), 2.53 (s, 3H), 2.80 (t, J = 7.20 Hz, 2H), 3.66 (s, 3H), 5.52 (s, 2H), 6.78 ~ 7.89 (m, 14H), 8.74 (s, 1H), 11.01 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 46.3, 56.2, 108.2, 111.8, 112.5, 118.3, 120.6, 124.7, 125.9, 126.7, 127.5, 128.2, 128.4, 129.4, 135.2,

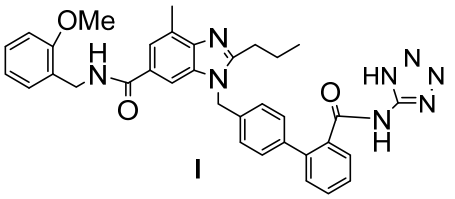
144.6, 151.2, 157.4, 165.8; MS (ESI), m/z: 600.3 (M+H); Anal. Calcd. $C_{34}H_{32}N_8O_3$: C, 68.01; H, 5.38; N, 18.66; Found: C, 68.00; H, 5.38; N, 18.66.



k

4'-[(6-N-benzylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]-[1,1'-biphenyl]-2-N-(1H-tetrazol-5-yl)amide **(1k)**.

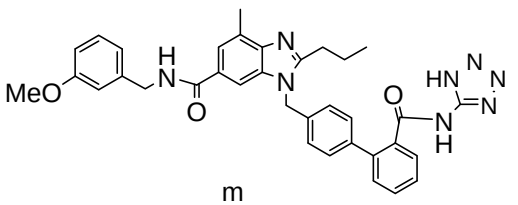
Light yellow solid (66.2 mg, 54.3 %), m.p. 112-114 ℃, ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.20 Hz, 3H), 1.75 (m, 2H), 2.55 (s, 3H), 2.83 (t, J = 7.17 Hz, 2H), 4.46 (d, J = 4 Hz, 2H), 5.53 (s, 2H), 7.07-7.92 (m, 15H), 8.83 (s, 1H), 11.09 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.2, 17.0, 21.0, 29.2, 43.2, 46.3, 107.8, 123.1, 124.3, 126.9, 127.1, 127.3, 128.3, 128.5, 128.5, 128.7, 129.6, 132.4, 132.7, 134.4, 135.1, 137.7, 138.2, 141.2, 141.7, 142.5, 155.9, 167.1 MS (ESI), m/z: 584.3 (M+H); Anal. Calcd. for $C_{34}H_{32}N_8O_2$: C, 69.85; H, 5.52; N, 19.17. Found: C, 69.86; H, 5.52; N, 19.16.



l

4'-[[6-(N-2-methoxybenzyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1l)**.

Light yellow solid **(** 65.5 mg, 53.7 %), m.p. 108-109 ℃. 1H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.23 Hz, 3H), 1.75 (m, 2H), 2.54 (s, 3H), 2.82 (t, J = 7.16 Hz, 2H), 3.69 (s, 3H), 4.39 (d, J = 6.4 Hz, 2H), 5.57 (s, 2H), 6.86 ~ 7.96 (m, 14H), 8.71 (s, 1H), 11.02 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 38.1, 49.0, 55.8, 107.8, 112.9, 118.8, 120.5, 121.7, 126.8, 127.6, 127.9, 128.2, 128.7, 129.3, 129.7, 130.5, 135.1, 135.1, 136.5, 139.0, 142.1, 144.2, 156.9, 157.1, 157.6, 167.2.; MS (ESI), m/z: 614.3 (M+H); Anal.Calcd.for $C_{35}H_{34}N_8O_3$: C, 68.45; H, 5.58; N, 18.21; Found:C, 68.44; H, 5.58; N, 18.23

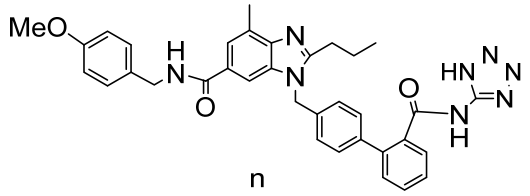


m

4'-[[6-(N-3-methoxybenzyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]

-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1m)**.

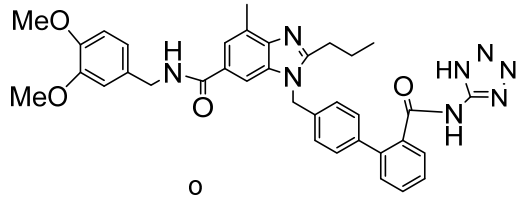
Light yellow solid**(**69.4 mg, 56.9 %), m.p. 106-107 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.22 Hz, 3H), 1.76 (m, 2H), 2.56 (s, 3H), 2.82 (t, J = 7.16 Hz, 2H), 3.80 (s, 3H), 4.42 (d, J = 6.4 Hz, 2H), 5.54(s, 2H), 6.83 ~ 7.69 (m, 14H), 8.67 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 14.2, 17.0, 21.0, 29.2, 43.1, 46.3, 55.4, 107.9, 112.4, 113.5, 119.9, 121.7, 126.8, 127.6, 127.9, 128.2, 128.7, 129.3, 129.7, 130.5, 135.1, 135.1, 136.5, 139.0, 142.1, 144.2, 157.1, 159.7, 167.1; MS (ESI), m/z: 614.3 (M+H); Anal.Calcd.for $C_{35}H_{34}N_8O_3$: C, 68.34; H, 5.58; N, 18.23; Found: C, 68.33; H, 5.57; N, 18.22



n

4'-[[6-(N-4-methoxybenzyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1n)**.

Light yellow solid**(** 65.4 mg, 53.6 %), m.p.103 ~ 105 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.96 (t, J = 7.21 Hz, 3H), 1.75 (m, 2H), 2.57 (s, 3H), 2.83 (t, J = 7.16 Hz, 2H), 3.77 (s, 3H), 4.41 (d, J = 6.4 Hz, 2H), 5.56 (s, 2H), 6.68 ~ 7.93 (m, 14H), 8.84 (s, 1H), 11.03 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 42.6, 46.2, 55.5, 107.8, 114.1, 121.7, 126.7, 127.6, 127.9, 128.4, 128.6, 129.1, 129.3, 130.3, 130.5, 132.4, 135.1, 136.5, 138.9, 139.6, 144.2, 157.1, 158.6, 166.9, 168.3; MS (ESI), m/z: 614.3 (M+H); Anal.Calcd.for $C_{35}H_{34}N_8O_3$: C, 68.42; H, 5.57; N, 18.21; Found: C, 68.44; H, 5.59; N, 18.22

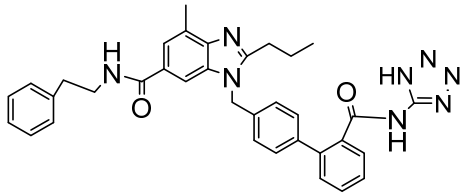


o

4'-[[6-(N-3,4-dimethoxybenzyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1o)**.

Light yellow solid**(**63.2 mg, 51.8 %), m.p.101 ~ 103 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.96 (t, J = 7.21 Hz, 3H), 1.75 (m, 2H), 2.57 (s, 3H), 2.84 (t, J = 7.19 Hz, 2H), 3.68 (s, 3H), 3.69 (s, 3H), 4.41 (d, J = 6.8 Hz, 2H), 5.55 (s, 2H), 6.82 ~ 7.93 (m, 13H), 8.83 (s, 1H), 11.06 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 16.9, 21.1, 29.3, 44.0, 46.9, 55.8, 56.2, 110.0, 112.6, 115.4, 120.6, 123.9, 125.2,

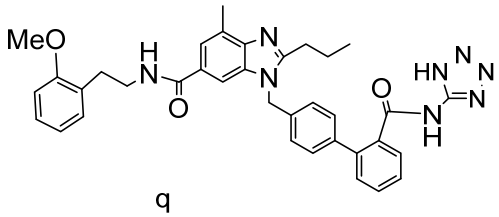
128.1, 129.1, 129.3, 129.4, 130.4, 133.3, 133.6, 134.7, 135.3, 136.0, 138.6, 139.0, 142.0, 143.4, 147.6, 149.6, 156.8, 165.3, 170.4; MS (ESI), m/z: 644.3 (M+H); Anal.Calcd.for $C_{36}H_{36}N_8O_4$: C, 67.05; H, 5.63; N, 17.40; Found: C, 67.05; H, 5.63; N, 17.39



p

4'-[(6-N-phenethylaminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl)methyl]-[1,1'-biphenyl] -2- N-(1H-tetrazol-5-yl)amide **(1p)**.

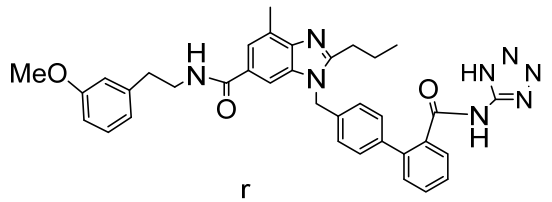
Light yellow solid**(**72.3 mg, 59.3 %), **(**m.p. 107-108 ℃). ^{1}H NMR (400MHz, DMSO) δ: 0.97 (t, J = 7.19 Hz, 3H), 1.73 (m, 2H), 2.58 (s, 3H), 2.86 (m, 4H), 3.49 (m, 2H), 5.57 (s, 2H), 7.10 ~ 7.88 (m, 15H), 8.46 (s, 1H), 11.09 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ14.2, 17.0 ,21.0, 29.2 , 35.7, 41.5, 46.2, 107.7 , 121.5, 126.5 , 126.8, 127.7, 127.9, 128.6, 128. 8, 129.1, 129.3, 130.7, 131.2, 135.1, 136.6, 140.1 , 144.2, 157.0, 167.0; MS (ESI), m/z: 598.3 (M+H); Anal.Calcd.for $C_{35}H_{34}N_8O_2$: C, 70.16; H, 5.73; N, 18.70; Found:C, 70.18; H, 5.74; N, 18.71



q

4'-[[6-(N-2-methoxyphenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1q)**.

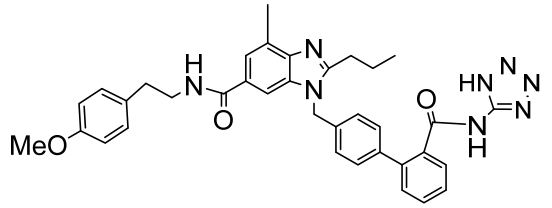
Light yellow solid**(** 70.0 mg, 57.4 %), m.p.105-107 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ0.90 (d, J = 7.5 Hz, 3H), 1.72 (q, J = 7.5 Hz, 2H), 2.56 (d, J = 3.7 Hz, 3H), 2.80 (dd, J = 36.2, 7.8 Hz, 4H), 3.49 – 3.33 (m, 2H), 3.77 (s, 3H), 5.51 (s, 2H), 7.89- 6.84 (0, 15H), 8.45 (s, 1H), 12.00 (s, 1H). ^{13}C NMR (126MHz, DMSO) δ: 14.2, 17.0, 21.0, 29.1, 46.2, 49.1, 55.7, 107.7, 110.1, 120.1, 126.8, 127.7, 127.8, 128.0, 128.7, 129.3, 130.5, 130.7, 131.1, 135.1, 136.6, 139.5, 144.1, 157.0, 157.7, 167.0; MS (ESI), m/z: 628.3 (M+H); Anal.Calcd.for $C_{36}H_{36}N_8O_3$: C, 68.77; H, 5.77; N, 17.82; Found:C, 68.81; H, 5.77; N, 17.83



r

4'-[[6-(N-3-methoxyphenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1r)**.

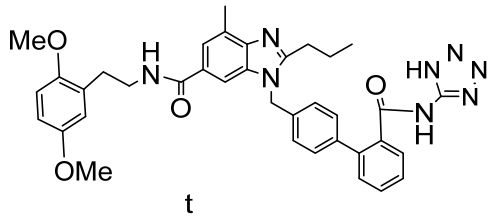
Light yellow solid(70.8 mg, 58.0 %), m.p.101-103 ℃. ^{1}H NMR (400MHz, DMSO) δ:1.01 (t, J = 7.22 Hz, 3H), 1.77 (m, 2H), 2.54 (s, 3H), 2.82 (m, 4H), 3.44 (m, 2H), 3.68 (s, 3H), 5.52 (s, 2H), 6.72 ~ 7.84 (m, 14H), 8.41 (s, 1H), 11.04 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 34.8, 41.7, 46.3, 55.4, 107.7, 111.4, 112.0, 120.4, 123.7, 124.9, 127.9, 128.9, 129.1, 129.2, 129.8, 130.2, 133.1, 133.3, 135.1, 135.8, 138.3, 138.8, 140.5, 141.8, 143.1, 156.6, 160.5, 167.0, 168.0; MS (ESI), m/z: 628.3 (M+H); Anal.Calcd.for $C_{36}H_{36}N_8O_3$: C, 68.83; H, 5.78; N, 17.84; Found:C, 68.81; H, 5.77; N, 17.83



s

4'-[[6-(N-4-methoxyphenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1s)**.

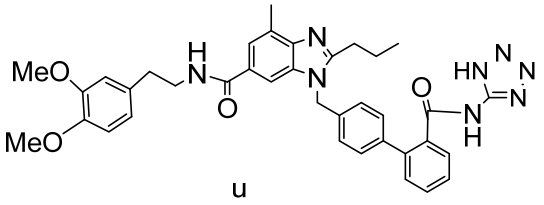
Light yellow solid(67.3 mg, 55.2 %), m.p.98-100 ℃. ^{1}H NMR (400MHz, DMSO) δ:1.07 (t, J = 7.23 Hz, 3H), 1.77 (m, 2H), 2.56 (s, 3H), 2.75 (t, J = 7.16 Hz, 2H), 2.82 (t, J = 7.11 Hz, 2H), 3.41 (m, 2H), 3.65 (s, 3H), 5.53 (s, 2H), 6.87 ~ 7.85 (m, 14H), 8.45 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 36.3, 41.6, 46.2, 56.0, 107.7, 112.3, 123.2, 124.4, 127.4, 128.4, 128.6, 128.6, 129.7, 131.4, 132.5, 132.8, 134.5, 135.2, 137.8, 138.3, 141.3, 142.6, 156.0, 157.9, 164.6, 169.6; MS (ESI), m/z: 628.3 (M+H); Anal.Calcd.for $C_{36}H_{36}N_8O_3$: C, 68.83; H, 5.77; N, 17.81; Found:C, 68.81; H, 5.77; N, 17.81



t

4'-[[6-(N-2,5-dimethoxyphenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl] methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1t)**.

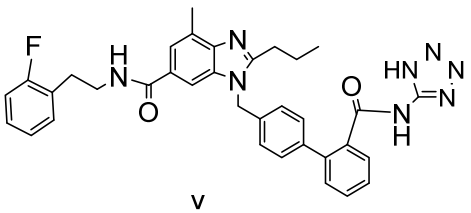
Light yellow solid**(** 68.7 mg, 56.3 %) ,m.p.95-96 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.25 Hz, 3H), 1.75 (m, 2H), 2.51 (s, 3H), 2.76~2.83 (m, 4H), 3.44 (m, 2H), 3.71 (s, 3H), 3.77 (s, 3H), 5.53 (s, 2H), 6.69 ~ 7.85 (m, 13H), 8.43 (s, 1H), 11.04 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.2, 16.7, 21.0, 29.1, 31.2, 36.3, 46.2, 55.7, 56.3, 107.9, 112.0, 121.4, 122.1, 122.7, 123.9, 126.9, 127.9, 128.1, 128.2, 129.2, 132.1, 132.3, 134.1, 134.8, 137.3, 137.8, 140.8, 142.1, 149.1, 149.8, 155.6, 162.7, 167.0; MS (ESI), m/z: 658.3 (M+H); Anal.Calcd.for $C_{37}H_{38}N_8O_4$: C, 67.44; H, 5.82; N, 17.03; Found:C, 67.46; H, 5.81; N, 17.02



u

4'-[[6-(N-3,4-dimethoxyphenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl] methyl]-[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1u)**.

Light yellow solid**(** 66.0 mg,54.1 %)，m.p. 96-97 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.93 (t, J = 7.22 Hz, 3H), 1.77 (m, 2H), 2.54 (s, 3H), 2.77 (t, J = 7.16 Hz, 2H), 2.83 (t, J = 7.21 Hz, 2H), 3.45 (m, 2H), 3.68 (s, 6H), 5.53 (s, 2H), 6.70 ~ 7.85 (m, 13H), 8.37 (s, 1H), 11.02 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 35.3, 41.7, 46.3, 55.8, 56.0, 107.5, 112.3, 112.8, 115.3, 123.0, 124.2, 127.2, 128.2, 128.4, 128.4, 129.5, 132.3, 132.6, 133.0, 134.3, 135.1, 137.6, 138.0, 141.1, 142.4, 147.6, 149.5, 157.0, 161.6, 167.0; MS (ESI), m/z: 658.3 (M+H); Anal.Calcd.for $C_{37}H_{38}N_8O_4$: C, 67.50; H, 5.81; N, 17.00; Found:C, 67.51; H, 5.81; N, 17.01

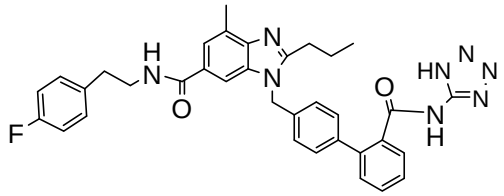


v

4'-[[6-(N-2-fluorophenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1v)**.

Light yellow solid **(**69.3 mg, 56.8 %), m.p.104-106 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.24 Hz, 3H), 1.74 (m, 2H), 2.52(s, 3H), 2.84 (t, J = 7.16 Hz, 2H), 2.87 (t, J = 7.14 Hz, 2H), 3.53 (m,

2H), 5.55 (s, 2H), 7.06 ~ 7.84 (m, 14H), 8.47 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2,31.3, 36.3, 46.3, 107.7, 115.7, 123.7, 124.1, 125.0, 127.9, 128.9, 129.1, 129.2, 130.2, 133.1, 133.4, 135.1, 135.8, 138.4, 138.8, 141.8, 143.2, 156.6, 160.4, 162.8, 167.1; MS (ESI), m/z: 616.3 (M+H); Anal.Calcd.for $C_{35}H_{33}FN_8O_2$: C, 68.14; H, 5.39; N, 18.17; Found:C, 68.15; H, 5.40; N, 18.18



w

4'-[[6-(N-4-fluorophenethyl)aminocarboxyl-4-methyl-2-n-propyl-1H-benzoimidazolyl]methyl] -[1,1'-biphenyl]-2- N-(1H-tetrazol-5-yl)amide **(1w)**.

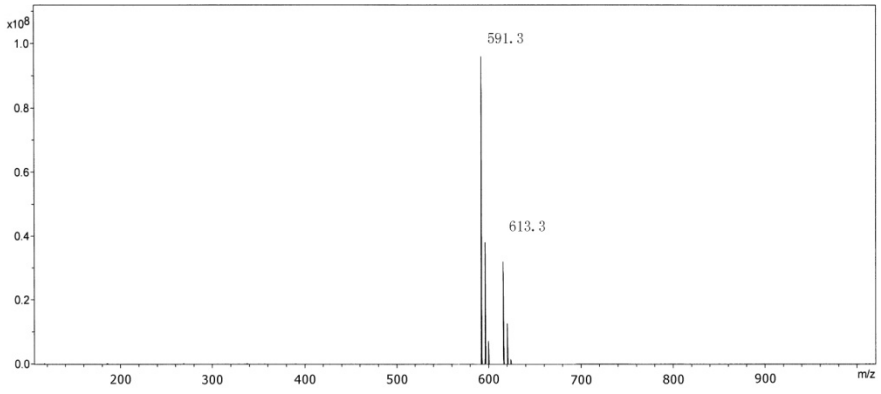
Light yellow solid **(**70.6 mg, 57.9 %), m.p.106-108 ℃. ^{1}H NMR (400MHz, DMSO) δ: 0.95 (t, J = 7.22 Hz, 3H), 1.76 (m, 2H), 2.54 (s, 3H), 2.81 (m, 4H), 3.45 (m, 2H), 5.55 (s, 2H), 7.03 ~ 7.83 (m, 14H), 8.40 (s, 1H), 11.03 (s, 1H); ^{13}C NMR (126MHz, DMSO) δ: 14.3, 17.0, 21.0, 29.2, 34.8, 41.4, 46.3, 107.7, 115.3, 115.5, 121.5, 126.4, 127.7, 127.9, 128.6, 129.2, 129.4, 130.8, 130.8, 130.9, 135.1, 136.2, 136.0, 140.7, 144.2, 157.0, 157.2, 167.1; MS (ESI), m/z: 616.3 (M+H); Anal.Calcd.for $C_{35}H_{33}FN_8O_2$: C, 68.14; H, 5.39; N, 18.16; Found:C, 68.15; H, 5.37; N, 18.16

2. MS

1A

样品编号： YWL-25d
操作者：BIODURO LC-MS C
进样日期：2009 07 20
仪器：Instrument 1

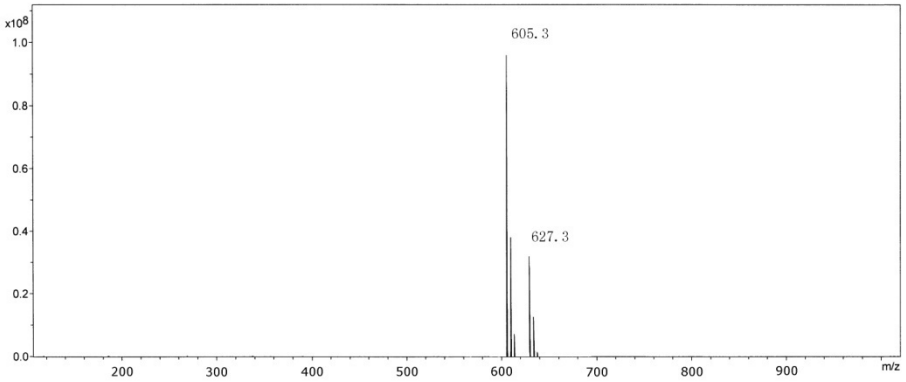
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1B

样品编号： YWL-25c
操作者：BIODURO LC-MS C
进样日期：2009 07 17
仪器：Instrument 1

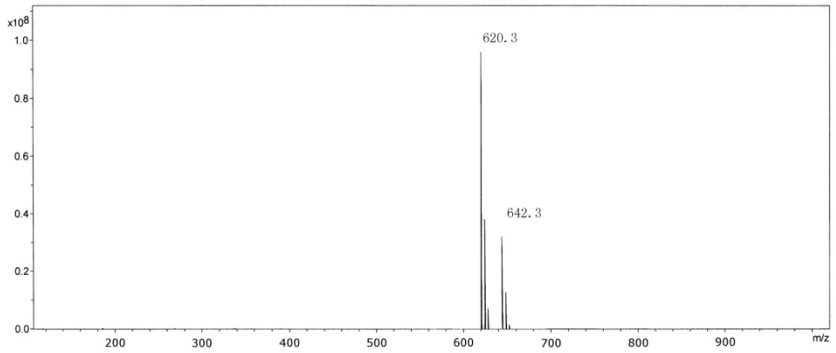
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1C

样品编号： YWL-25b
操作者：BIODURO LC-MS C
进样日期：2009 07 17
仪器：Instrument 1

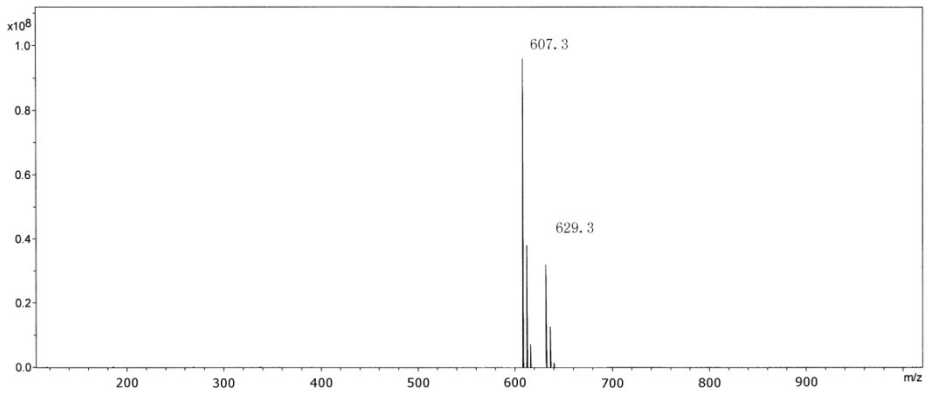
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1D

样品编号： YWL-25a
操作者：BIODURO LC-MS C
进样日期：2009 07 17
仪器：Instrument 1

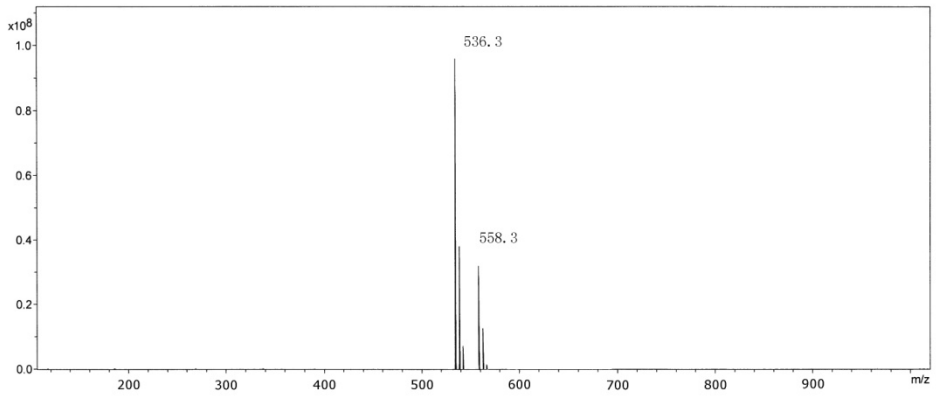
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1E

样品编号： YWL-25e
操作者：BIODURO LC-MS C
进样日期：2009 07 20
仪器：Instrument 1

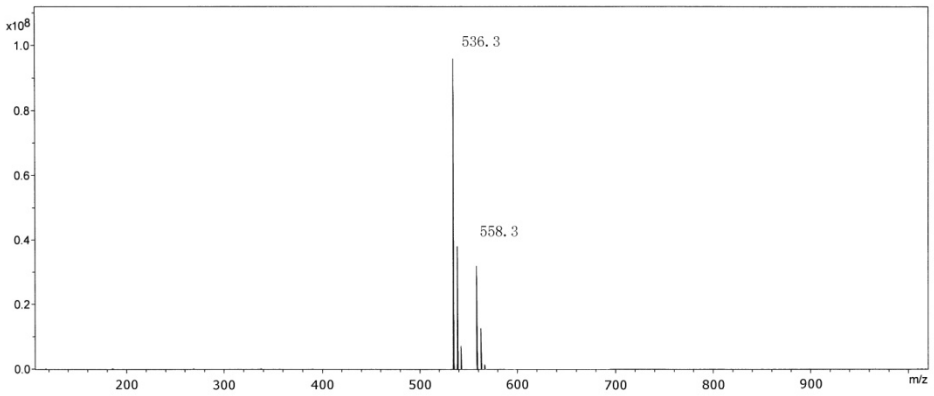
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1F

样品编号： YWL-25f
操作者：BIODURO LC-MS C
进样日期：2009 07 20
仪器：Instrument 1

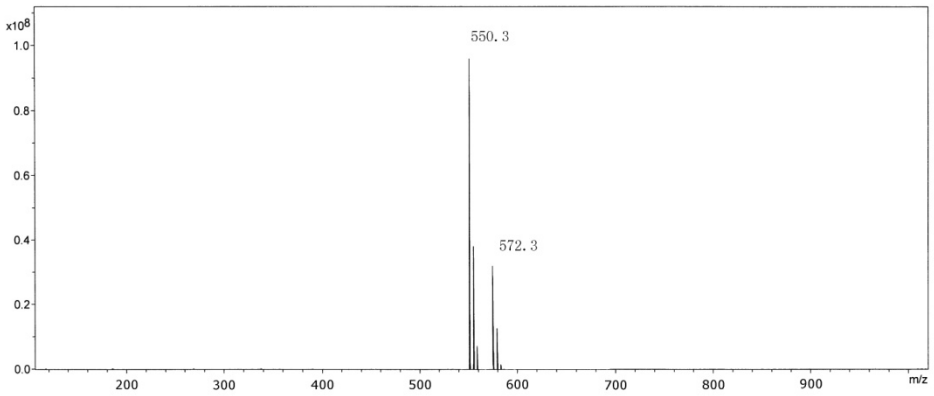
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1G

样品编号： YWL-25g
操作者：BIODURO LC-MS C
进样日期：2009 07 21
仪器：Instrument 1

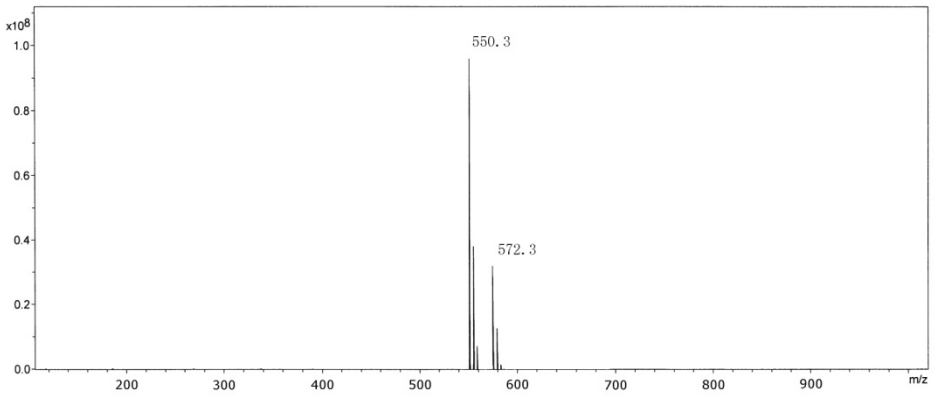
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1H

样品编号： YWL-25h
操作者：BIODURO LC-MS C
进样日期：2009 07 21
仪器：Instrument 1

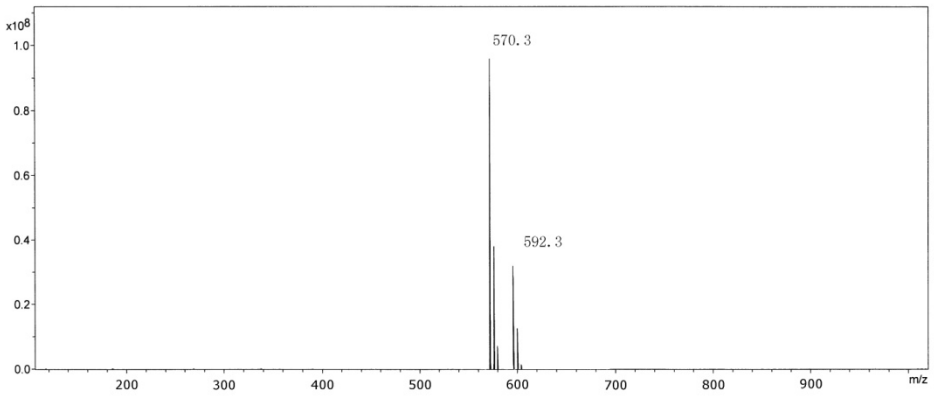
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1I

样品编号： YWL-25i
操作者：BIODURO LC-MS C
进样日期：2009 07 21
仪器：Instrument 1

Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



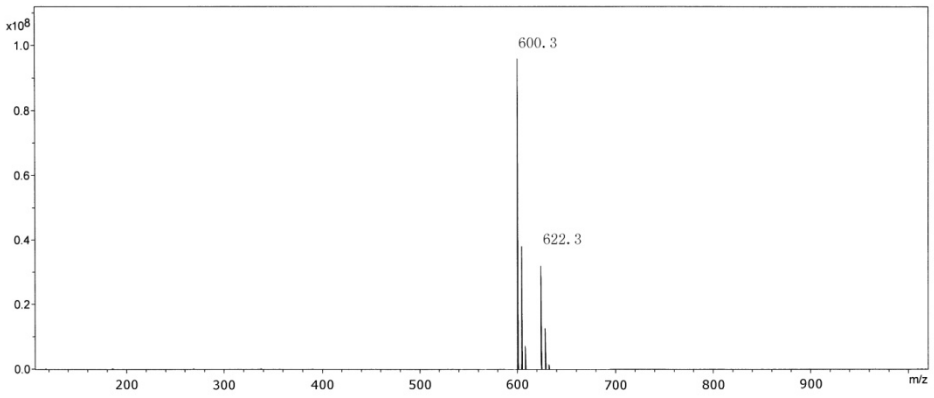
1J

样品编号： YWL-25k

操作者：BIODURO LC-MS C　　Pos ESI

进样日期：2009 07 22　　进样俩： 0.4 ul

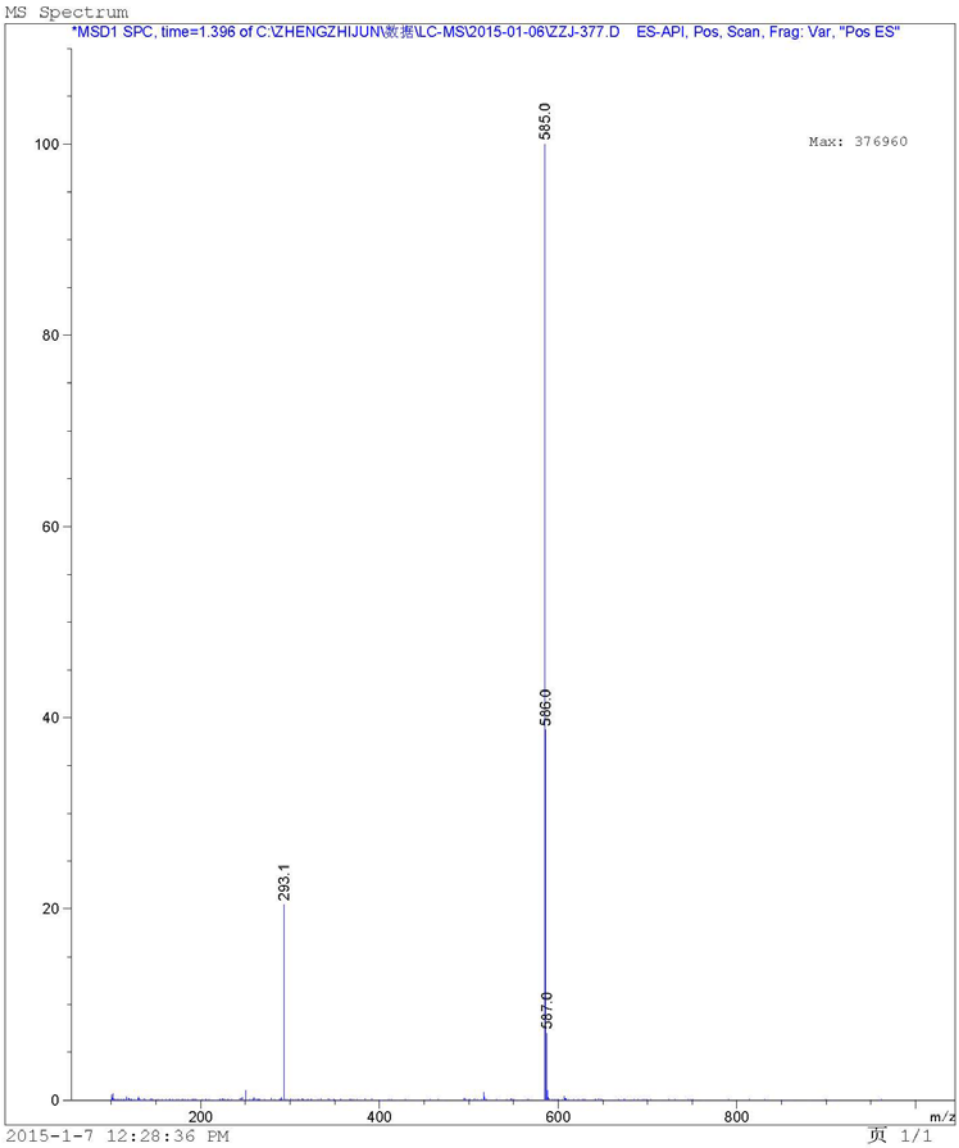
仪器：Instrument 1　　采集方法：D:\Chemstation\DATA\P100-p10000.M



1K

打印窗口 79: MS Spectrum
数据文件: : C:\ZHENGZHIJUN\数据\LC-MS\2015-01-06\ZZJ-377.D
样品名称 : ZZJ-377

操作者 : LARGESCALE
仪器 : Agilent LCMS C 位置 : P1-D-09
进样日期 : 2015-1-6 11:06:07 AM 进样次数 : 1
进样量 : 0.8 µl
采集方法 : D:\CHEM32\METHODS\P100-1000.M
最后修改 : 2015-1-6 11:05:29 AM : LARGESCALE
(调用后修改)
分析方法 : C:\CHEM32\1\METHODS\DEF_LC.M
最后修改 : 2015-1-4 06:37:19 PM
(调用后修改)
样品信息 : Easy-Access Method: 'P100-1000'

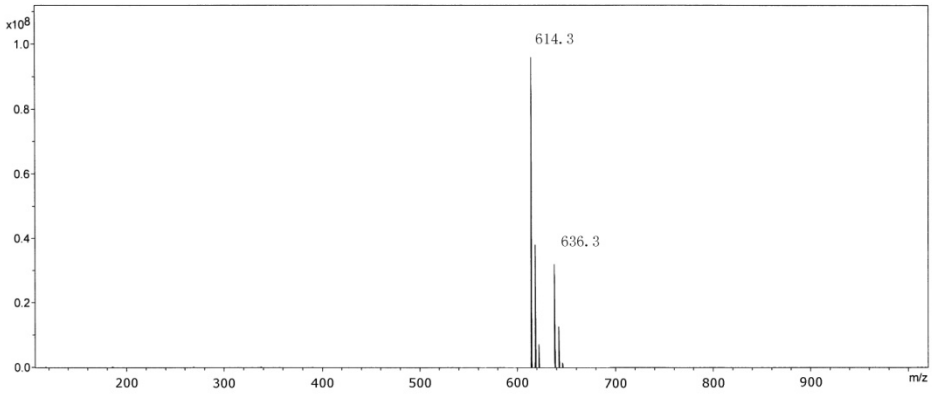


仪器 1 2015-1-7 12:28:36 PM 页 1/1

1L

样品编号： YWL-251
操作者：BIODURO LC-MS C
进样日期：2009 07 22
仪器：Instrument 1

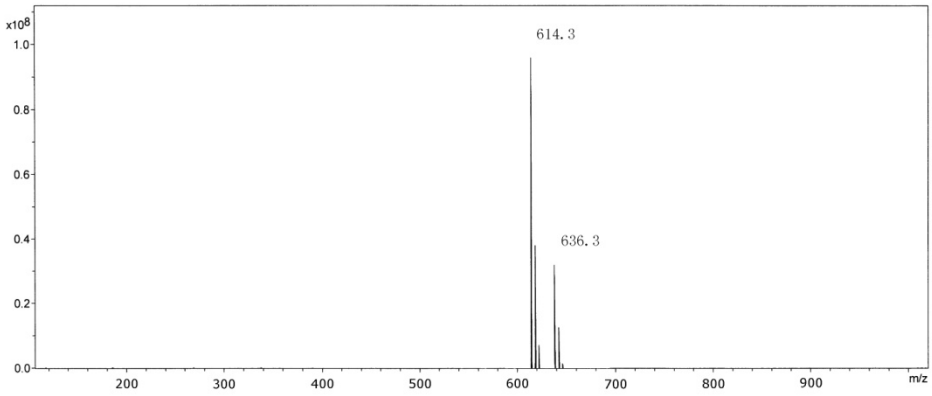
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1M

样品编号： YWL-25m
操作者：BIODURO LC-MS C
进样日期：2009 07 23
仪器：Instrument 1

Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1N

样品编号： YWL-25n
操作者：BIODURO LC-MS C
进样日期：2009 07 23
仪器：Instrument 1

Pos ESI
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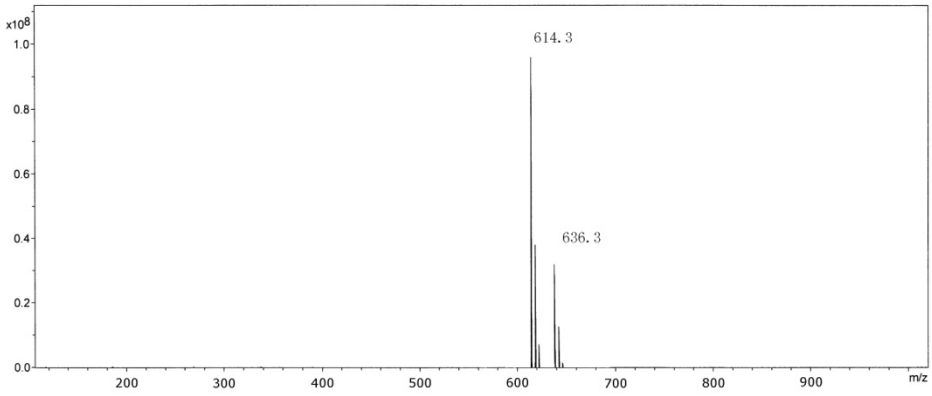
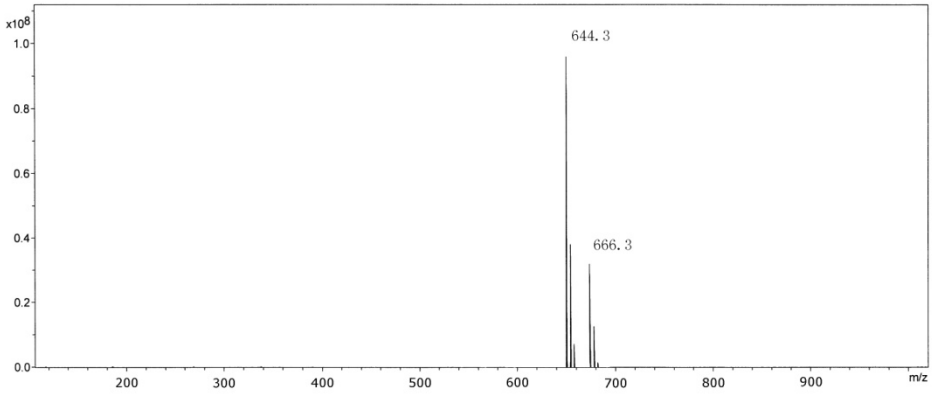


1O

样品编号： YWL-25o
操作者：BIODURO LC-MS C
进样日期：2009 07 23
仪器：Instrument 1

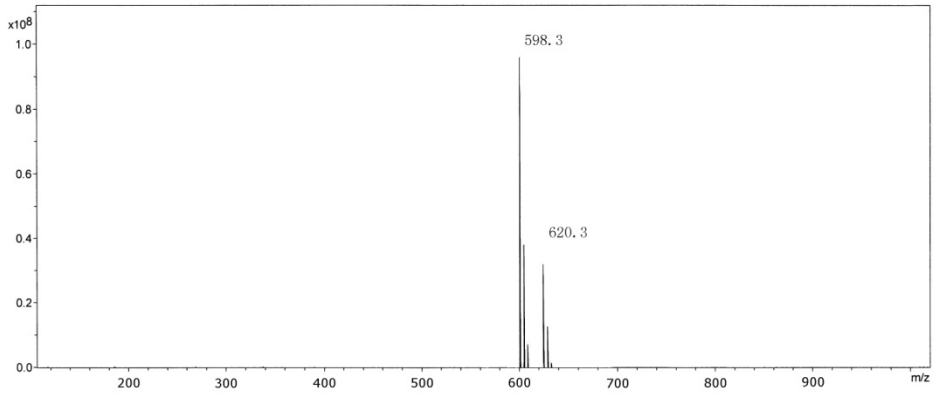
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1P

样品编号： YWL-25p
操作者：BIODURO LC-MS C
进样日期：2009 07 24
仪器：Instrument 1

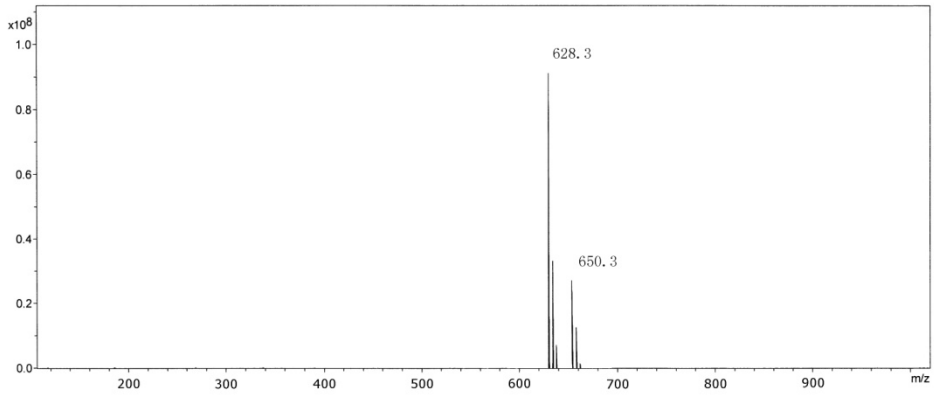
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1Q

样品编号： YWL-25q
操作者：BIODURO LC-MS C
进样日期：2009 07 27
仪器：Instrument 1

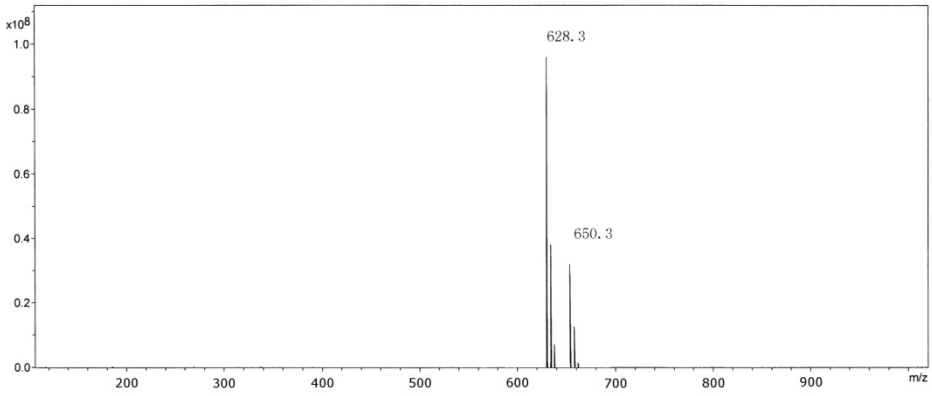
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1R

样品编号： YWL-25r
操作者：BIODURO LC-MS C
进样日期：2009 07 24
仪器：Instrument 1

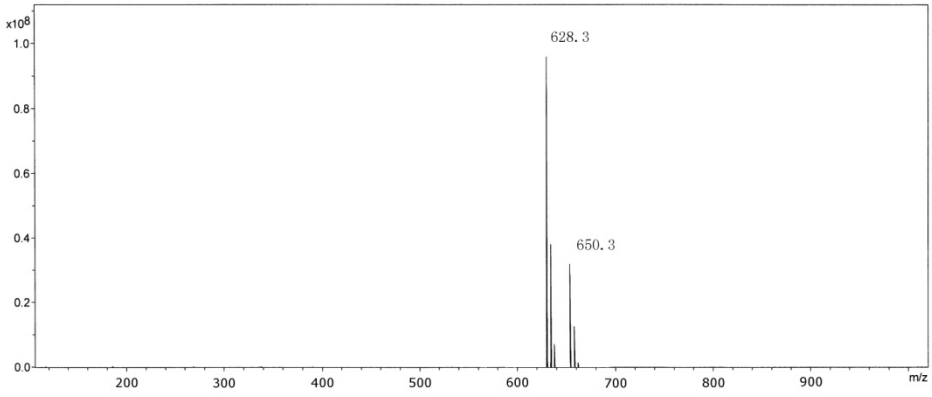
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1S

样品编号： YWL-25s
操作者：BIODURO LC-MS C
进样日期：2009 07 27
仪器：Instrument 1

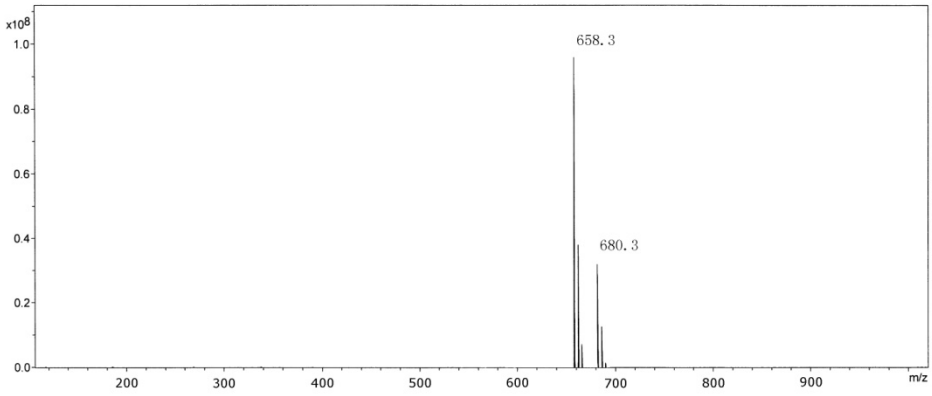
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1T

样品编号： YWL-25t
操作者：BIODURO LC-MS C
进样日期：2009 07 27
仪器：Instrument 1

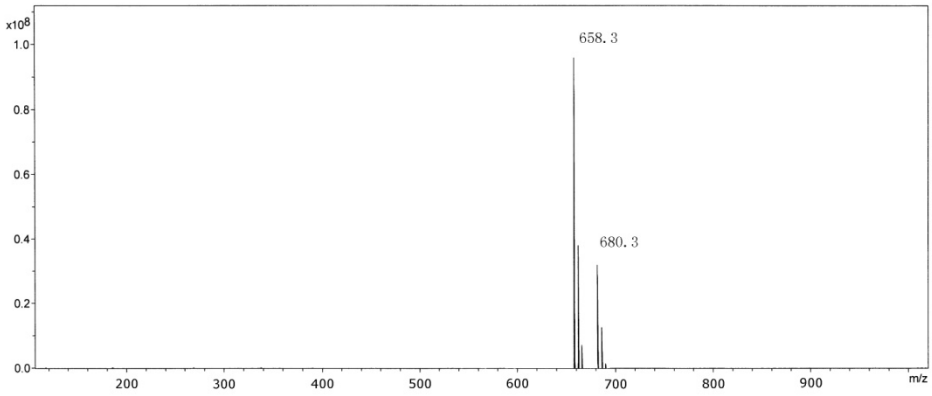
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1U

样品编号： YWL-25u
操作者：BIODURO LC-MS C
进样日期：2009 07 27
仪器：Instrument 1

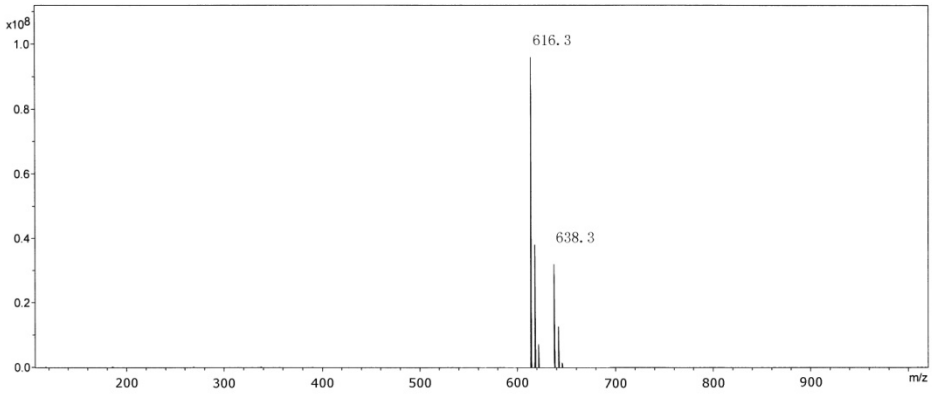
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1V

样品编号： YWL-25v
操作者：BIODURO LC-MS C
进样日期：2009 08 03
仪器：Instrument 1

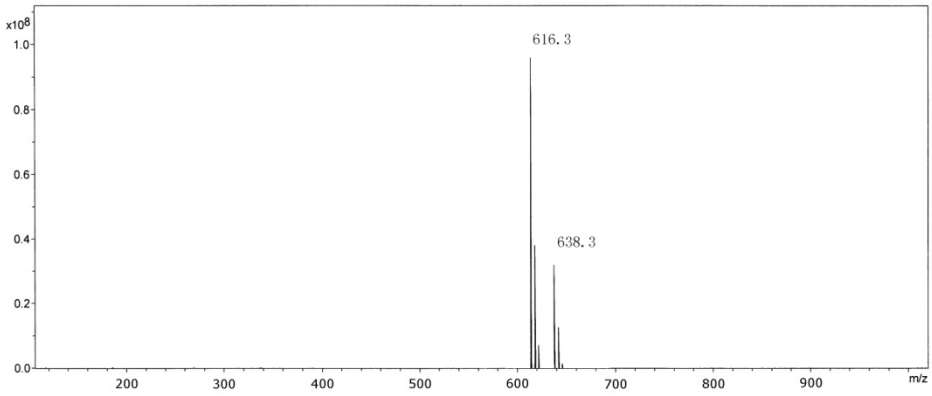
Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M



1w

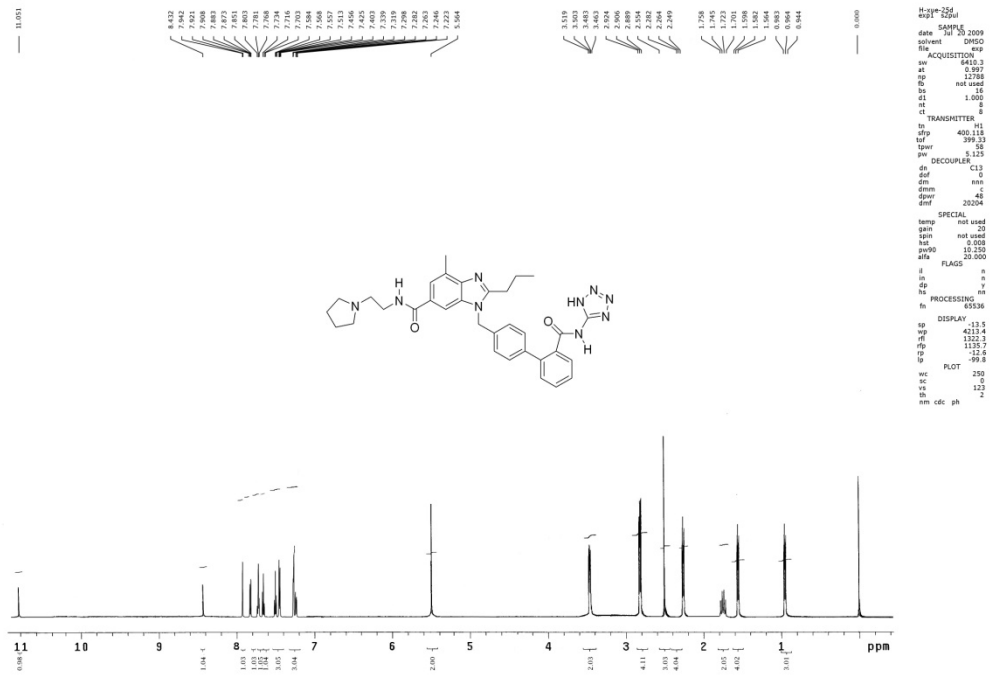
样品编号： YWL-25w
操作者：BIODURO LC-MS C
进样日期：2009 08 03
仪器：Instrument 1

Pos ESI
进样俩： 0.4 ul
采集方法：D:\Chemstation\DATA\P100-p10000.M

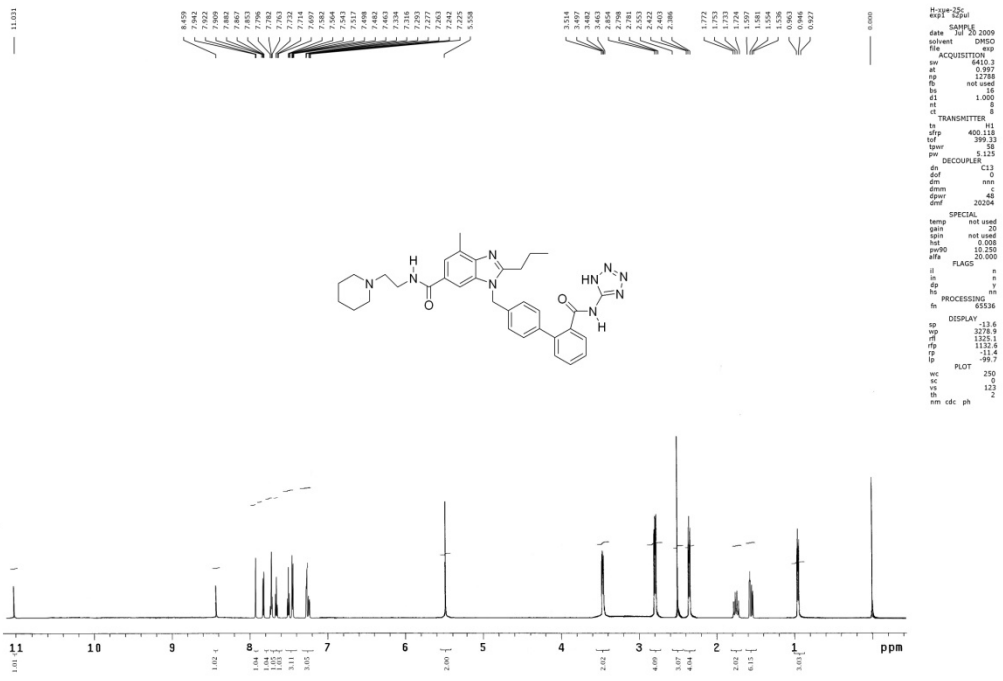


3.[1]HNMR

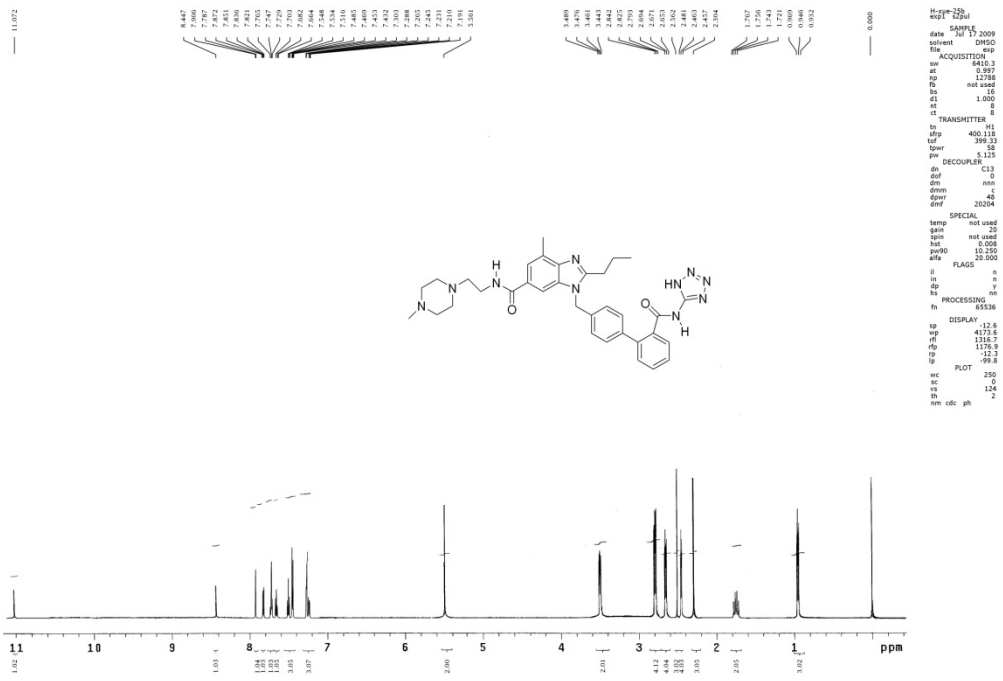
[1]HNMR 1a



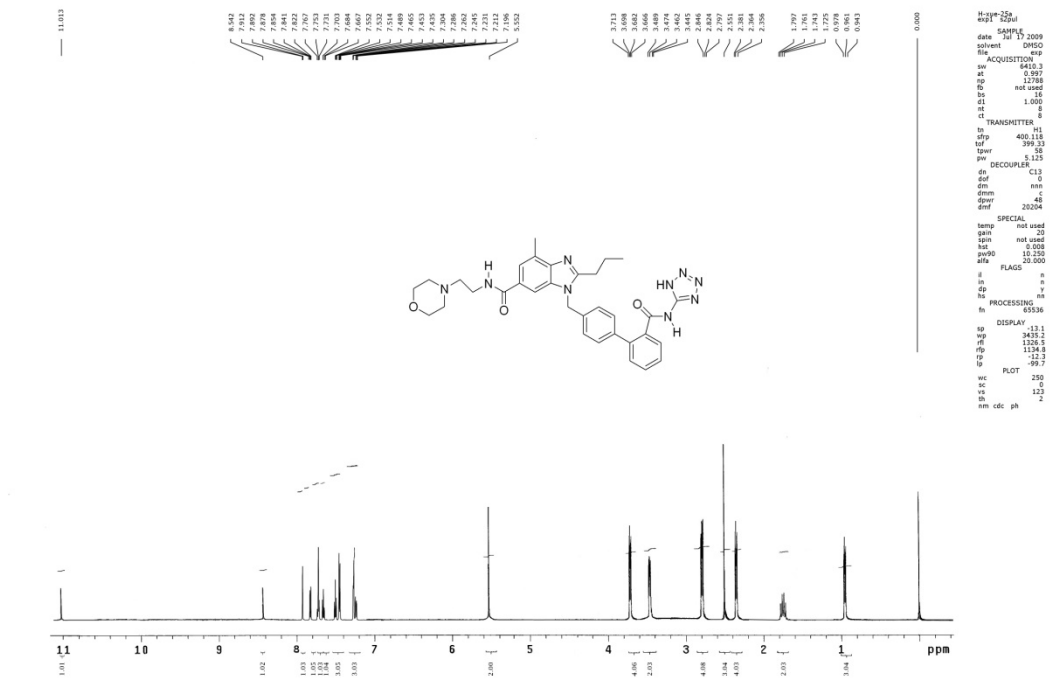
[1]HNMR 1b



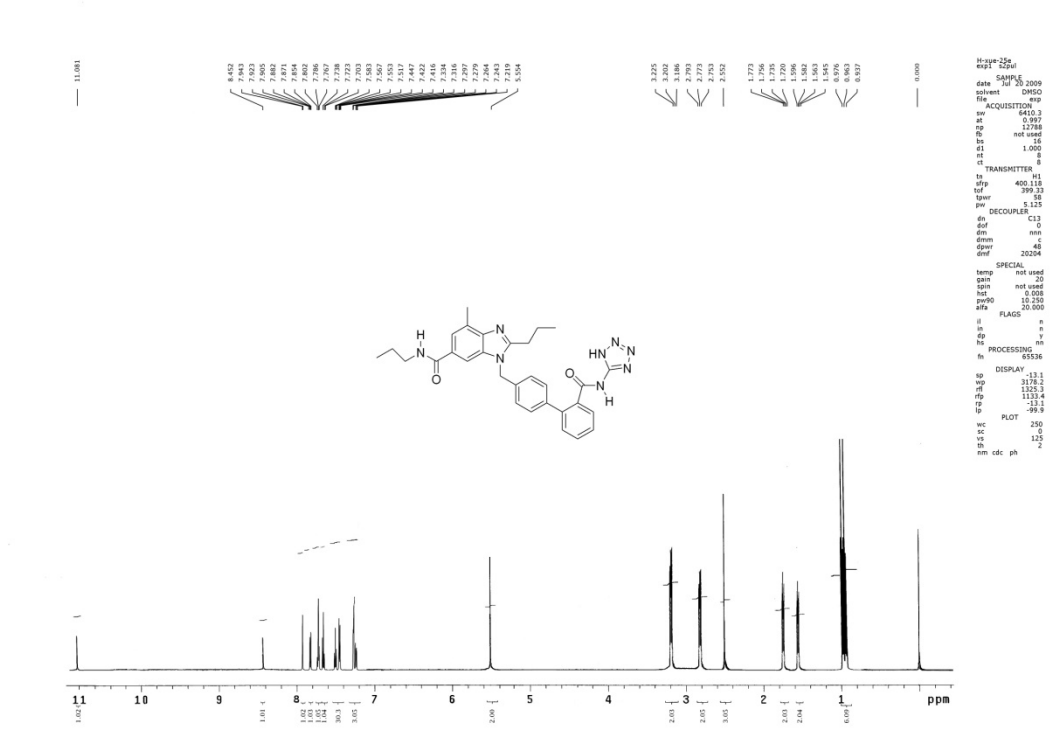
^{1}HNMR 1c



^{1}HNMR 1d



^{1}HNMR 1e



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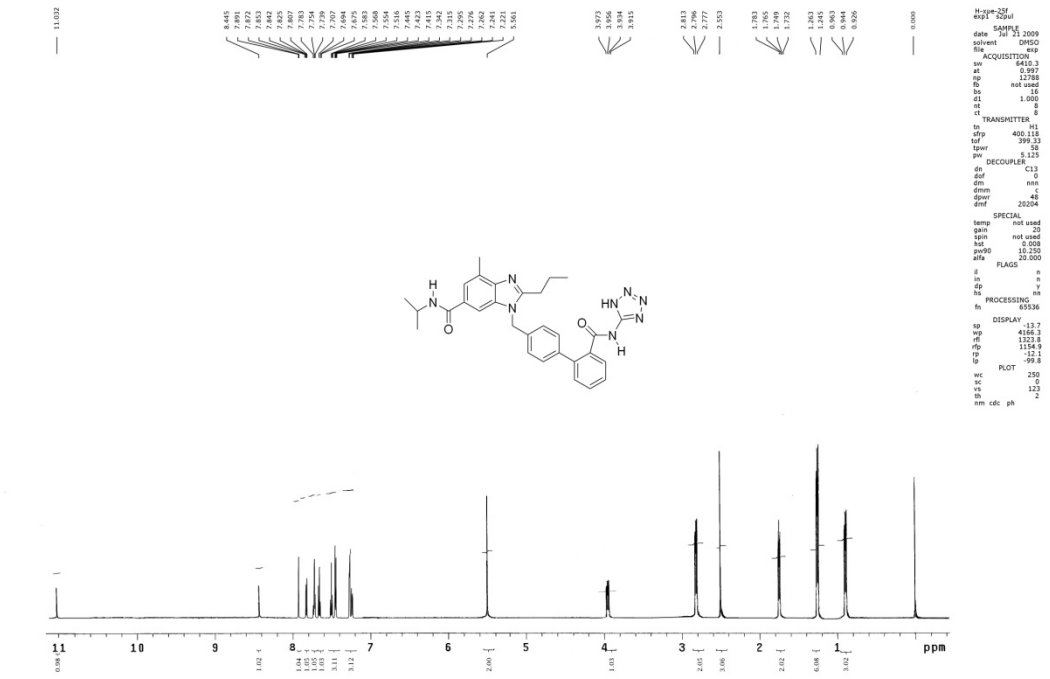
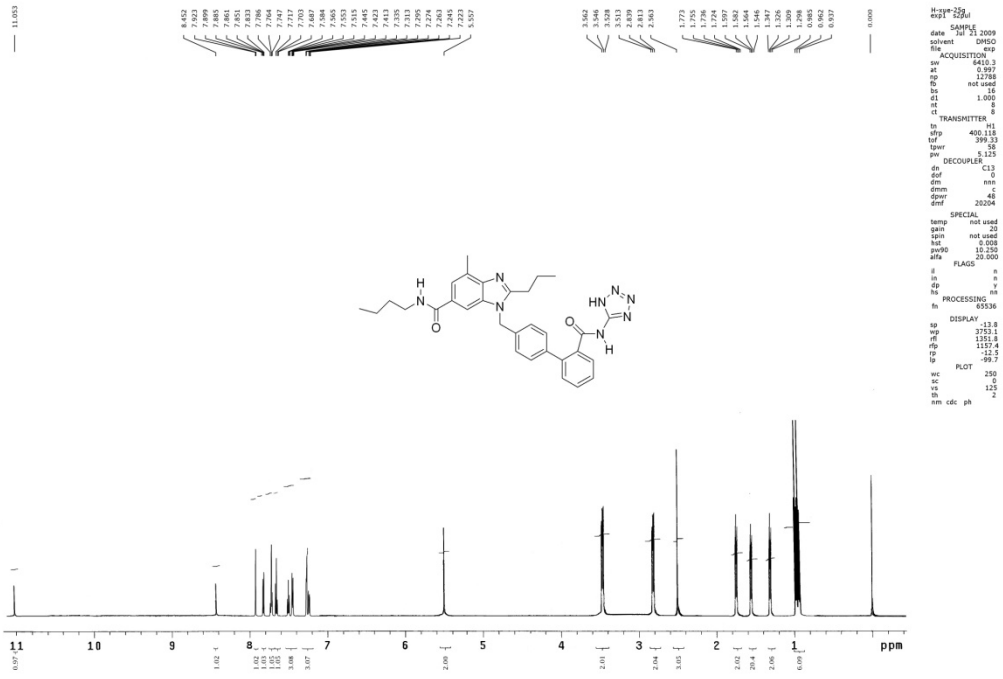


^{1}HNMR 1g



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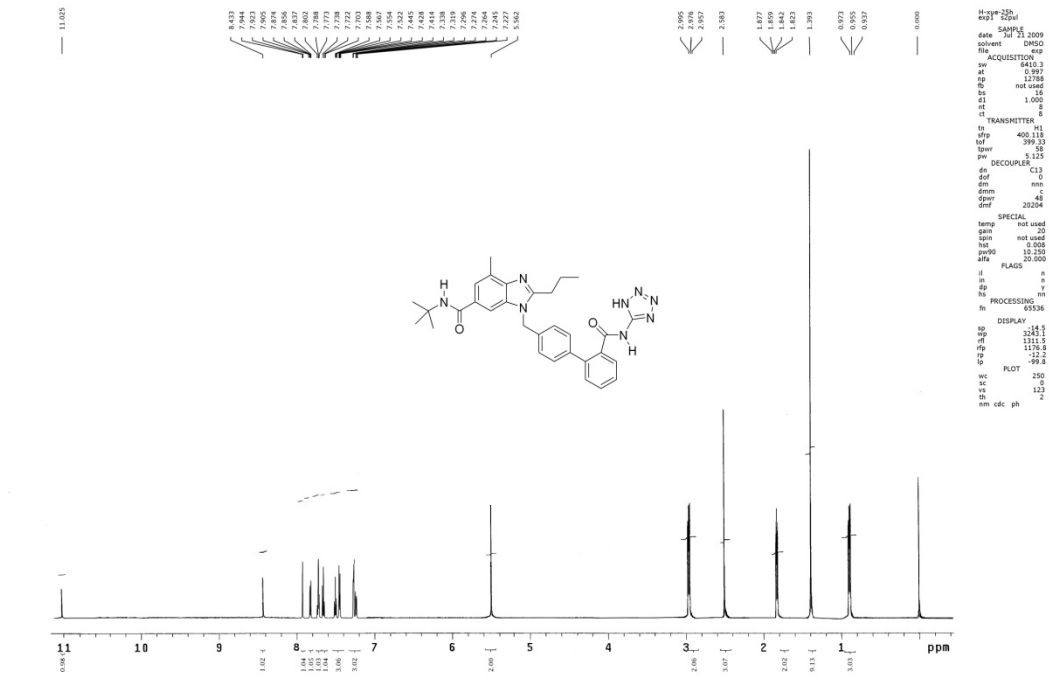
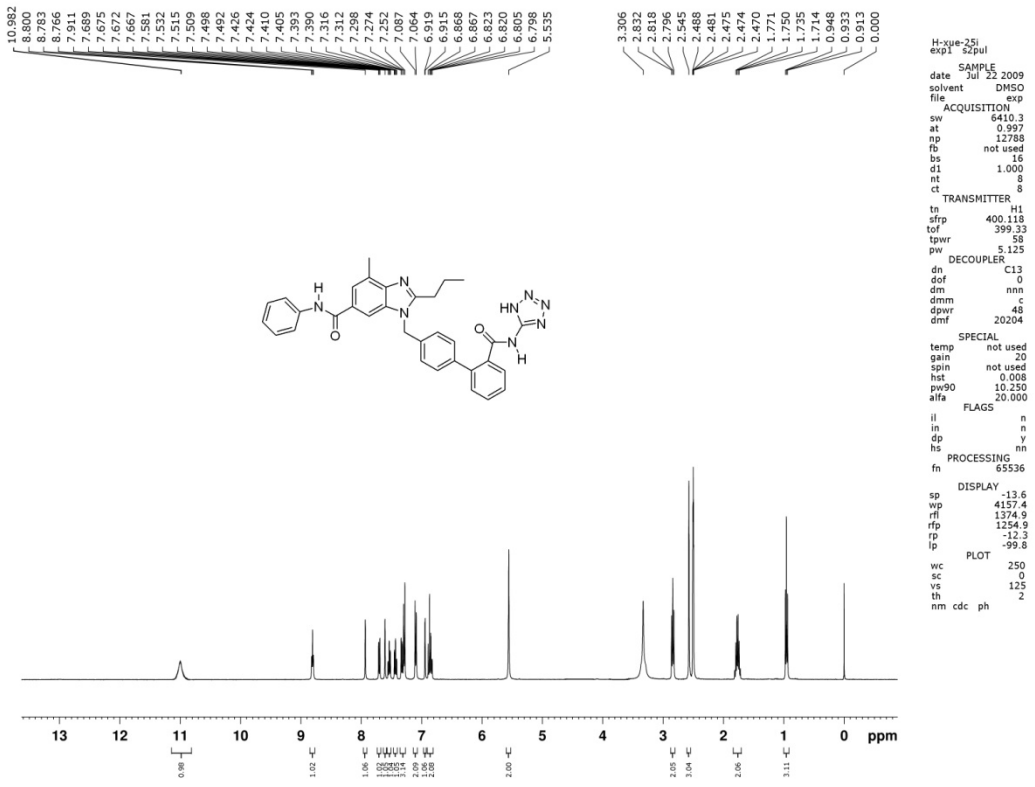
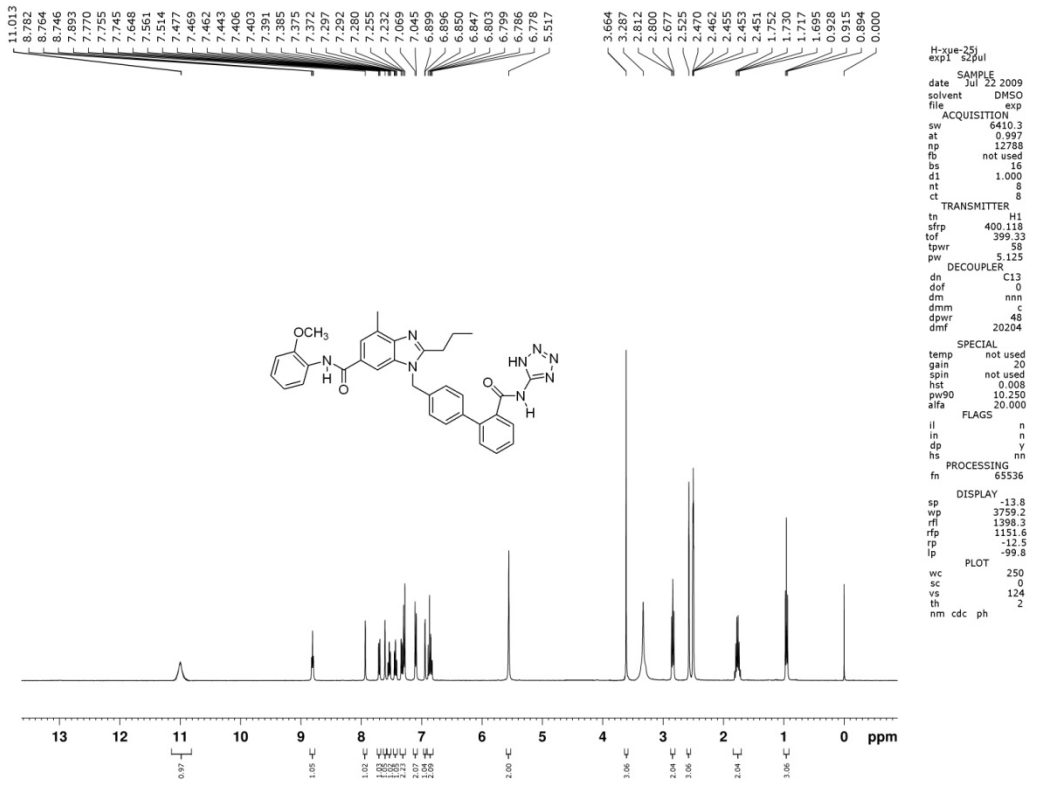


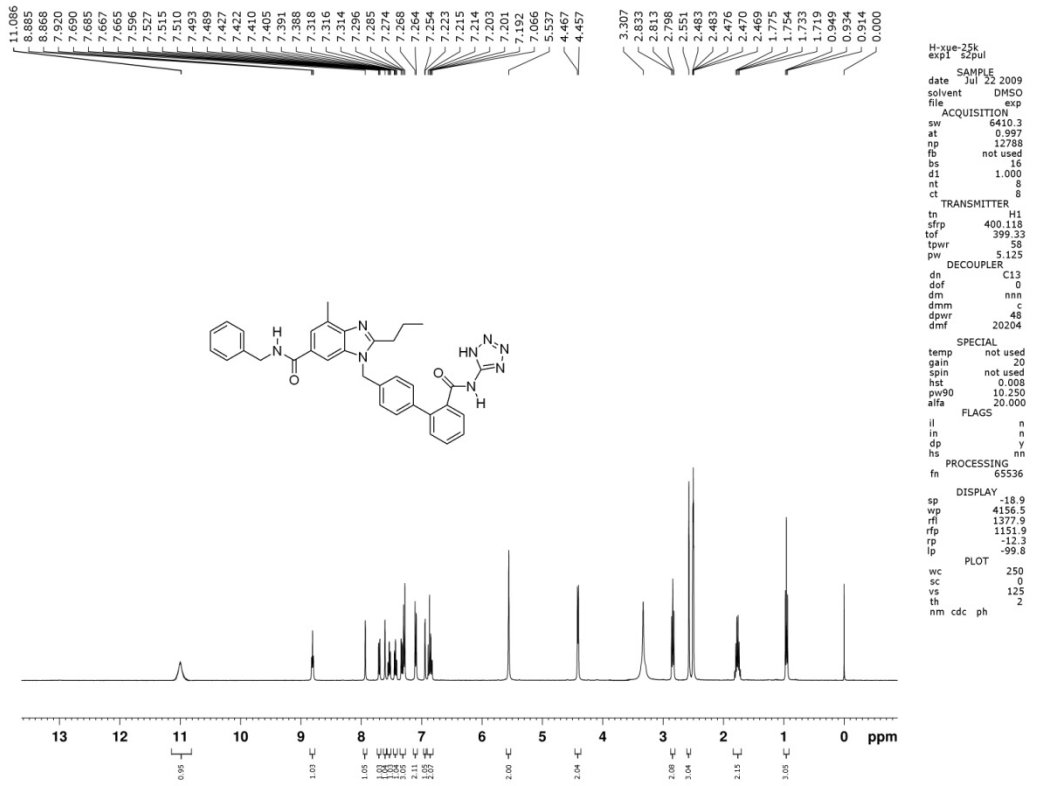
^{1}HNMR 1i



^{1}HNMR 1j

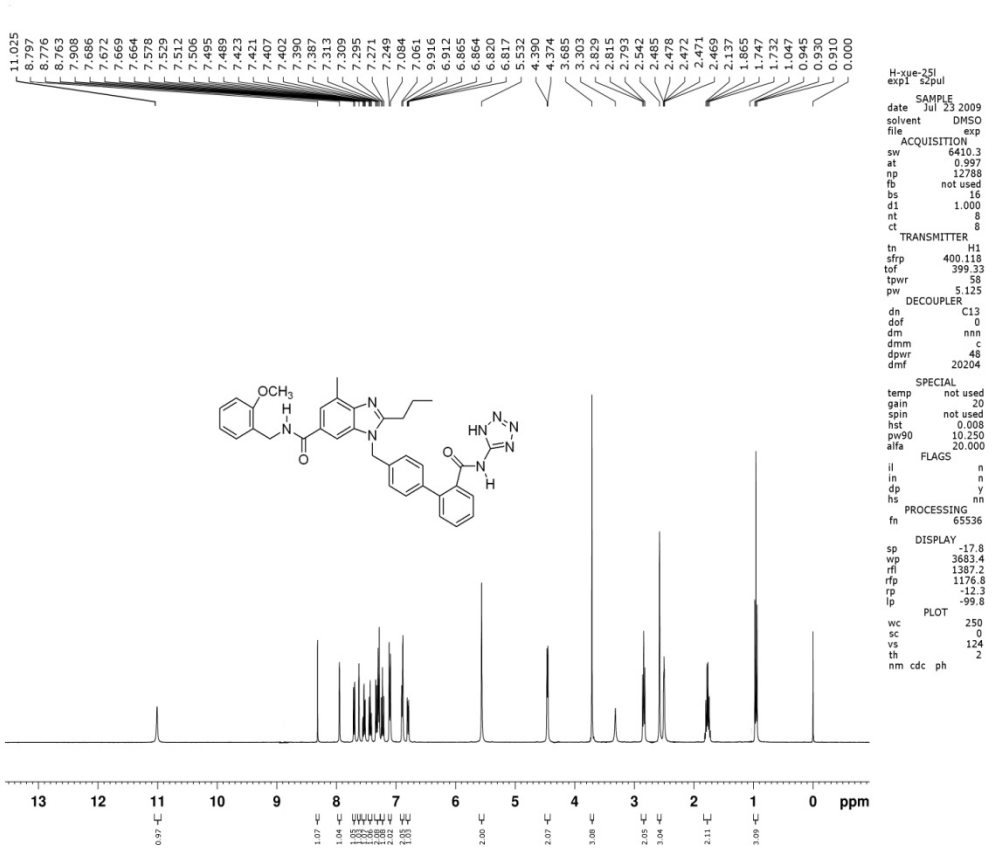


^{1}HNMR 1k



^{1}HNM

R 1l



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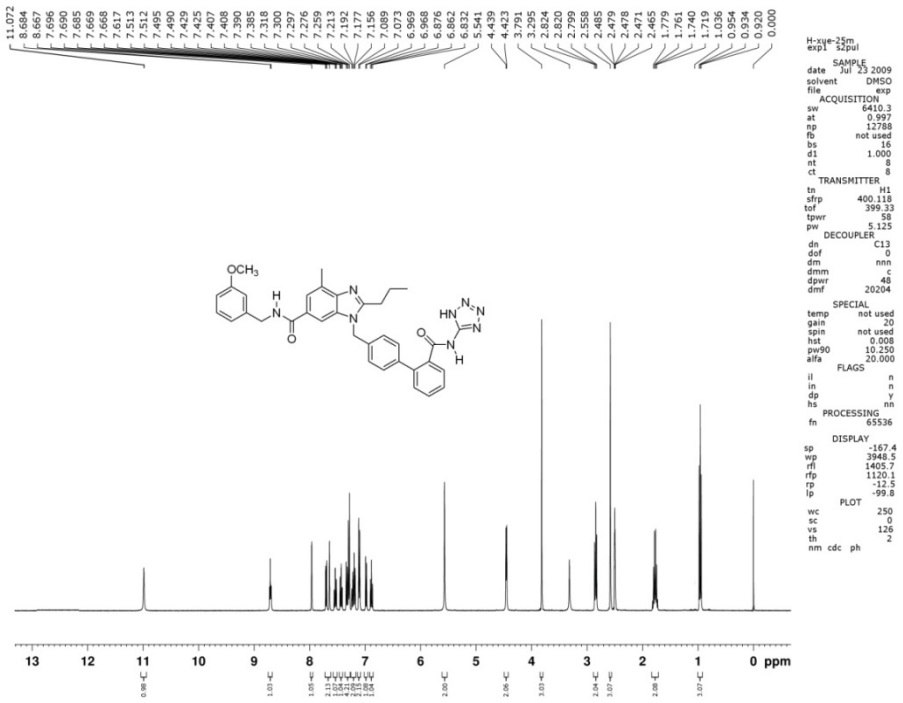

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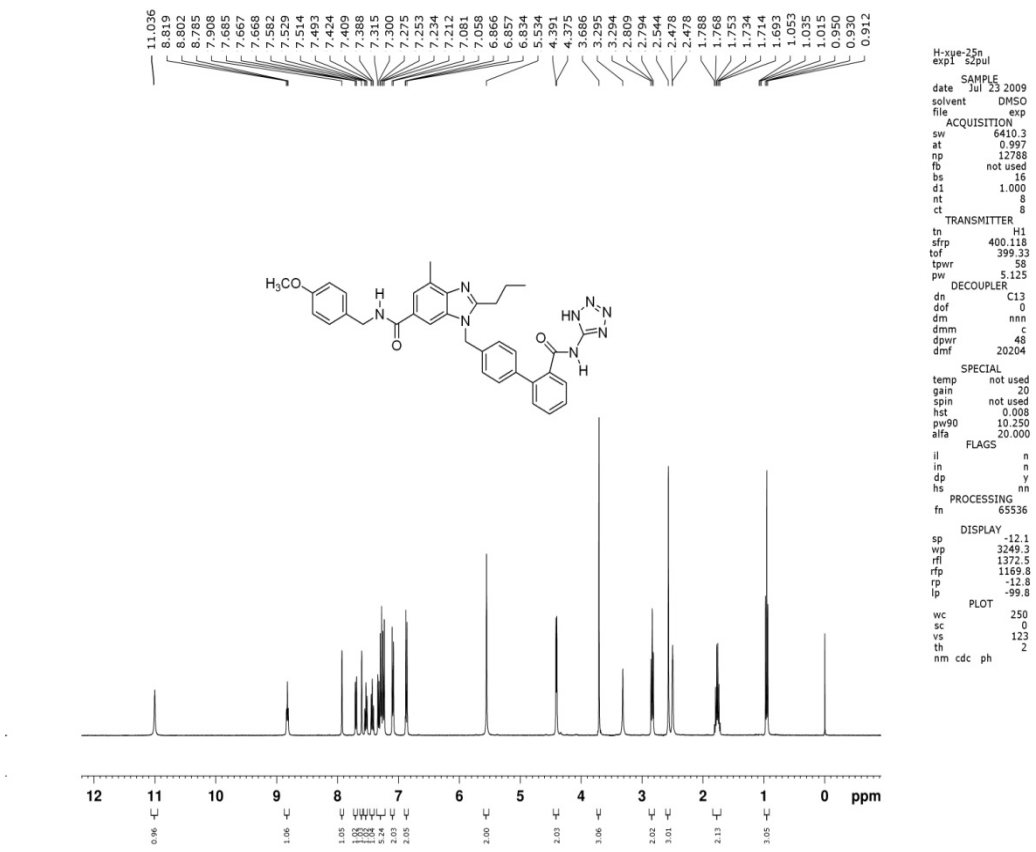


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solvent       DMSO
file           exp
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sw          6410.3
at           0.997
np           12788
fb        not used
bs             16
d1          1.000
nt              8
ct              8
    TRANSMITTER
tn             H1
sfrp       400.118
tof         399.33
tpwr            58
pw           5.125
    DECOUPLER
dn            C13
dof             0
dm            nnn
dmm             c
dpwr           48
dmf         20204
        SPECIAL
temp      not used
gain            20
spin      not used
hst         0.008
pw90        10.250
alfa        20.000
        FLAGS
il              n
in              n
dp              y
hs             nn
    PROCESSING
fn          65536

        DISPLAY
sp          -12.1
wp         3249.3
rfl        1372.5
rfp        1169.8
rp          -12.8
lp          -99.8
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sc              0
vs            123
th              2
nm  cdc   ph
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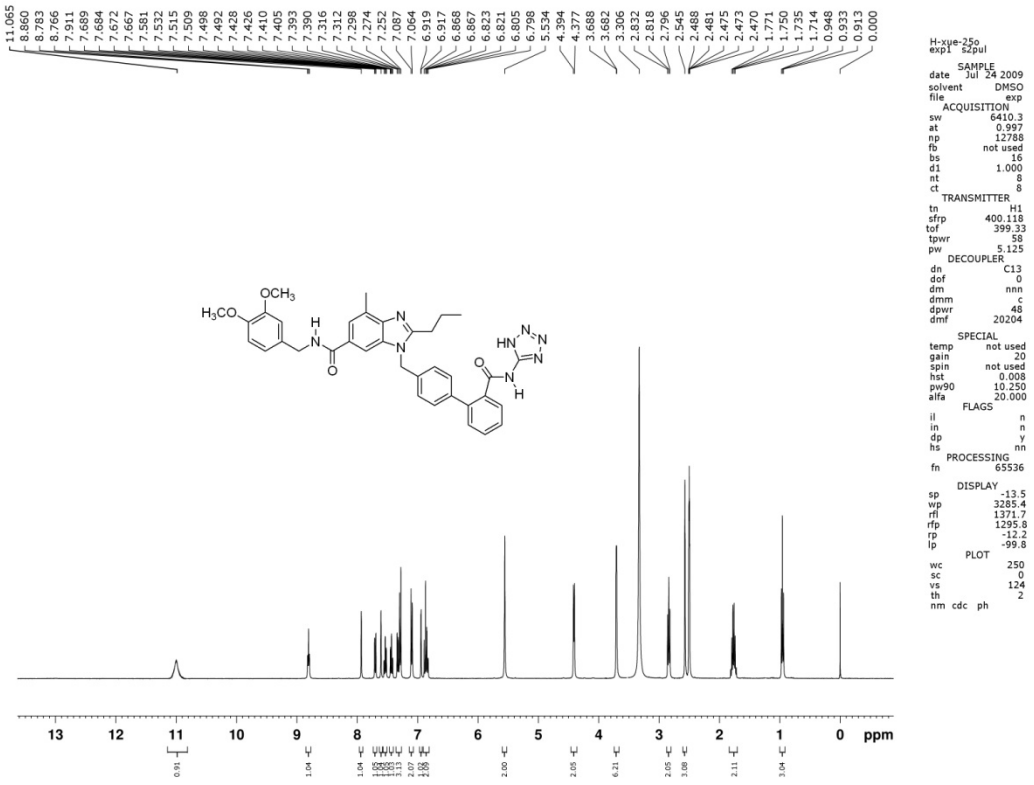
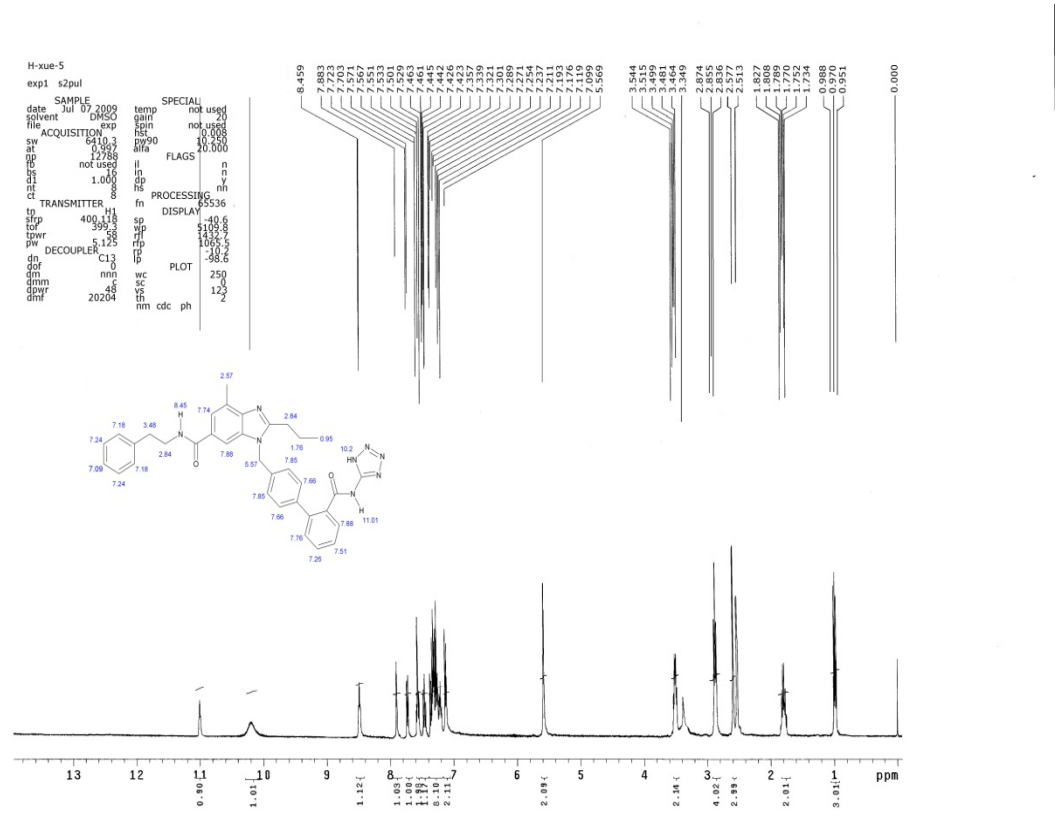
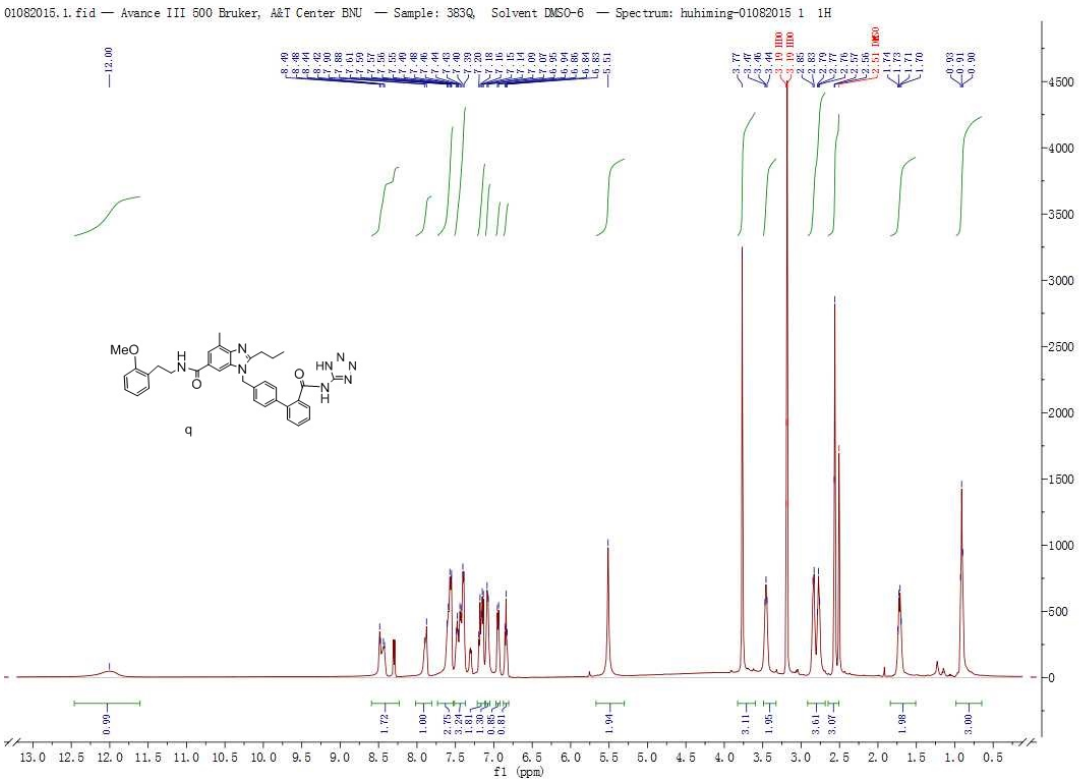
^{1}HNMR 1o



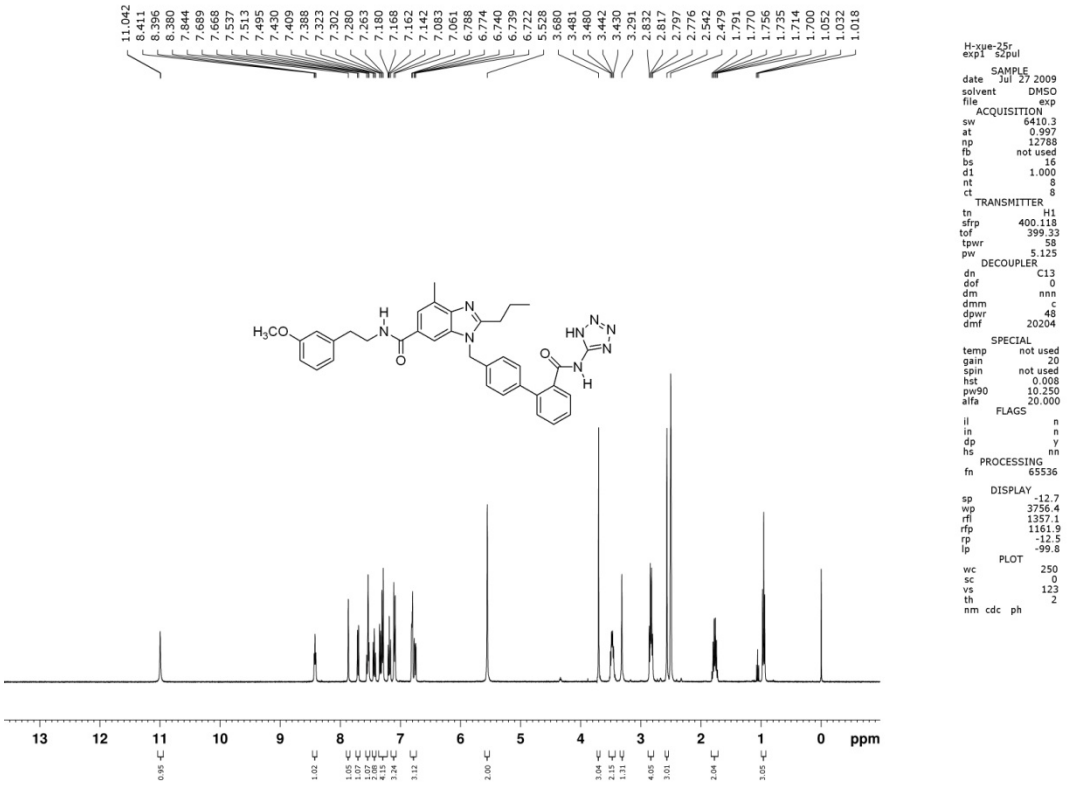
^{1}HNMR 1p



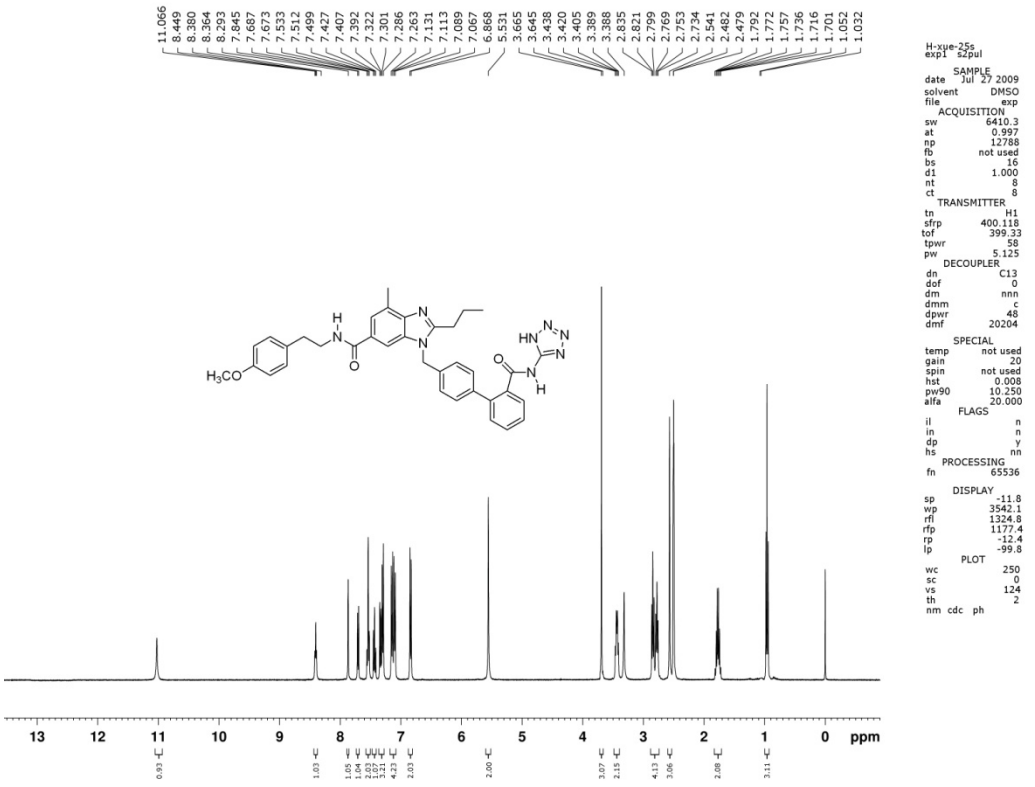
^{1}HNMR 1q



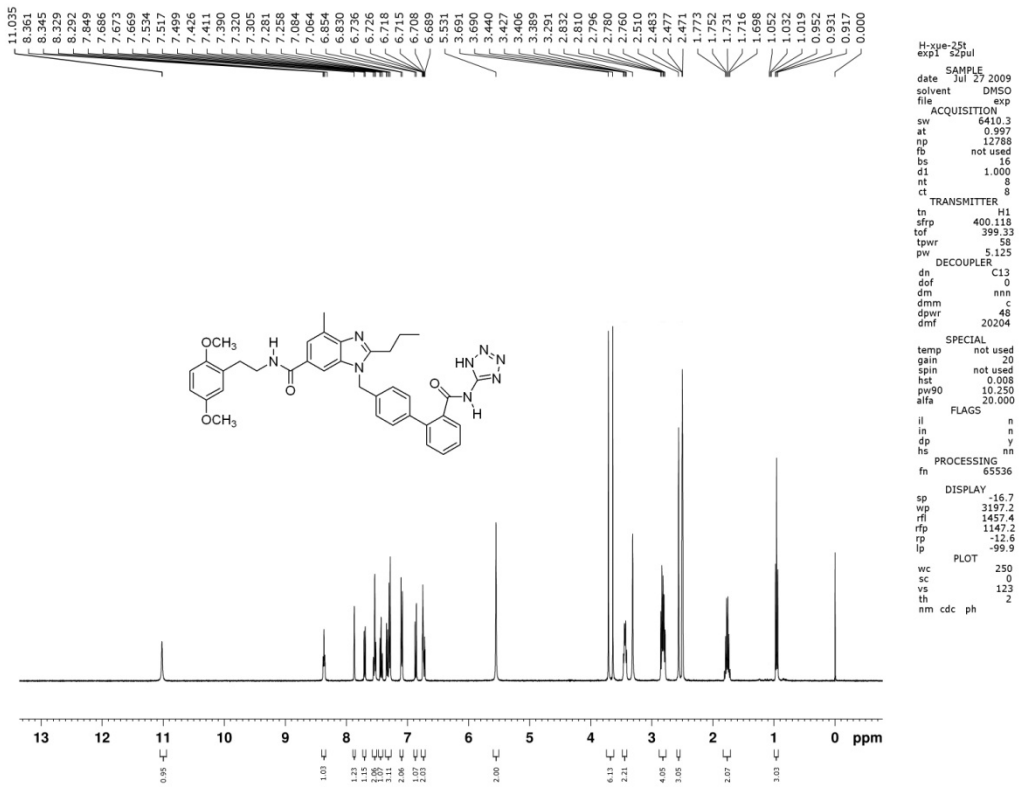
^{1}HNMR 1r



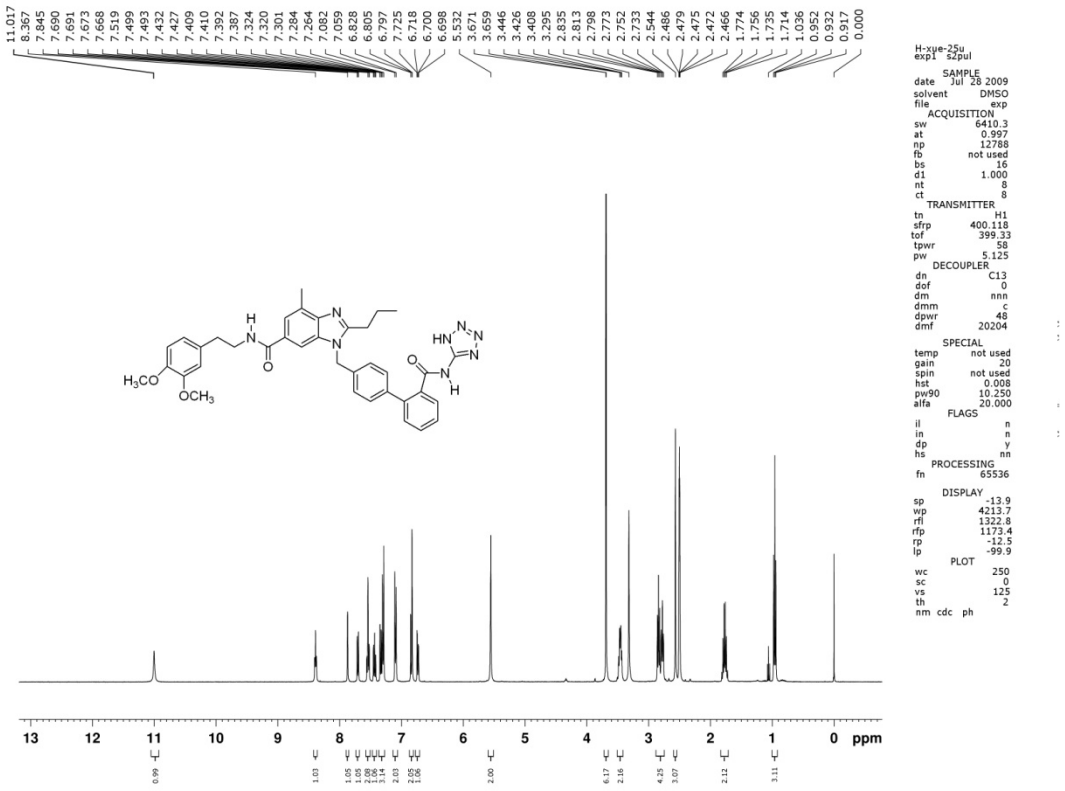
^{1}HNMR 1s



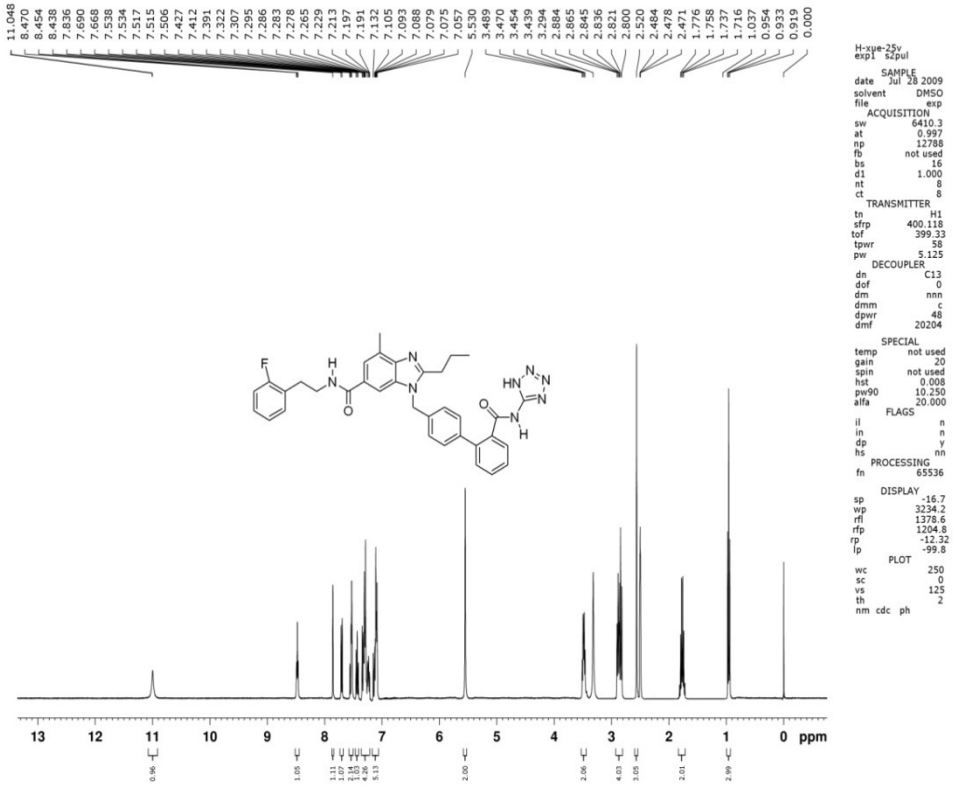
^{1}HNMR 1t



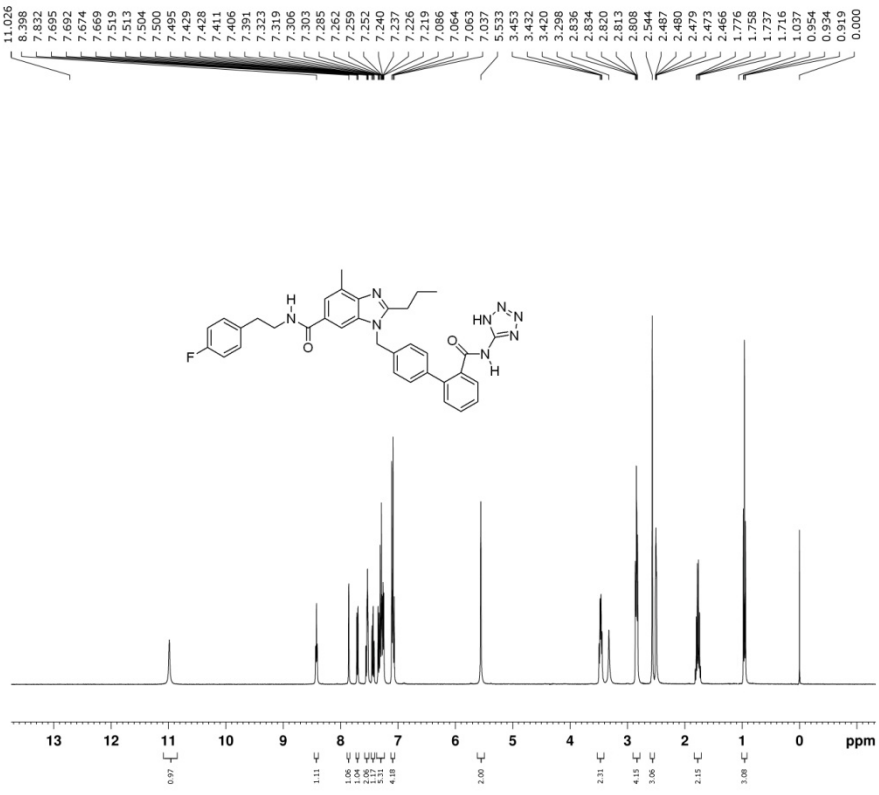
^{1}HNMR 1u



^{1}HNMR 1v



^{1}HNMR 1w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H-xye-25w
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SAMPLE
date Jul 28 2009
solvent DMSO
file exp
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sw 6410.3
at 0.997
np 12788
fb not used
bs 16
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrp 400.118
tof 399.33
tpwr 58
pw 5.125
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 48
dmf 20204
SPECIAL
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gain 20
spin not used
hst 0.008
pw90 10.250
alfa 20.000
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in n
dp y
hs nn
PROCESSING
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DISPLAY
sp -14.9
wp 3273.5
rfl 1353.4
rfp 1246.8
rp -12.6
lp -99.8
PLOT
wc 250
sc 0
vs 122
th 2
nm cdc ph
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12
11
10
9
8
7
6
5
4
3
2
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ppm
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1.11
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1.04
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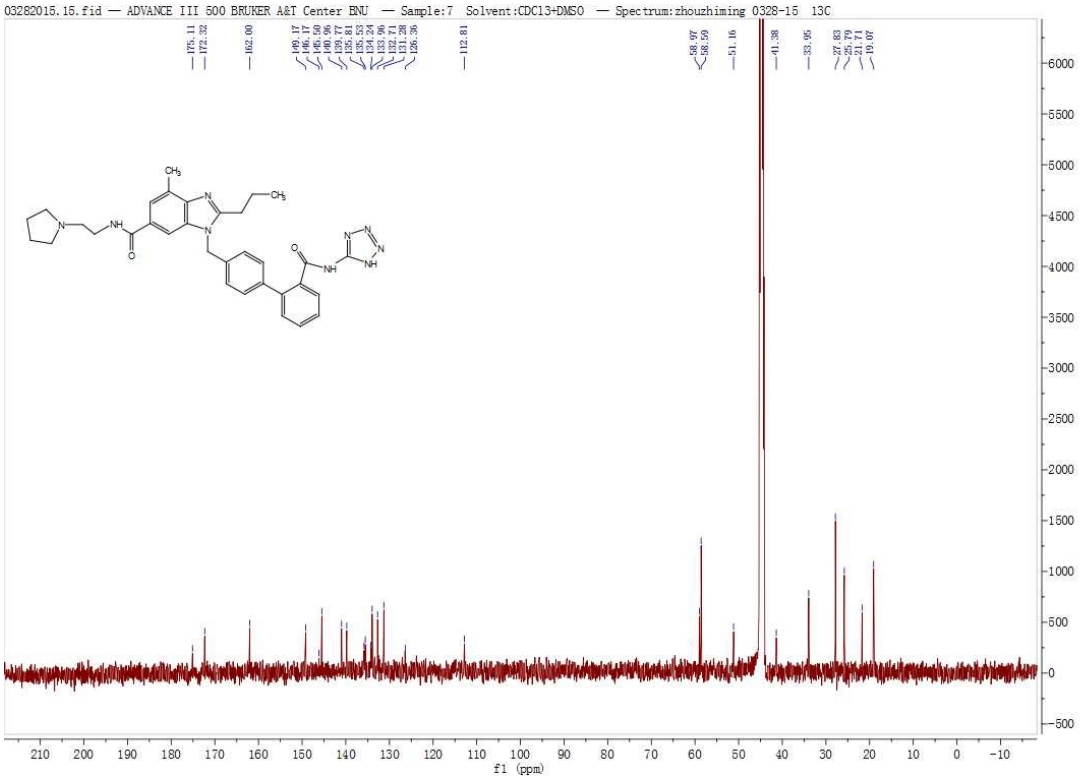
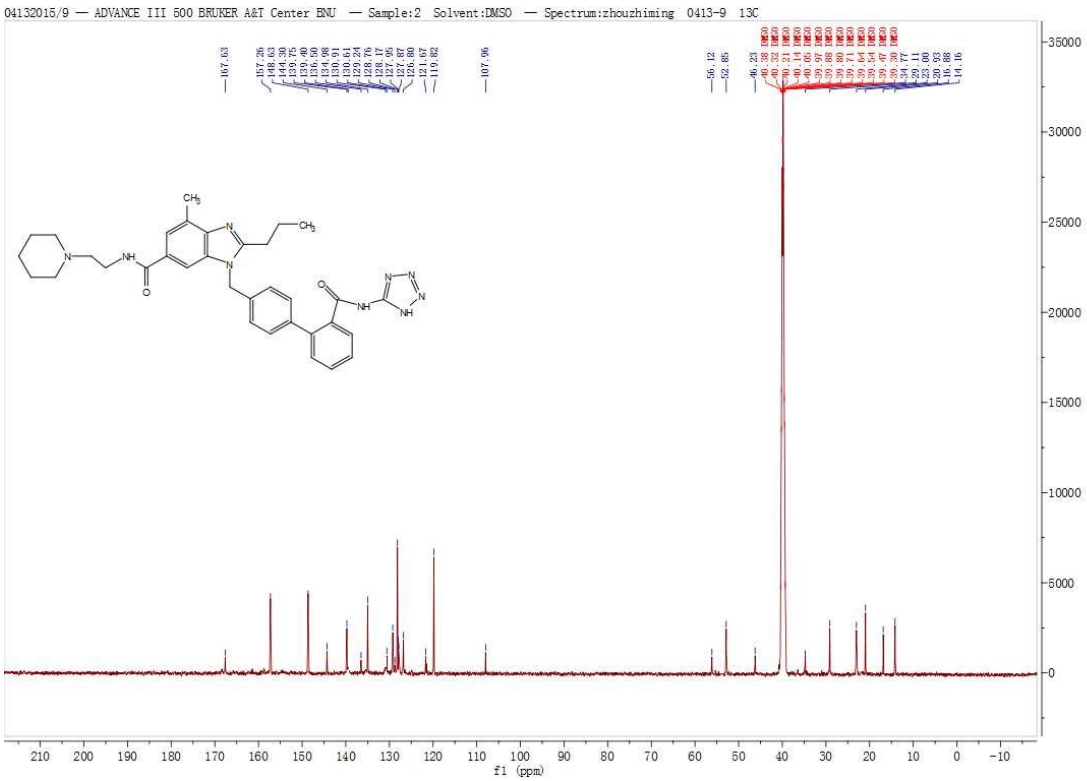
2.15
3.09

4. ^{13}CNMR

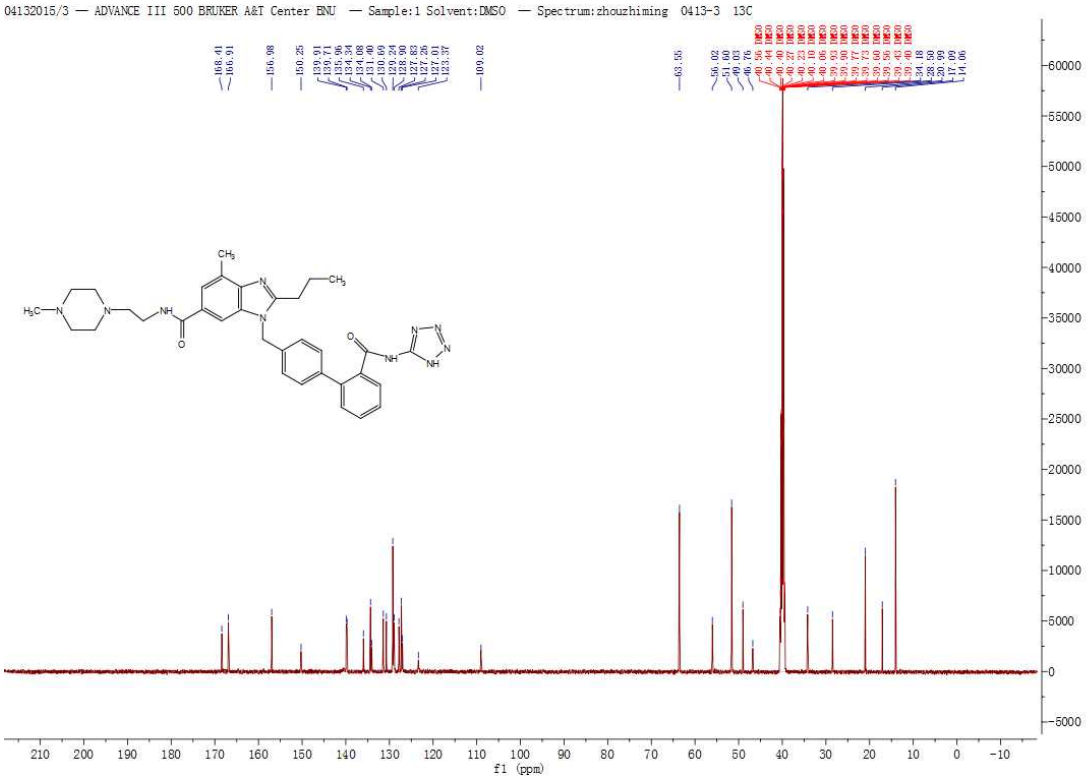
^{13}CNMR 1a



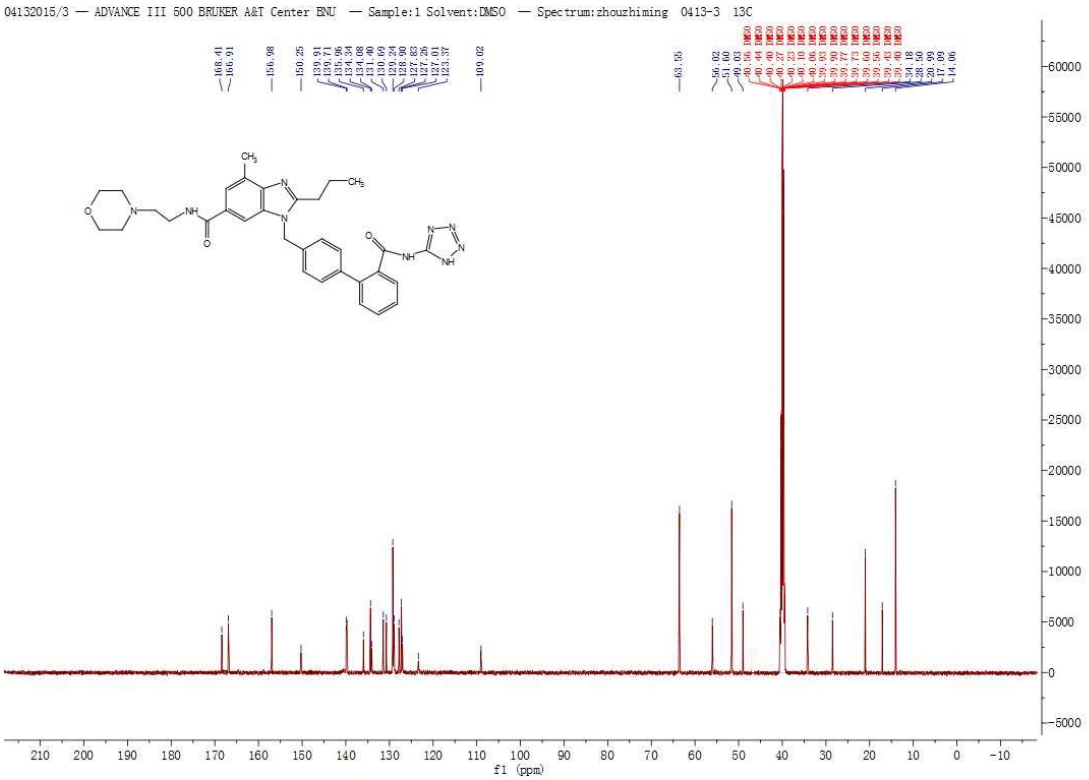
^{13}CNMR 1b



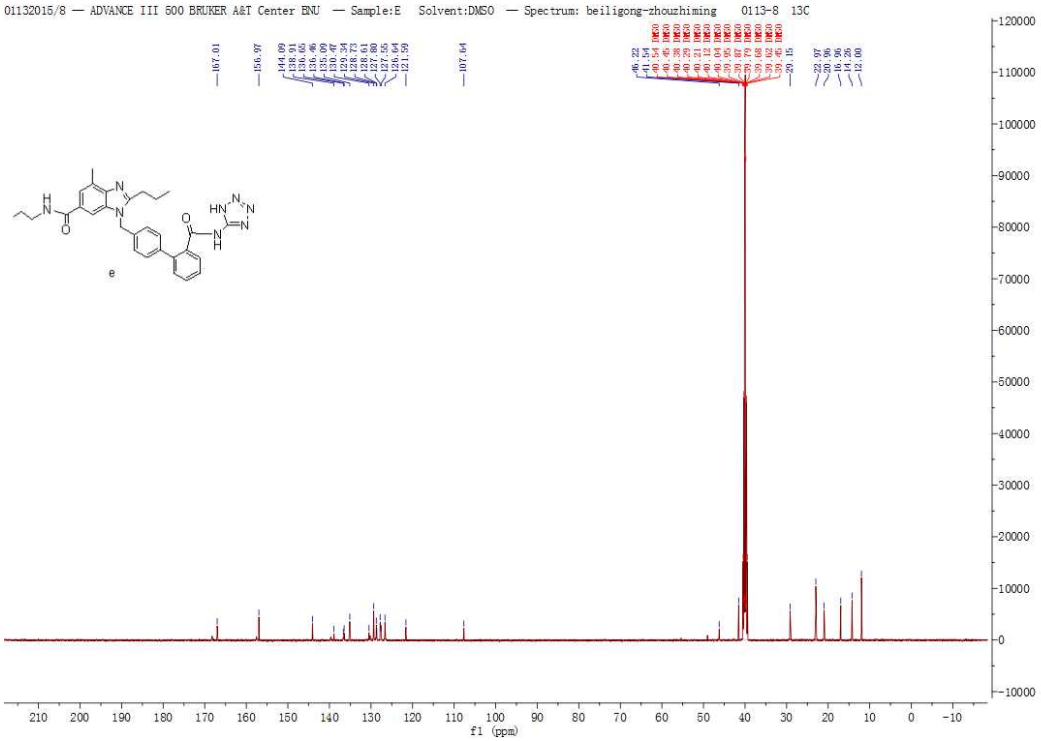
^{13}CNMR 1c



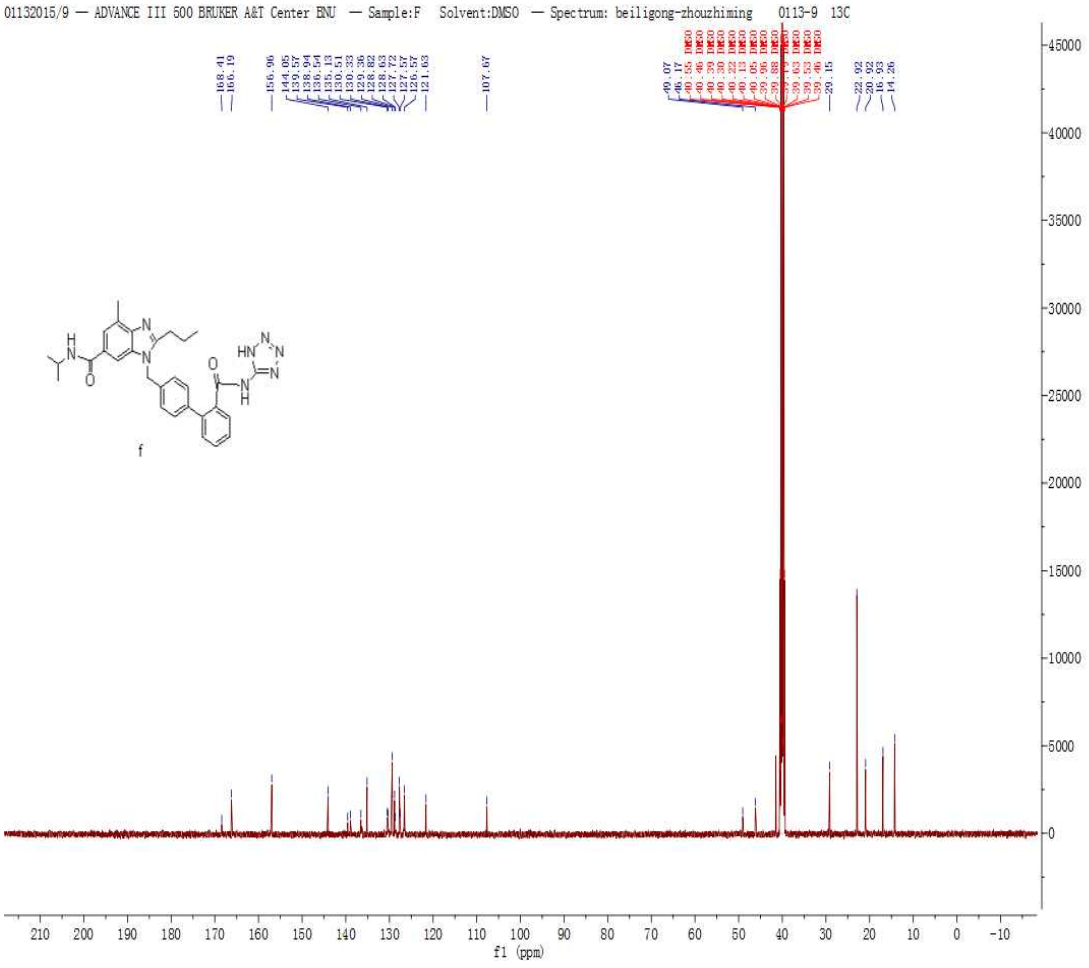
^{13}CNMR 1d



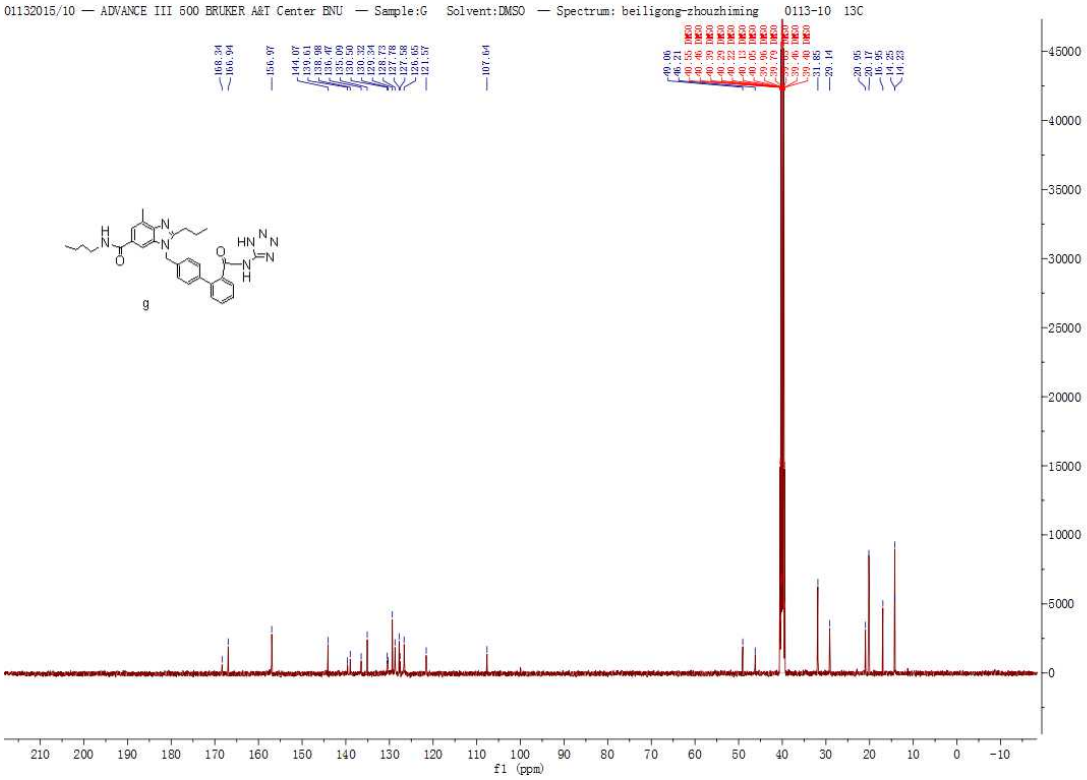
^{13}CNMR 1e



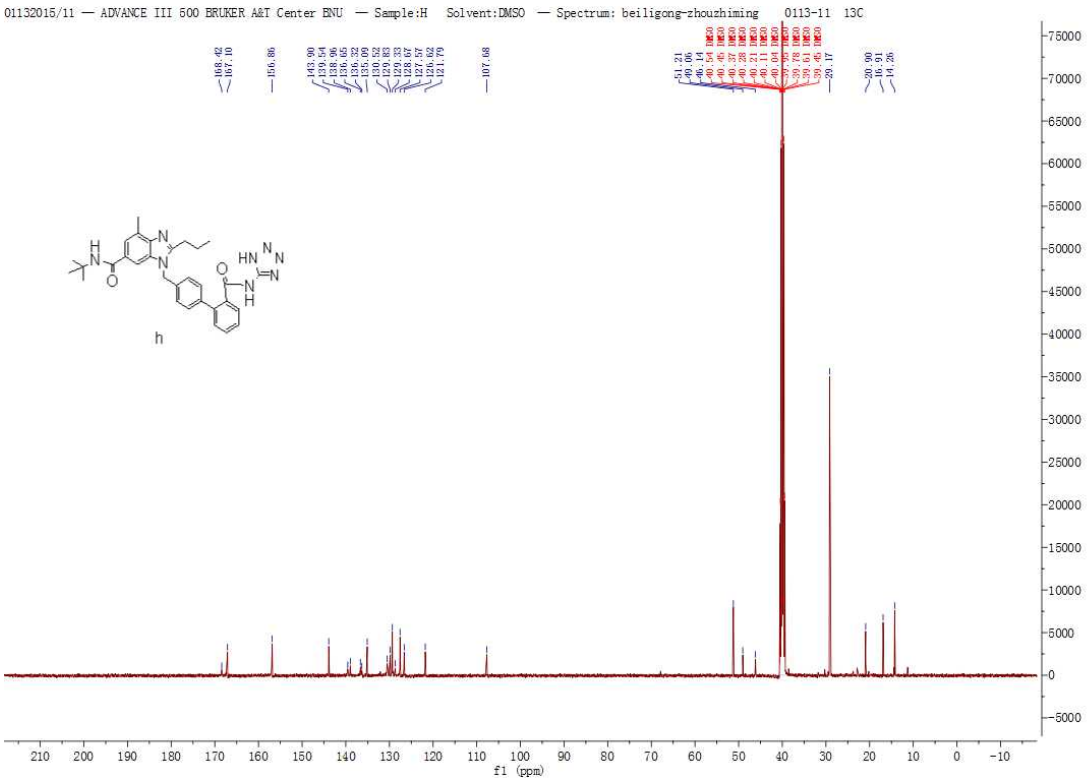
^{13}CNMR 1f



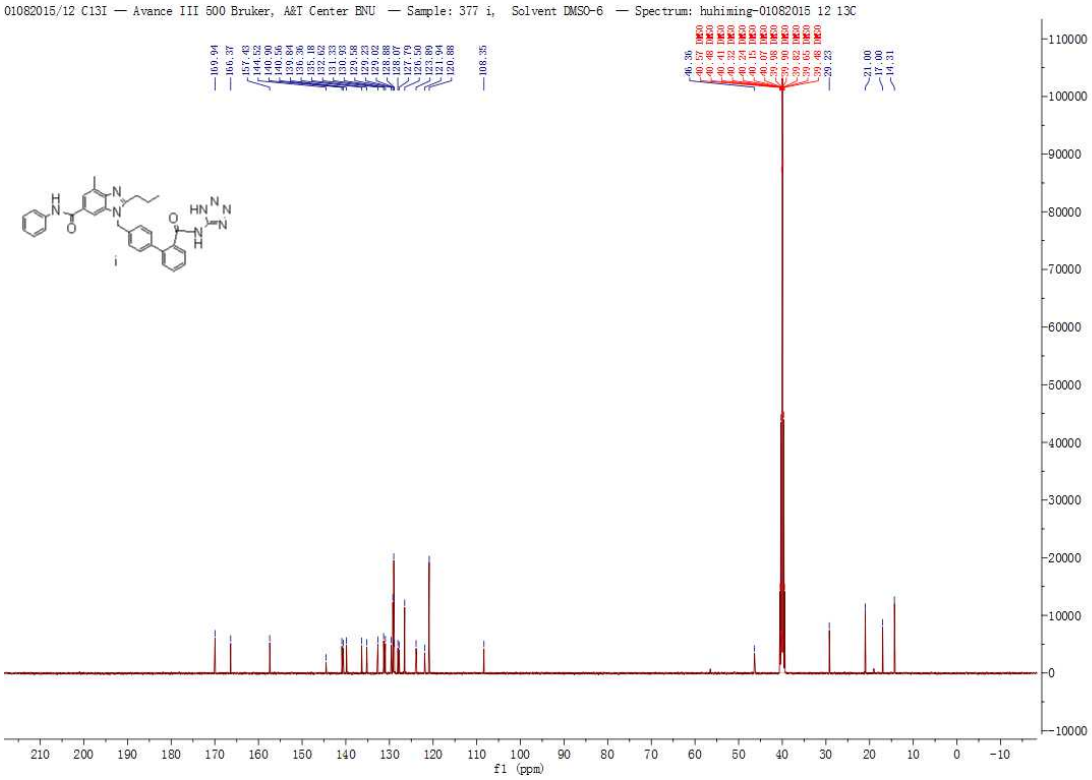
^{13}CNMR 1g



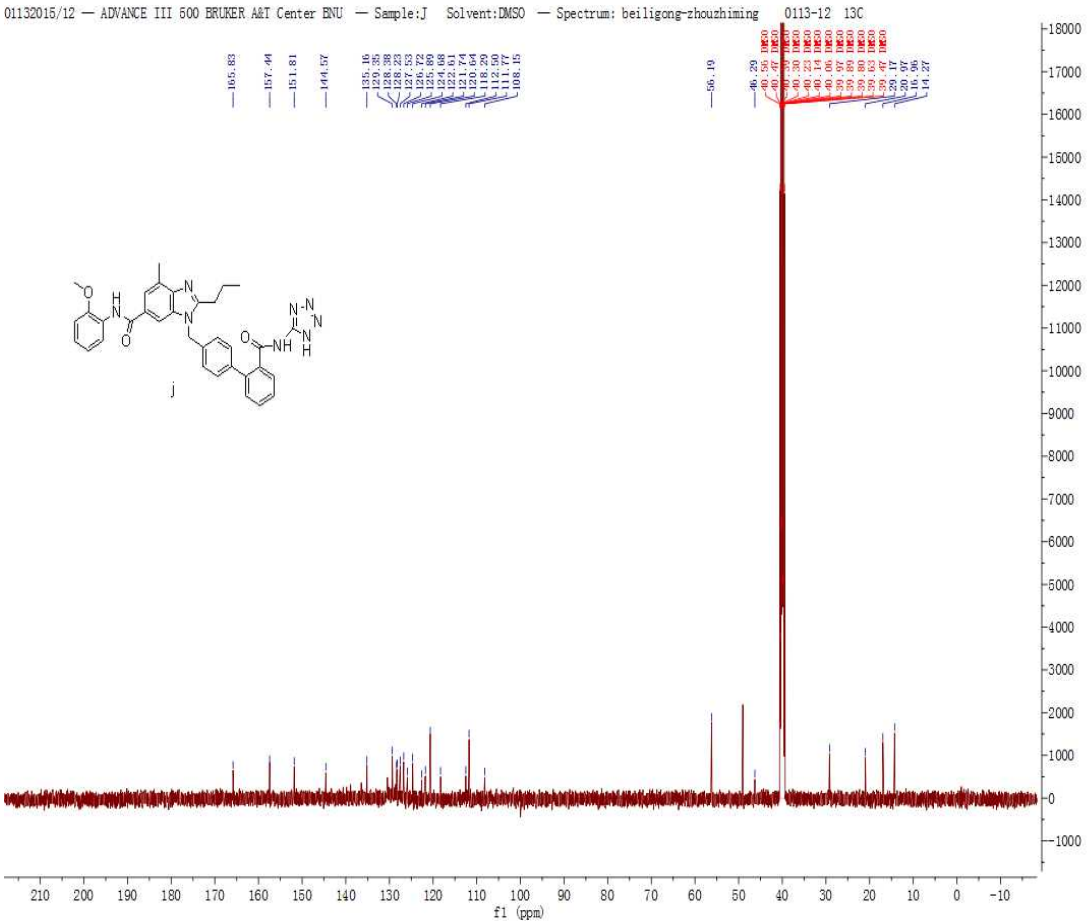
^{13}CNMR 1h



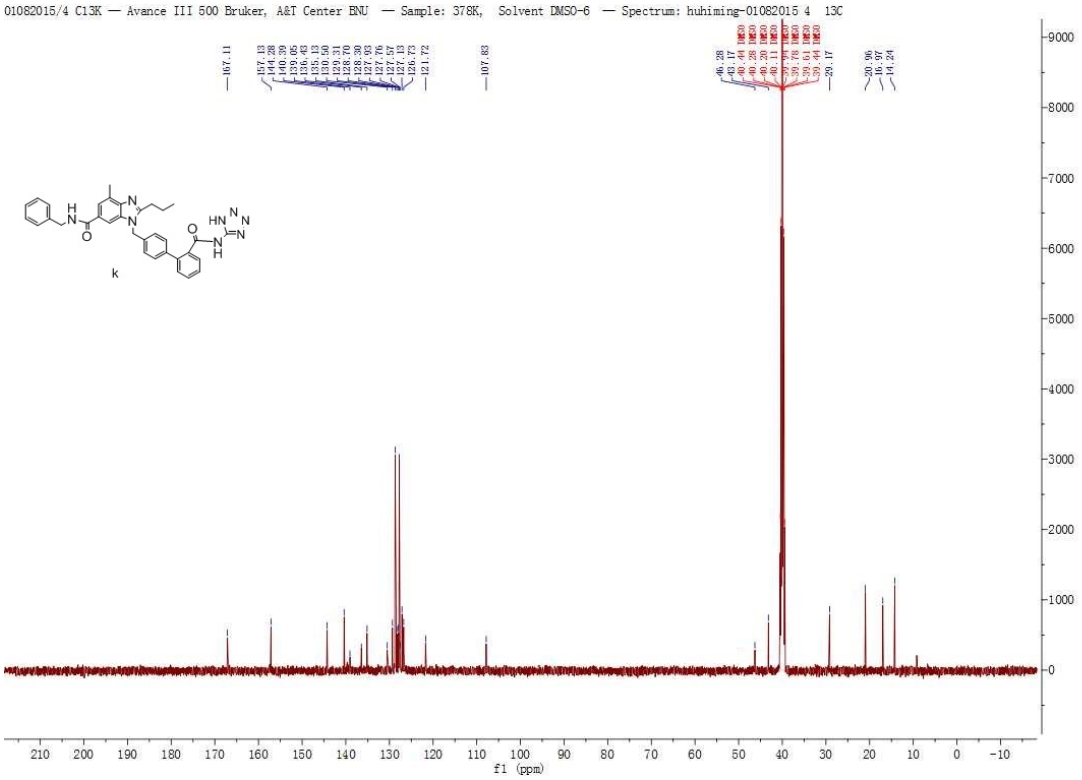
^{13}CNMR 1i



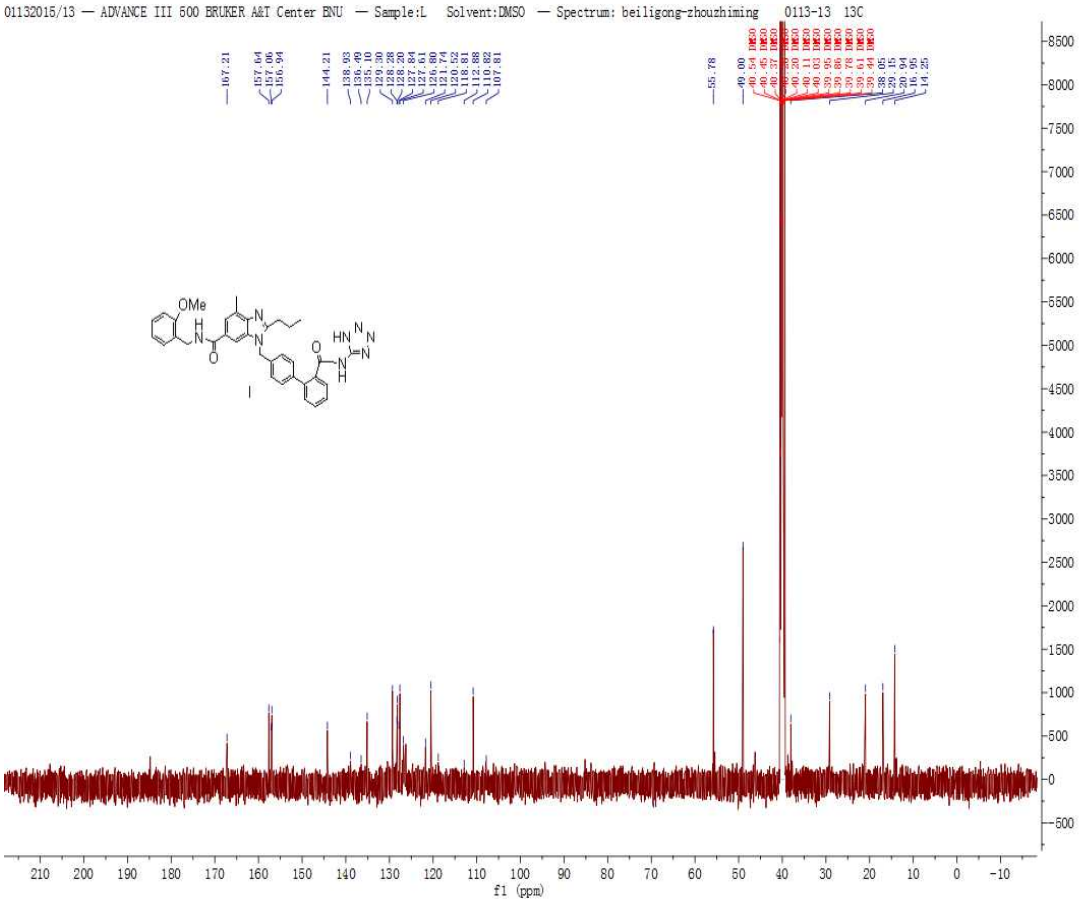
^{13}CNMR 1j



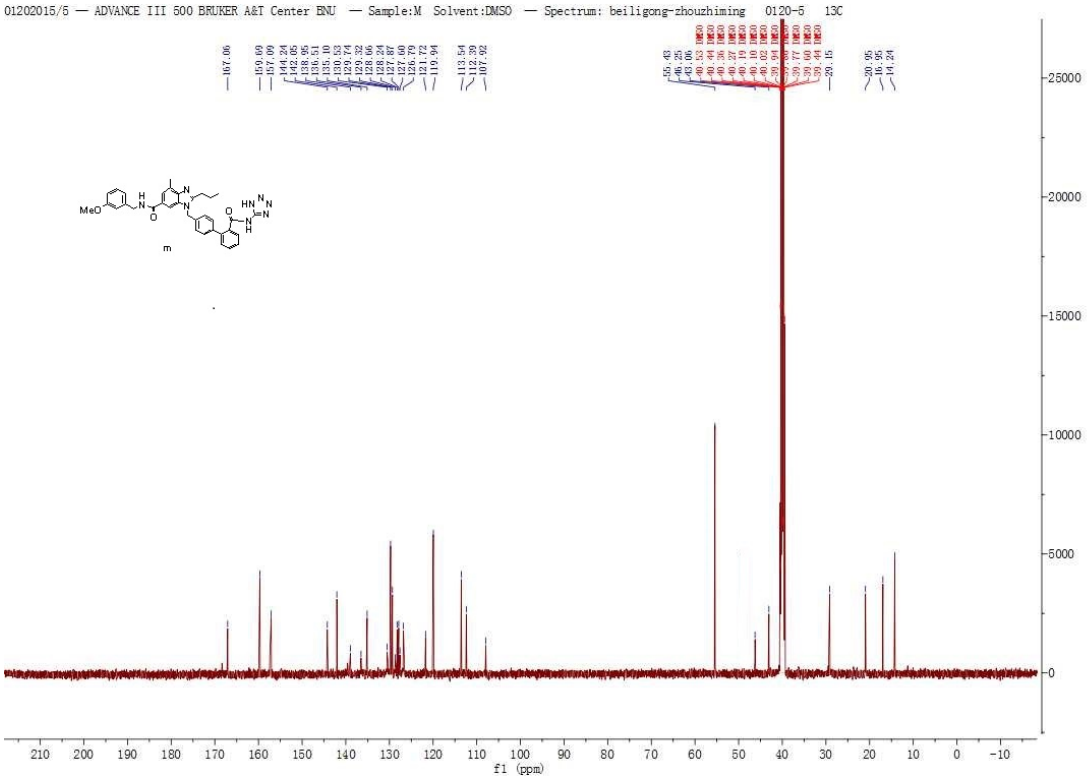
^{13}CNMR 1k



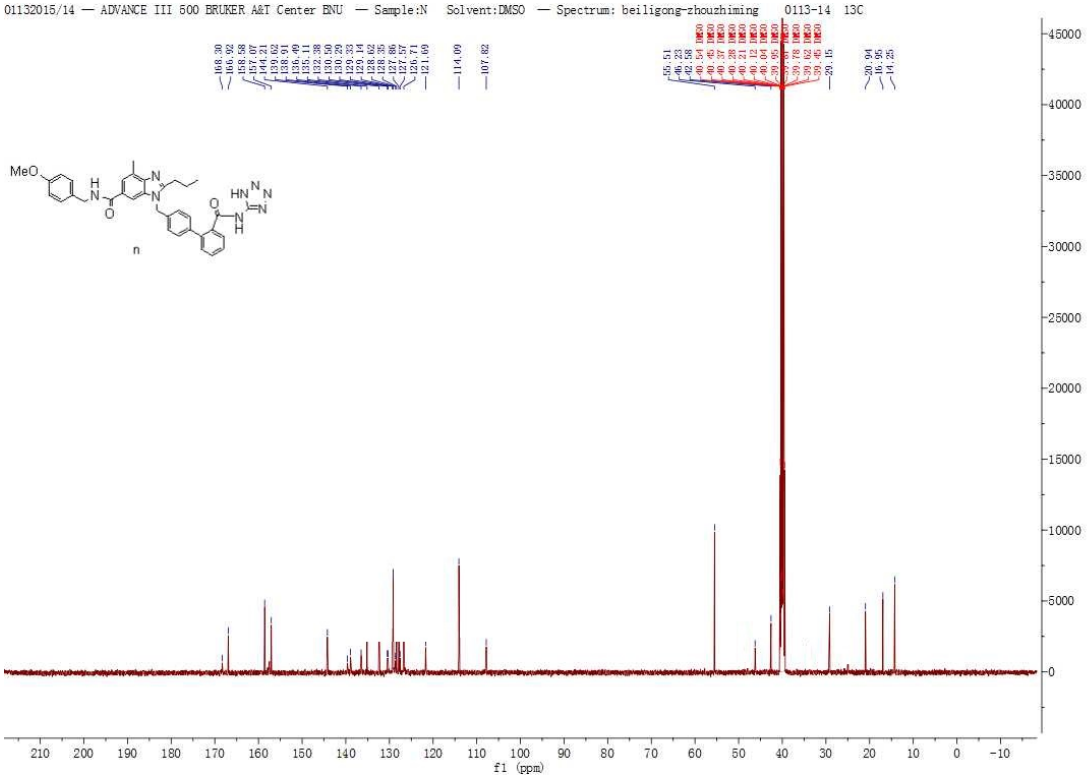
^{13}CNMR 1l



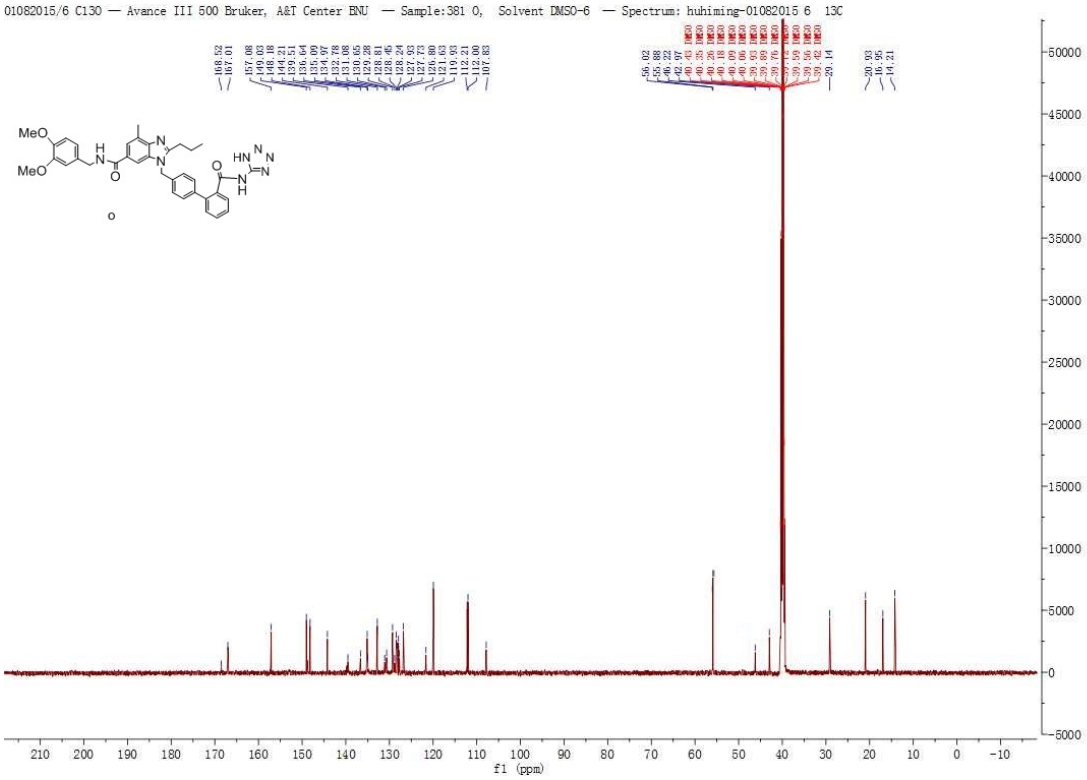
^{13}CNMR 1m



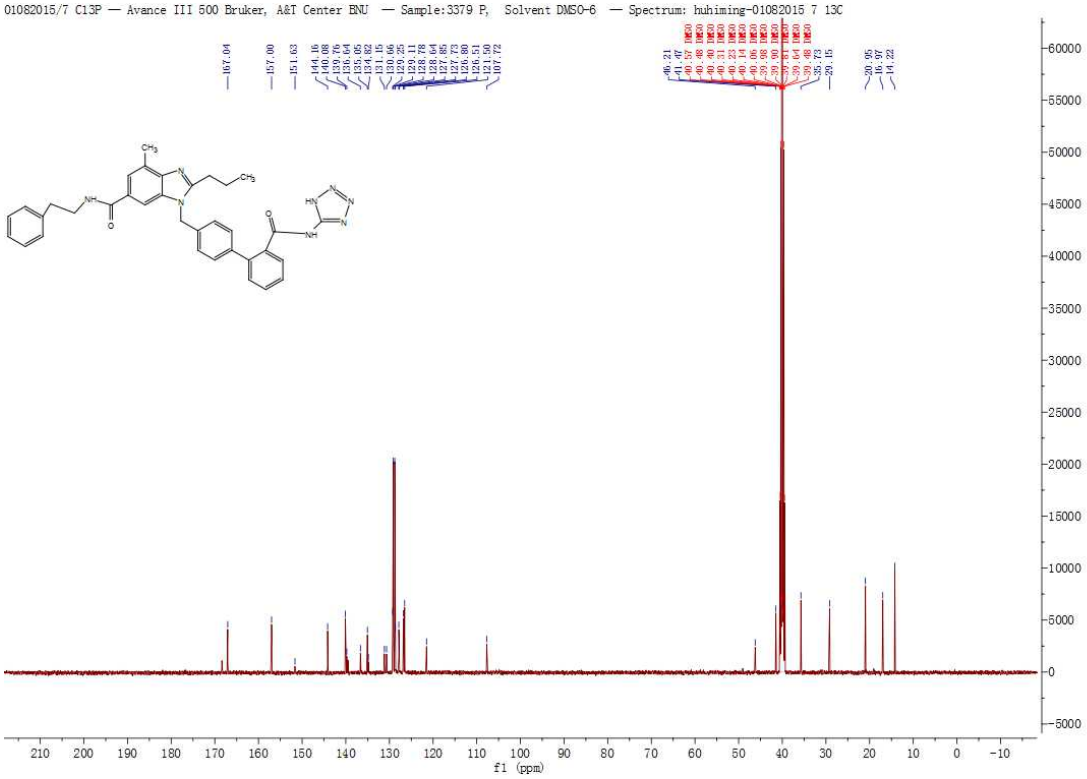
^{13}CNMR 1n



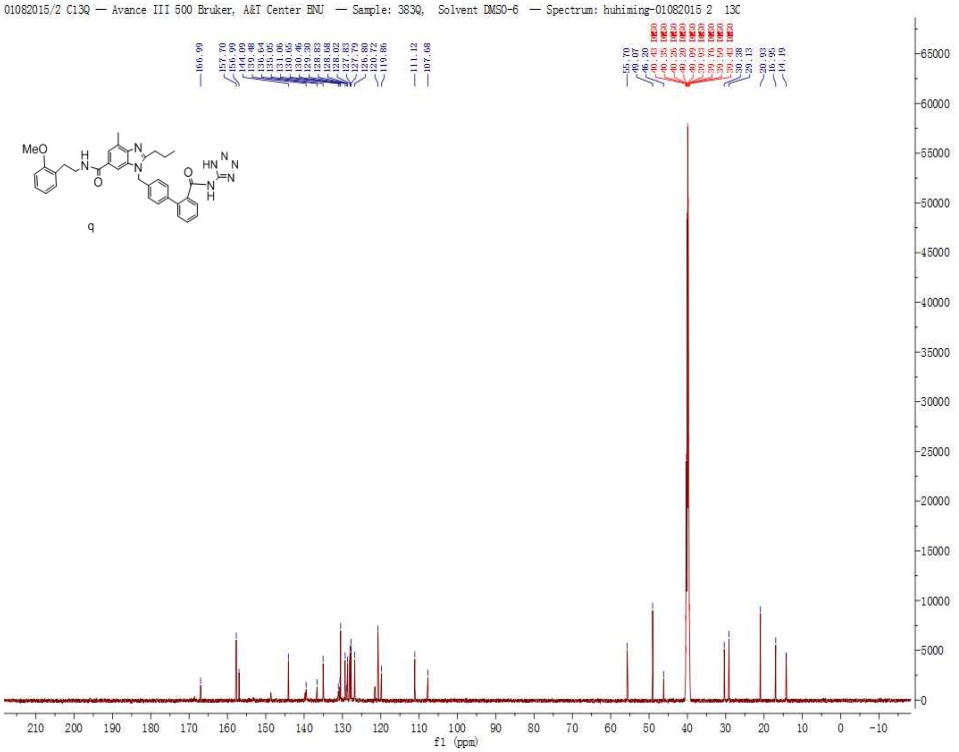
^{13}CNMR 1o



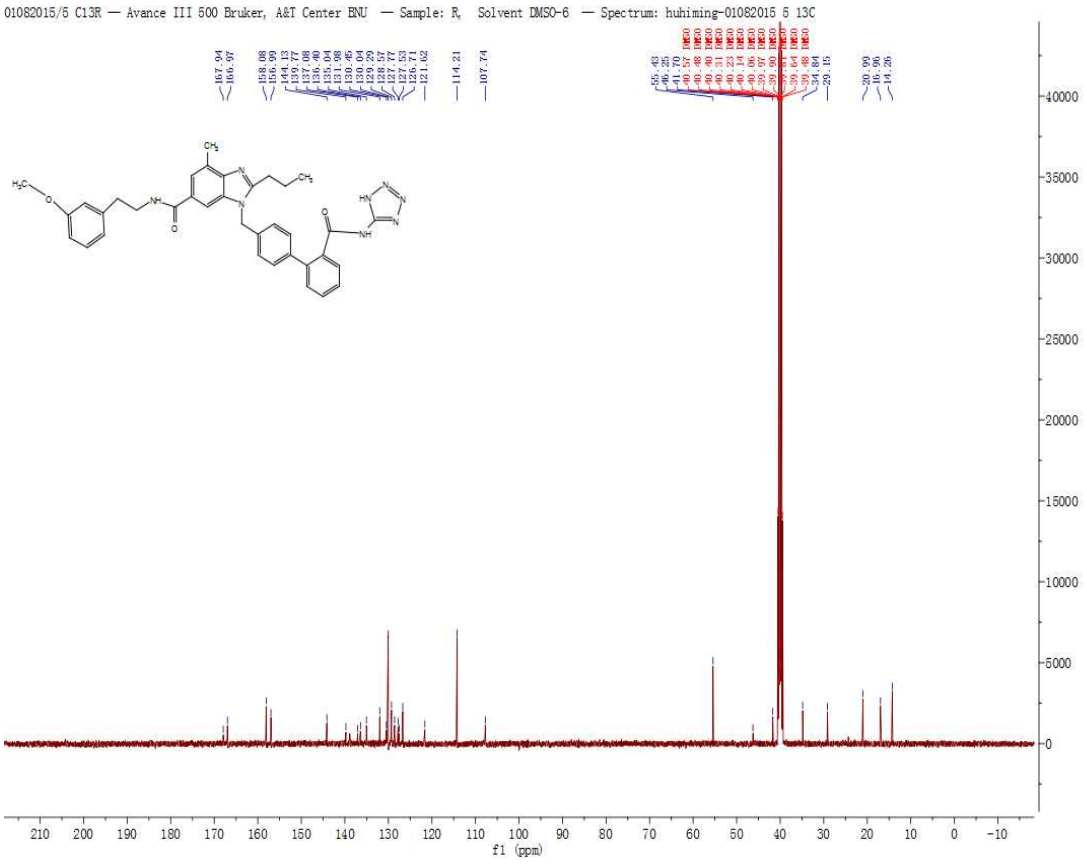
^{13}CNMR 1p



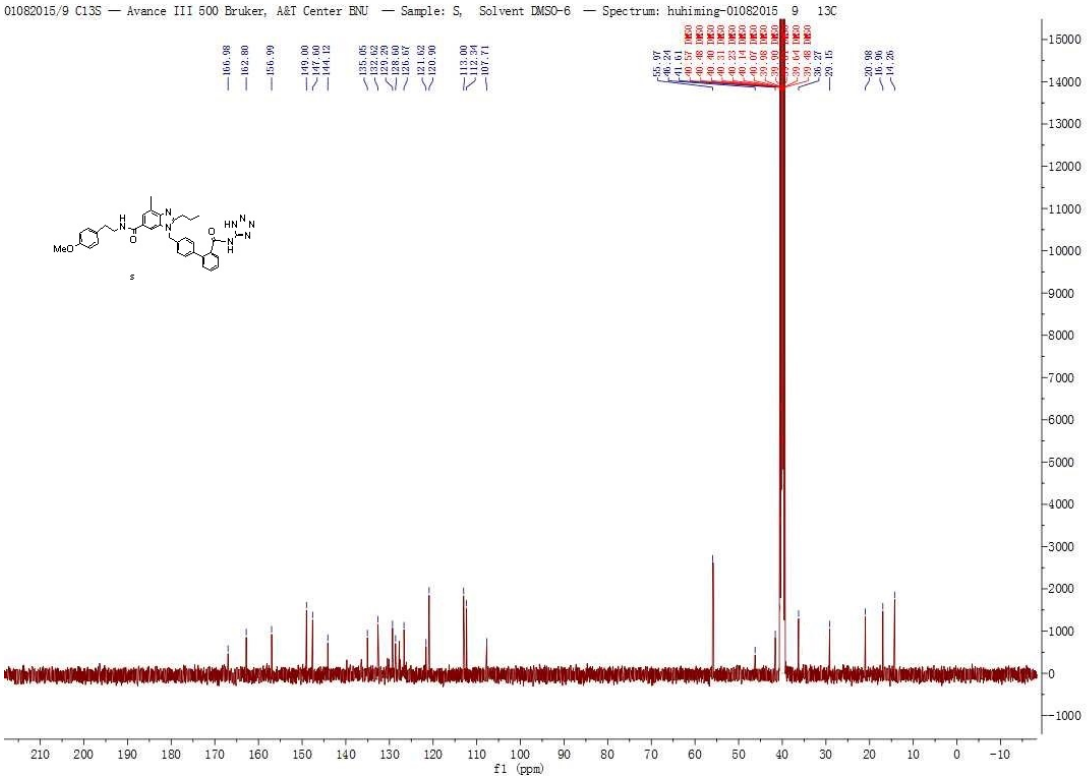
^{13}CNMR 1q



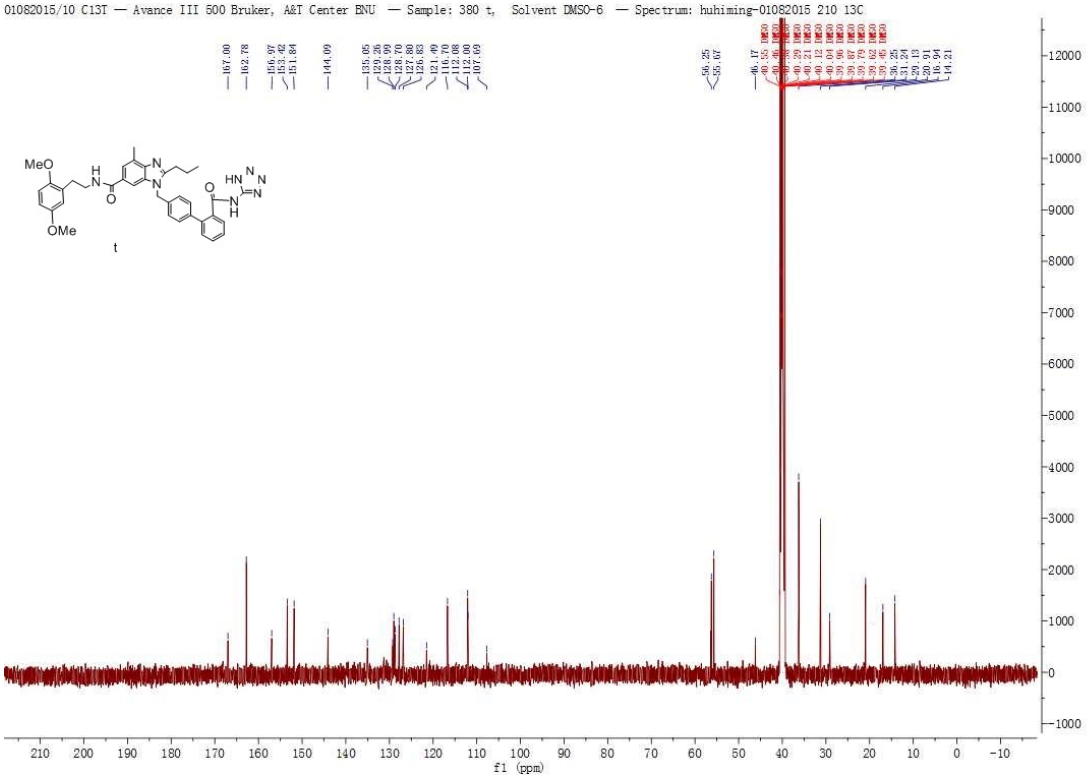
^{13}CNMR 1r



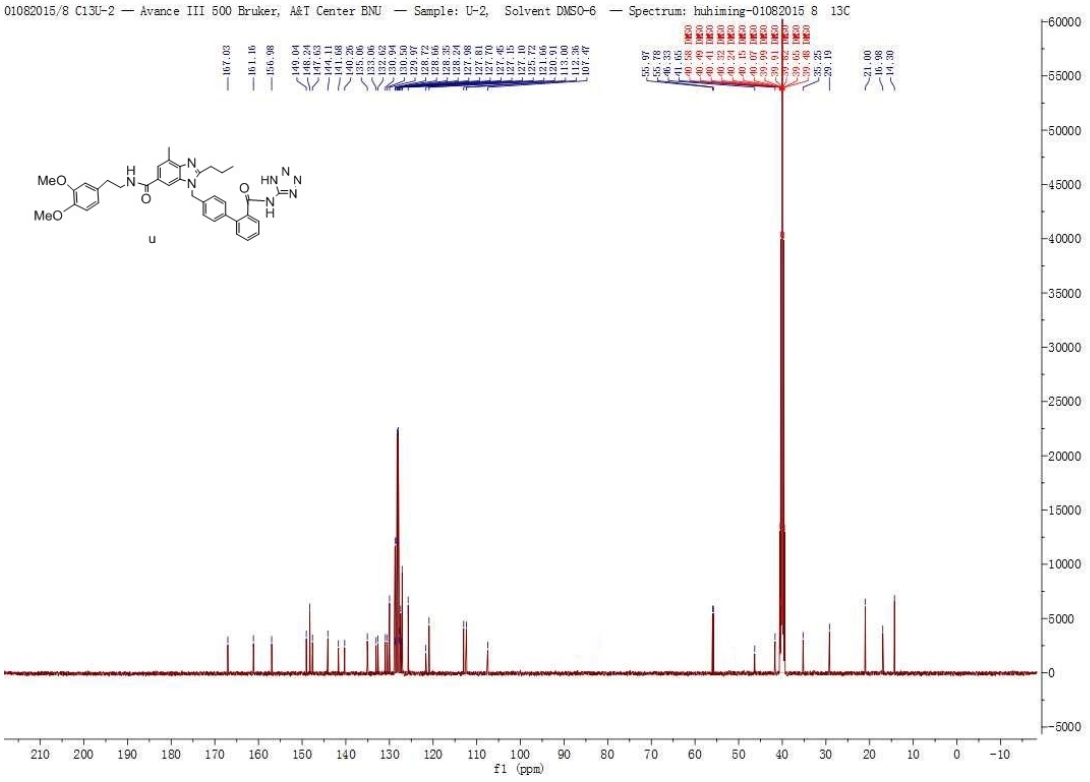
^{13}CNMR 1s



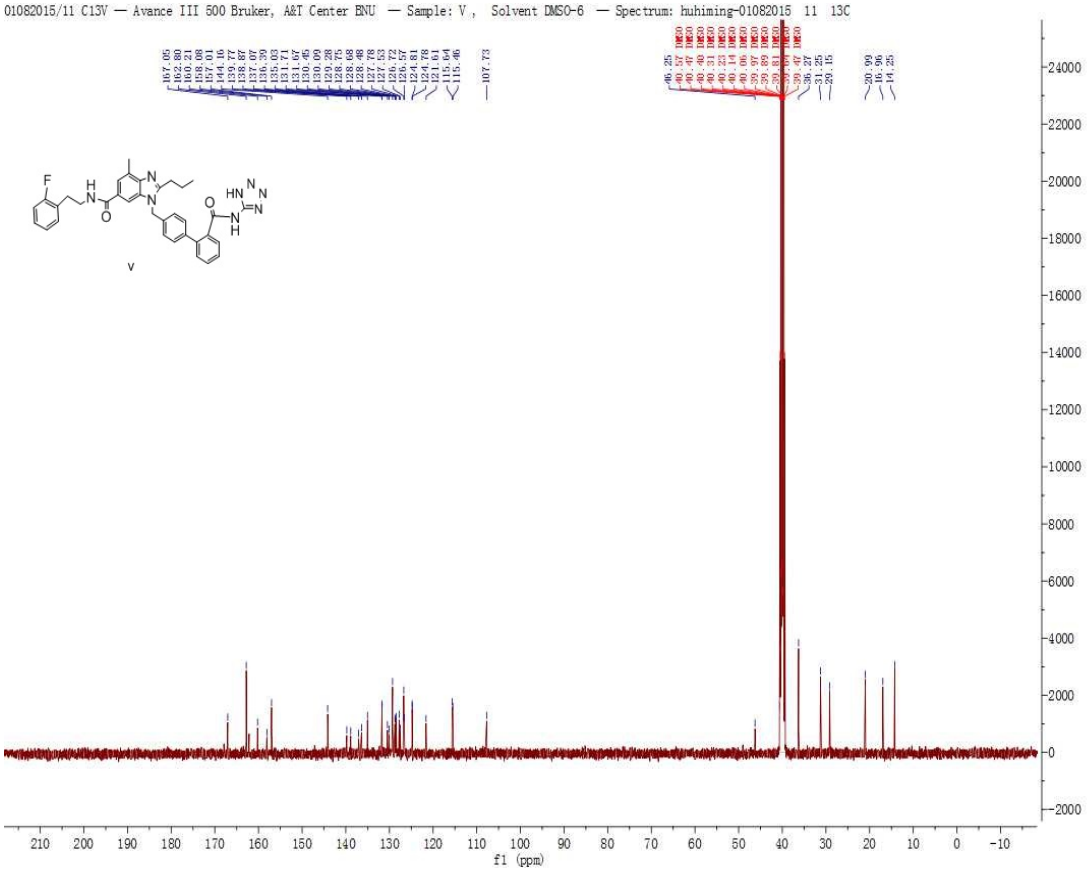
^{13}CNMR 1t



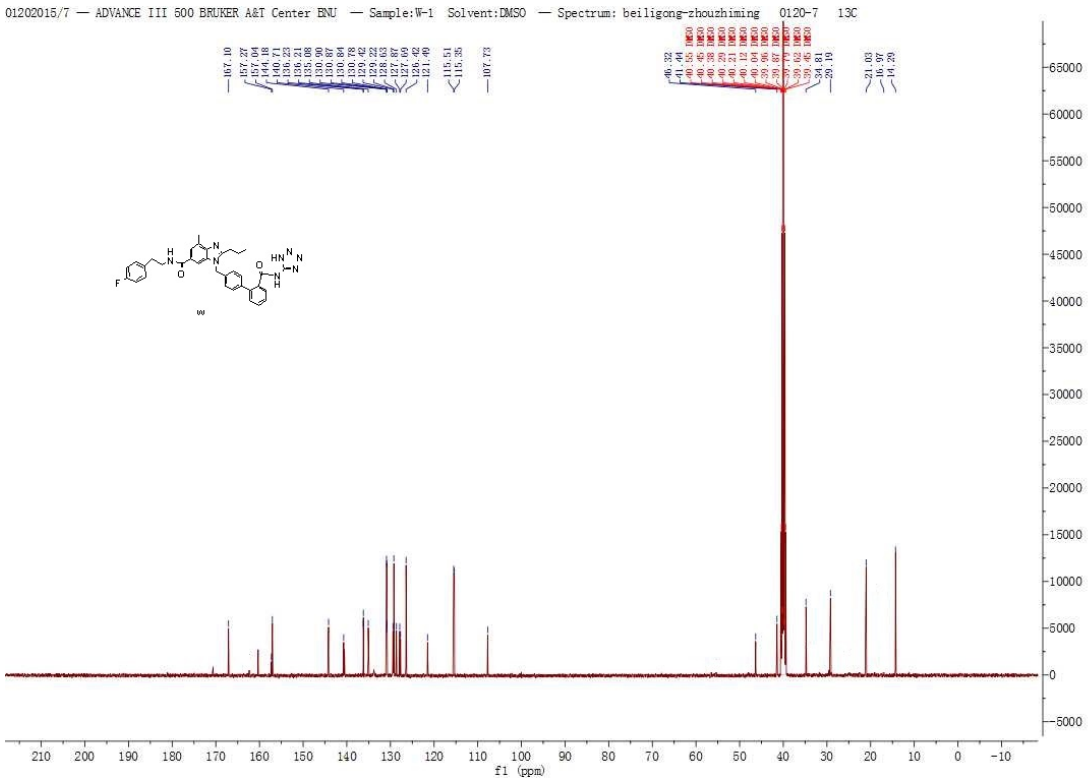
^{13}CNMR 1u



^{13}CNMR 1v



^{13}CNMR 1w


01202015/7 — ADVANCE III 500 BRUKER A&T Center ENU — Sample:W-1 Solvent:DMSO — Spectrum: beiligong-zhouzhiming 0120-7 13C
f1 (ppm)

5. Elemental analysis

1a

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25d	百分含量（%）				
	C	H	N	S	O
1	67.00	6.29	21.31		
1	67.01	6.29	21.32		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 10 2009

1b

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25c	百分含量（%）				
	C	H	N	S	O
1	67.44	6.48	20.79		
1	67.45	6.49	20.79		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 9 2009

1c

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25b	百分含量（%）				
	C	H	N	S	O
1	65.73	6.50	22.53		
1	65.74	6.50	22.54		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 8 2009

1d

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25a	百分含量（%）				
	C	H	N	S	O
1	65.16	6.14	20.73		
1	65.18	6.14	20.74		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 7 2009

1e

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25e	百分含量（%）				
	C	H	N	S	O
1	67.18	6.01	22.87		
1	67.19	6.01	22.88		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 13 2009

1f

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25f	百分含量（%）				
	C	H	N	S	O
1	67.11	6.01	20.87		
1	67.13	6.01	20.88		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 14 2009

1g

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25g	百分含量（%）				
	C	H	N	S	O
1	67.65	6.22	20.34		
1	67.66	6.22	20.35		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 15 2009

1h

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25h	百分含量（%）				
	C	H	N	S	O
1	67.58	6.21	20.35		
1	67.60	6.22	20.37		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 16 2009

1i

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25i	百分含量（%）				
	C	H	N	S	O
1	69.46	5.30	19.63		
1	69.47	5.30	19.64		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 17 2009

1j

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25k	百分含量（%）				
	C	H	N	S	O
1	68.00	5.37	18.66		
1	68.01	5.38	18.66		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 20 2009

1k

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25j	百分含量（%）				
	C	H	N	S	O
1	69.86	5.52	19.16		
1	69.85	5.52	19.17		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 20 2009

11

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25l	百分含量（%）				
	C	H	N	S	O
1	68.44	5.58	18.23		
1	68.45	5.58	18.21		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 21 2009

1m

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25m	百分含量（%）				
	C	H	N	S	O
1	68.33	5.57	18.22		
1	68.34	5.58	18.23		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 22 2009

1n

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25n	百分含量（%）				
	C	H	N	S	O
1	68.44	5.59	18.22		
1	68.42	5.57	18.21		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Jul 23 2009

1o

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25o	百分含量（%）				
	C	H	N	S	O
1	67.06	5.63	17.39		
1	67.05	5.63	17.40		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 24 2009

1p

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25p	百分含量（%）				
	C	H	N	S	O
1	70.18	5.74	18.71		
1	70.16	5.73	18.70		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 27 2009

1q

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25q	百分含量（%）				
	C	H	N	S	O
1	70.27	5.72	18.70		
1	70.28	5.72	18.71		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 28 2009

1r

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25r	百分含量（%）				
	C	H	N	S	O
1	68.81	5.77	17.83		
1	68.83	5.78	17.84		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 29 2009

1s

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25s	百分含量（%）				
	C	H	N	S	O
1	68.81	5.77	17.81		
1	68.83	5.77	17.81		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 30 2009

1t

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25t	百分含量（%）				
	C	H	N	S	O
1	67.46	5.81	17.02		
1	67.44	5.82	17.03		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Jul 31 2009

1u

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25u	百分含量（%）				
	C	H	N	S	O
1	67.50	5.81	17.00		
1	67.51	5.81	17.01		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Aug 3 2009

1v

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25v	百分含量（%）				
	C	H	N	S	O
1	68.15	5.40	18.18		
1	68.14	5.39	18.17		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日期：Aug 3 2009

1w

保诺科技分析测试中心

微量有机元素分析报告

送样人：YWL　　仪器型号 Elementar Vario MICRO CUBE (Germany)

样品编号 YWL-25w	百分含量（%）				
	C	H	N	S	O
1	68.15	5.37	18.16		
1	68.14	5.39	18.16		
2					
2					
3					
3					
4					
4					

分析人：Zhou　日 期：Aug 4 2009

6. In vitro receptor binding assay

AT_1 receptor binding assay was carried out by competitive displacement of the binding of 0.2 nM ^{125}I-labelled Sar^1-Ile^8-angiotensin II with human angiotensin AT_1 receptor, according to ref. 1. Binding to the human ET_A receptor was evaluated by incubating test compounds with CHO-K1 cells and expressing the human ET_A receptor in the presence of 0.05 nM ^{125}I-labelled endothelin 1 according to ref. 1. IC_{50} values were estimated from the linear portion of the competition curves. Each reported IC_{50} value is the mean of at least three individual experiments.

7. In vivo study of anti-hypertensive activity

Rule of Laboratory Animal and the Guide for Care and Use of Laboratory Animals

All experiments involving the use of live animals in this study were performed in compliance with Beijing Administration Rule of Laboratory Animal and the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Policy of Animal Care and Use Committee of Institute of Chinese Materia

Medica China Academy of Chinese Medical Science (Institute of Chinese Materia Medica CACMS).

Members of Expert Committees of Institution to Approve This Experiment

Professor Dr Aihua Liang, Institute of Chinese Materia Medica CACMS

Associate Professor Ting Liu, Institute of Chinese Materia Medica CACMS

Dr Lifang Wang, Institute of Chinese Materia Medica CACMS

Dr Baoqiang Dai, Institute of Chinese Materia Medica CACMS

Spontaneous hypertensive rats (SHRs) and Wistar-Kyoto (WKY) rats came from Vital River Laboratories (VRL) of Beijing, China. All the animals were male rats aged 12 weeks to 13 weeks with 200-250 g. Six WKY rats and six SHRs served as normal and control group given a vehicle treatment (water) individually. The other SHRs were divided into two groups (n=6). Group 1 received Irbesartan (20 mg/kg); group 2 was given the same dose of the tested compound **1p**. Both the vehicle and test compound were orally administered. Blood pressure and heart rate were measured by tail plethysmography (BP-98A, Softron, Japan), after a warming period in non-anaesthetised rats. The blood pressure measurements required only few minutes per individual rat. All data were processed analytically by **SPSS Statistics 17.0** and expressed as mean ± SEM (standard error of the mean)[2].

Table S1 Effect on blood pressure in spontaneous hypertensive rats.

Groups	Dose (mg/kg)	Index	Time of observation									
			0h	1h	2h	3h	4h	5h	6h	7h	8h	24h
Control	--	HR(BPM)	430.54±21.8	462.53±21.1	403.33±33.5	399.56±28.3	435.33±24.6	458.50±24.3	469.00±23.5	473.67±26.8	454.73±39.0	427.33±8.8
		SBP(mmHg)	188.33±5.5	181.07±9.3	184.50±9.3	189.72±7.5	193.58±11.8	190.39±7.9	182.00±8.0	185.47±4.0	188.73±6.8	187.08±2.6
		DBP(mmHg)	144.33±6.9	147.67±4.7	142.08±6.2	145.11±4.7	145.92±8.2	151.00±4.5	149.00±5.3	150.33±2.1	144.40±5.9	140.33±6.8
		MBP(mmHg)	159.17±2.9	158.93±6.2	156.33±4.1	160.06±3.9	162.08±7.9	164.17±5.1	160.00±1.7	162.13±1.9	159.20±4.4	156.00±3.9
Irbesartan	20	HR(BPM)	476.44±11.7	454.72±31.2	413.00±31.3	419.94±28.9	388.94±37.3	385.22±17.9***	391.17±16.3***	383.27±26.2***	374.93±25.9***	393.83±45.7**
		SBP(mmHg)	190.67±6.5	179.94±7.5	178.14±8.0	171.67±5.4***	168.11±6.5***	173.56±3.8***	172.83±5.9*	169.80±5.6***	171.27±4.9***	178.28±9.7
		DBP(mmHg)	148.67±4.7	140.61±7.4	137.03±4.3	134.67±6.1**	130.06±2.3***	135.28±3.8***	135.17±8.1***	129.87±1.8***	130.80±3.6**	130.67±8.9*
		MBP(mmHg)	162.89±2.8	153.83±6.4	150.81±4.4	147.06±5.4***	142.83±3.3***	148.22±2.7***	147.67±7.1***	143.20±1.8***	144.40±3.7***	146.72±6.9**
1P	20	HR(BPM)	445.83±14.3	439.89±32.0	423.22±43.3	391.78±27.9	403.67±32.0	384.50±25.7***	389.25±20.8***	378.20±4.6***	384.60±9.1***	390.50±24.8**
		SBP(mmHg)	187.17±4.4	178.72±6.1	175.28±6.7*	169.39±8.6***	168.00±5.5***	171.72±10.3***	169.58±12.6**	170.33±2.5***	176.53±11.2**	172.56±11.1*
		DBP(mmHg)	146.83±3.7	140.94±7.1	135.61±8.7	130.39±6.3***	126.33±7.0***	123.11±2.5***	119.25±1.9***	120.53±6.7***	128.87±10.3***	132.78±4.4***
		MBP(mmHg)	160.39±3.7	153.61±6.3	148.83±7.4*	143.39±4.8***	140.27±5.6***	139.33±2.9***	136.08±3.9***	137.20±4.9***	144.73±9.1***	146.06±4.7**
Normal	--	HR(BPM)	341.44±26.7	339.00±40.3	331.56±9.7	310.44±27.3	307.56±10.4	313.44±3.7	305.22±7.7	328.11±26.3	314.22±15.2	323.67±8.7
		SBP(mmHg)	131.22±5.1	132.00±11.8	127.56±3.7	130.44±5.1	125.89±0.2	123.78±5.2	125.56±6.4	122.56±1.2	119.78±1.7	123.89±2.0
		DBP(mmHg)	90.67±6.7	93.89±9.2	94.67±3.3	86.89±7.8	88.22±1.8	88.22±1.8	87.67±3.2	90.11±1.3	88.78±3.3	86.44±1.0
		MBP(mmHg)	104.11±6.2	106.56±7.8	105.67±1.9	101.67±4.2	100.89±1.5	100.00±0.6	100.33±1.7	101.22±0.7	99.22±2.0	99.00±0.6

Each value represents the mean±SEM (n=6).

* Significance levels $p< 0.05$, ** Significance levels $p< 0.01$, *** Significance levels $p< 0.001$ as compared with the corresponding control.

8. Computation studies

8.1 Molecular modeling experiments

Molecular modeling studies were performed using a Silicon Graphics desktop (SGI) Fuel work station. The training set was selected as described above, and the pharmacophore model for DARAs was generated using the HipHop module in Discovery Studio, version 2.0, from Accelrys, Inc. Molecules were built in a 3D window, and conformational models for each molecule were generated using the diverse conformation module. Then, the resulting sd files were used for common features hypothesis generation using the HipHop module by default. Through these experiments, we specified the features that are crucial for binding with AT_1 and ET_A receptors, which are in agreement with the literatures 3, 4, 5, 6.

8.2 Comparison of AT_1 antagonist, ET_A antagonist and DARA pharmacophore models

We had confirmed two pharmacophore models (named Hypo-AT_1-7 and Hypo-ET_A-1) in previous work, and the present study was continued to compare with DARA models. The comparison to generated Hypo-DARA identified the important features for the compounds to be highly active and selective toward their corresponding receptors. Hypo-AT_1-7 and Hypo-DARA were firstly superimposed. **Fig. S1A** shows the four key features of Hypo-AT_1-7 (A, R, Y and N) mapped on the 'left side' of Hypo-DARA. Secondly, Hypo-ET_A-1 and Hypo-DARA were superimposed. **Fig. S1B** shows the four features of Hypo-ET_A-1 (A, R, Y and N) mapped on the 'right side' of Hypo-DARA. The aromatic ring (R) feature matched the five- and six-member aromatics by considering the orientation of the aromatic moiety. The hydrophobic aromatic (Y) feature included only the centre of mass for aromatic groups. The Y and R features were assumed to be matched because of their correspondence to the aromatic groups of the molecules. Thus, the results showed that the features hydrophobic aromatic (Y), negative ionisable (N) and ring aromatic (R) were common for the three hypotheses. These features were important to AT_1 receptor antagonists, ET_A receptor antagonists and DARAs. However, the hydrogen bond acceptor feature ($A_{2\text{-}DARA}$) in Hypo-DARA was commonly shared by DARAs but could not be mapped by Hypo-AT_1-7. Therefore, this feature was one of the key areas for ET_A selectivity. $A_{2\text{-}DARA}$ involving the isoxazole sulphonamide group was required in

any biphenyl DARA. The key feature differentiating ET_A receptor antagonists from DARAs was the hydrogen bond acceptor feature ($A_{1\text{-}DARA}$) in Hypo-DARA, because most ET_A selective antagonists lacked this feature. Thus, $A_{1\text{-}DARA}$ is an important part of AT_1 receptor antagonists.

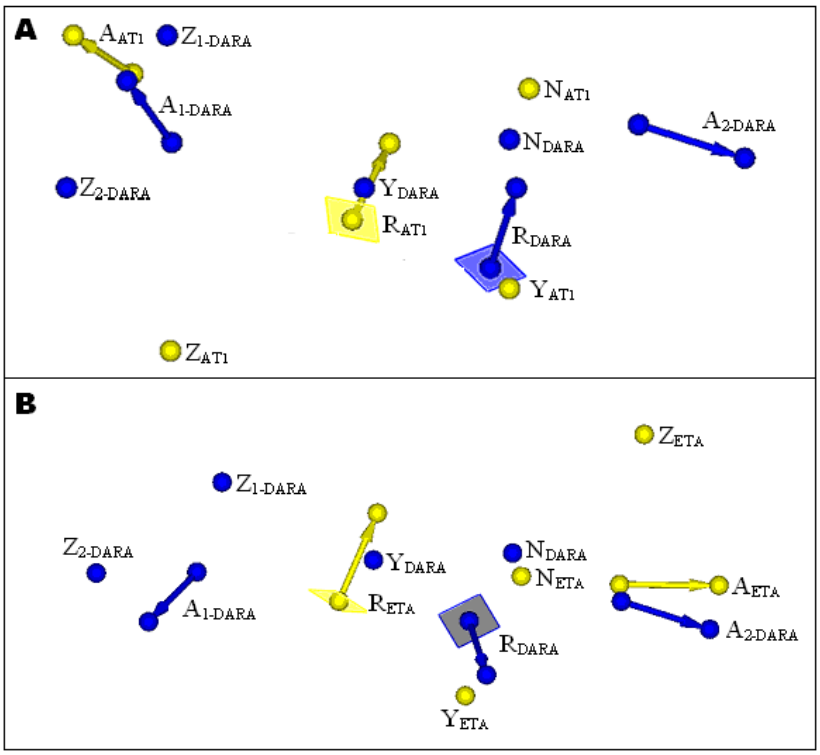


Fig. S1 (A) The comparison of Hypo-AT_1-7 (yellow) with Hypo-DARA (blue). (B) The comparison of Hypo-ET_A-1 (yellow) with Hypo-DARA (blue). (R: ring aromatic; N: negative ionizable; Y: hydrophobic aromatic; Z: hydrophobic aliphatic; A: hydrogen bond acceptor.)

8.3 Homology modeling

The model structure of ET_A receptor was generated with Insight II as previously described[7]. The model structure of ET_A was constructed using the crystal structure of bovine rhodopsin (1HZX) as the template[8]. The primary sequence of ET_A receptor was obtained from the NCBI protein sequence database (NP_001948). Sequences of ET_A and bovine rhodopsin GPCR were aligned to determine the structurally conservative regions (SCR) by using the mutation score function. After alignment, the model structure of ET_A was constructed. The obtained structures were further refined with the minimisation and MD simulations. All minimisation and MD simulations were carried out using the CHARMm force field. The final structure was evaluated using Profile-3D module in Insight II.

8.3 Molecular docking

The affinity programme within Insight II was used to dock the compounds into the AT_1 and ET_A receptor models. Consistent valence force field was selected prior to docking calculations. The binding site for the models was defined as the residues that are within 5 Å of the active site, which was found using the Binding Site Analysis module in Insight II. The centred complexes were dissolved in a sphere of TIP3P water molecules with a radius of 10 Å to consider the solvent effect. The initial position of the compound within the AT_1 or ET_A receptor model was found using a Monte Carlo–type procedure, which was used to determine the conformational and Cartesian space. The resulting structure was accepted based on an energy check. A simulated annealing phase optimised the compound placement, and the structures were subjected to energy minimisation on the basis of molecular dynamics. The final conformations were obtained through a simulated annealing procedure from 500 K to 300 K, and 5000 rounds of energy minimisation were performed to reach convergence.

8.4 Receptor–ligand interaction of DARA-3

Fig. S2A shows the binding mode of DARA-3 at the AT_1 receptor. Hydrophobic groups were positioned in a lipophilic cavity pocket formed by Val108, Ser109, Leu112, Tyr113, Val179, Trp253 and Tyr292. The anionic group interacted with Tyr184 and His256. The imidazole formed a hydrogen bond with Tyr113. **Fig. S2B** shows the binding mode of DARA-3 at the ET_A receptor. Hydrophobic groups were positioned in a lipophilic cavity pocket formed by Val85, Ile86, Val93, Leu134 and Ile355. The anionic group interacted with Tyr129 and Lys166. Isoxazole formed a hydrogen bond with Ser362. These results are consistent with those of previous studies. Moreover, imidazole and isoxazole did not form hydrogen bonds in the ET_A and AT_1 receptors, respectively. The docking results indicated that the $A_{1\text{-DARA}}$ and $A_{2\text{-DARA}}$ of Hypo-DARA were important for AT_1 and ET_A receptor antagonist activities, respectively.

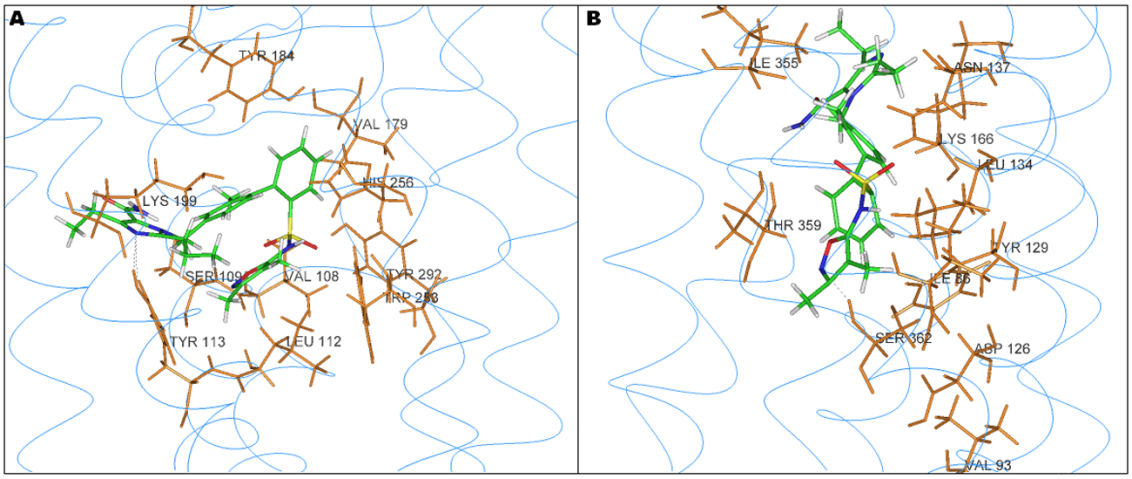


Fig. S2 (A) Model of the compound DARA-3 bound to AT_1 receptor. (B) Model of the compound DARA-3 bound to ET_A receptor. Compound DARA-3 are represented in sticks and colored by atom types (carbon: green, oxygen: red, nitrogen: blue, sulfur: yellow; hydrogen bonds are shown in black).

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