Isoxazole derivatives of 6-fluoro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)benzo[*d*]thiazol-2-amine and *N*-(pyrimidin-2-yl)benzo[*d*]thiazol-2-amine: regulation of cell cycle and apoptosis by p53 activation via mitochondrial-dependent pathways

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Supporting Information

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General

(A) Chemistry

All chemicals and solvents employed in the synthesis were supplied by Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. All the reactions were monitored by TLC performed on 2.0-6.0 cm aluminium sheets precoated with silica gel 60 (HF-254, E, Merck, India) and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H spectra were recorded in $CDCl_3$ and DMSO on Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are given in ppm relative to TMS. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

General procedure for synthesis of compound 11

Compound **10** (4.3 mmol) were dissolved in dry *N*,*N*-dimethyl formamide (10 ml), K_2CO_3 (8.7 mmol) was added and the reaction mixture was stirred for 15 min at room temperature. Propargyl bromide (4.38 mmol) was slowly added drop wise to the above mixture over a period of 15 min and stirring was continued for 4 hrs. The reaction was quenched with water and extracted with EtOAc (3×20 ml). The combined extracts were washed with water (3×25 ml) and brine (20 ml). The organic layer was dried over Na_2SO_4, filtered, evaporated under reduced pressure and

purified by silica gel chromatography (5% ethyl acetate in hexane) which gave the desired product **11**.

6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)-N-(prop-2-ynyl)benzo[d]thiazol-2-amine (11)

White solid, yield 1.4 g, 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.37 (t, 1H, *J* = 2.2 Hz, -C≡H), 3.98 (s, 3H, $-OCH_3$), 5.26 (d, 2H, *J* = 2.2 Hz, $-CH_2$-N-), 7.02 -7.10 (m, 1H, Ar-H), 7.24 -7.34 (m, 2H, Ar-H), 7.38 -7.50(m, 1H, Ar-H), 7.71-7.84 (m, 2H, Ar-H); ESI-MS: *m/z* 370 $[M+1]^+$.

General procedure for synthesis of compound 18

Compound **17** (4.3 mmol) were dissolved in dry *N*,*N*-dimethyl formamide (10 ml), K_2CO_3 (8.7 mmol) was added and the reaction mixture was stirred for 15 min at room temperature. Propargyl bromide (4.38 mmol) was slowly added drop wise to the above mixture over a period of 15 min and stirring was continued for 4 hrs. The reaction was quenched with water and extracted with EtOAc (3×20 ml). The combined extracts were washed with water (3×25 ml) and brine (20 ml). The organic layer was dried over Na_2SO_4, filtered, evaporated under reduced pressure and purified by silica gel chromatography (5% ethyl acetate in hexane) which gave the desired product **18**.

N-(prop-2-ynyl)-N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine (18)

White solid, yield 1.0 g, 93%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (t, 1H, *J* = 2.3 Hz, -C≡H), 5.54 (d, 2H, *J* = 2.3 Hz, $-CH_2$-N-), 6.98 – 7.04 (m, 1H, Ar-H), 7.23 – 7.29 (m, 1H, Ar-H), 7.42 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.78 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.85 – 7.90 (m, 1H, Ar-H), 8.70 (d, 2H, *J* = 4.7 Hz, Pyrimidine-H); ESI-MS: *m/z* 267 $[M+1]^+$.

General Procedure for the Synthesis of isoxazole derivatives 13

To a mixture of dipolarophile **11** (1mmol) and Et_3N (1mmol) in CH_2Cl_2 were added, a 9-12% aqueous solution of NaOCl (1.6 mmol) dropwise over a period of 1 hr at 0 ^{0}C and the appropriate oxime (1 mmol) in CH_2Cl_2. After being stirred at room temperature for 24 hrs, CH_2Cl_2 was removed under reduced pressure, water was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. This was further purified by column chromatography using ethyl acetate: n-hexane, (15:85) as eluent to obtain the pure product.

General Procedure for the Synthesis of isoxazole derivatives 20

To a mixture of dipolarophile **18** (1mmol) and Et_3N (1mmol) in CH_2Cl_2 were added, a 9-12% aqueous solution of NaOCl (1.6 mmol) dropwise over a period of 1 hr at 0 ^{0}C and the appropriate oxime (1 mmol) in CH_2Cl_2. After being stirred at room temperature for 24 hrs, CH_2Cl_2 was removed under reduced pressure, water was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. This was further purified by column chromatography using ethyl acetate: n-hexane, (15:85) as eluent to obtain the pure product.

6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)-N-((3-(4-(trifluoromethylthio)phenyl)isoxa zol-5-yl)methyl)benzo[d]thiazol-2-amine (13a)

White solid, yield 370 mg, 63%; mp 170 - 172 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2931, 1605, 1538, 1459, 1428, 1263, 1116, 845; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 3H, -OCH_3), 5.79 (s, 2H, -CH_2-N-), 6.60 (s, 1H, Isoxazol-H), 6.93 - 7.56 (m, 4H, Ar-H), 7.60 - 7.94 (m, 6H, Ar-H); ^{13}C NMR

(75 MHz, $CDCl_3$): δ 47.0, 56.3, 101.5, 104.4, 107.2, 107.6, 109.5, 114.3, 115.0, 118.8, 120.2, 121.7, 127.7, 129.7, 131.0, 133.1, 136.4, 144.0, 146.0, 156.7, 158.9, 160.3, 161.5, 167.9; ESI-MS: *m/z* 589 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{26}H_{17}O_2N_4F_4S_3$ Calcd: 589.0422, found: 589.0444 $[M+1]^+$.

6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)-N-((3-(4-(trifluoromethoxy)phenyl)isoxa zol-5-yl)methyl)benzo[d]thiazol-2-amine (13b)

White solid, yield 389 mg, 68 %; mp 120 - 122 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2937, 1606, 1537, 1459, 1433, 1263, 1054, 852; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H, $-OCH_3$), 5.77 (s, 2H, $-CH_2$-N-), 6.50 (s, 1H, Isoxazol-H), 6.93 - 7.07 (m, 2H, Ar-H), 7.09 - 7.19 (m, 1H, Ar-H), 7.29 - 7.49 (m, 2H, Ar-H), 7.65 - 7.84 (m, 5H, Ar-H); ^{13}C NMR (75 MHz,$CDCl_3$): δ 46.9, 55.9, 101.5, 104.3, 104.6, 107.2, 107.5, 109.3, 110.5, 111.3, 114.3, 115.0, 120.1, 121.7, 127.7, 130.3, 130.9, 136.5, 143.9, 145.9, 156.6, 158.6, 161.5, 166.9, 173.0; ; ESI-MS: *m/z* 573 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{26}H_{17}O_3N_4F_4S_2$ Calcd: 573.0650, found: 573.0672 $[M+1]^+$.

6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)-N-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)benzo[d]thiazol-2-amine (13c)

White solid, yield 373 mg, 72 %; mp 162 - 164 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2930, 1608, 1532, 1458, 1429, 1252, 1027, 836; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 5.77 (s, 2H, $-CH_2$-N-), 6.52 (s, 1H, Isoxazol-H), 6.84 - 7.54 (m, 7H, Ar-H), 7.61 - 7.88 (m, 3H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.8, 55.6, 56.8, 101.3, 104.4, 107.0, 107.4, 109.4, 109.5, 110.5, 111.3, 114.0, 114.8, 119.7, 121.5, 126.4, 128.0, 132.9, 152.2, 157.3, 160.9, 162.2, 166.7; ESI-MS: *m/z* 519 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{26}H_{20}O_3N_4FS_2$ Calcd: 519.0937, found: 519.0955 $[M+1]^+$.

6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)-N-((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methyl)benzo[d]thiazol-2-amine (13d)

White solid, yield 404 mg, 70 %; mp 174 - 176 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2931, 1604, 1538, 1505, 1461, 1421, 1264, 1130, 842; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.77 – 3.99 (m, 12H, -OCH_3), 5.77 (s, 2H, -CH_2-N-), 6.45 (s, 1H, Isoxazol-H), 6.87 - 7.04 (m, 3H, Ar-H), 7.10 - 7.29 (m, 2H, Ar-H), 7.41 - 7.50 (m, 1H, Ar-H), 7.65 - 7.85 (m, 2H, Ar-H); ^{13}C NMR (75MHz,$CDCl_3$): δ 47.3,55.9, 56.8, 59.9, 101.1, 104.1, 105.0, 108.4, 112.0, 114.2, 115.0, 120.3, 121.0, 123.4, 132.8, 139.0, 143.2, 145.5, 153.1, 156.1, 156.8, 159.1, 160.7, 162.0, 167.3; ESI-MS: *m/z* 579 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{28}H_{24}O_5N_4FS_2$ Calcd: 579.1145, found: 579.1166 [*M*+1]$^+$.

N-((3-(4-bromophenyl)isoxazol-5-yl)methyl)-6-fluoro-N-(6-methoxybenzo[d]thiazol-2-yl)benzo[d]thiazol-2-amine (13e)

White solid, yield 339 mg, 60 %; mp 114 - 116 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2929, 1603, 1538, 1457, 1426, 1262, 1161, 853; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H, -OCH_3), 5.63 (s, 2H, -CH_2-N-), 6.50 (s, 1H, Isoxazol-H), 6.91 - 7.06 (m, 2H, Ar-H), 7.08 - 7.19 (m, 2H, Ar-H), 7.29 - 7.36 (m, 1H, Ar-H), 7.56 - 7.64 (m, 3H, Ar-H), 7.66 - 7.78 (m, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.8, 56.3, 100.6, 103.6, 104.6, 107.5, 107.9, 111.5, 114.5, 119.8, 120.6, 122.9, 132.3, 138.6, 142.7, 145.0, 152.66, 155.6, 160.2, 161.5, 166.6; ESI-MS: *m/z* 567 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{25}H_{17}BrFN_4O_2S_2$ Calcd: 567.9882, found: 567.9900 [*M*+1]$^+$.

6-fluoro-N-((3-(2-fluorophenyl)isoxazol-5-yl)methyl-N-(6-methoxybenzo[d]thiazol-2-yl)benzo[d]thiazol-2-amine (13f)

White solid, yield 303 mg, 60 %; mp 162 - 164 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2931, 1606, 1538, 1460, 1260, 1055, 761; ^{1}H NMR (300 MHz, DMSO): δ 3.91 (s, 3H, -OCH_3), 5.80 (s, 2H, -CH_2-

N-), 6.74 (s, 1H, Isoxazol-H), 6.89 - 7.26 (m, 5H, Ar-H), 7.28 - 7.57 (m, 3H, Ar-H), 7.60 - 7.75 (m, 1H, Ar-H), 7.83 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, DMSO): δ 47.3, 57.4, 103.6, 103.7, 104.7, 105.0, 107.6, 107.9, 111.7, 114.7, 115.4, 119.8, 120.6, 122.1, 128.1, 130.7, 131.3, 136.9, 144.3, 146.3, 156.9, 162.0, 173.4; ESI-MS: *m/z* 507 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{25}H_{17}O_2N_4F_2S_2$ Calcd: 507.0733, found: 507.0755 [*M*+1]$^+$.

N-(pyrimidin-2-yl)-N-((3-(4-(trifluoromethylthio)phenyl)isoxazol-5-yl)methyl)benzo [d]thiazol-2-amine (20a)

White solid, yield 412 mg, 85 %; mp 112 - 114 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2951, 1572, 1496, 1439, 1251, 1119, 837; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.14 (s, 2H, -CH_2-N-), 6.42 (s, 1H, Isoxazol-H), 6.96 - 7.05 (m, 2H, Ar-H), 7.20- 7.28 (m, 2H, Ar-H), 7.38 (t, 1H, *J* = 7.9 Hz, Ar-H), 7.65 (d, 1H, *J* = 7.9 Hz, Ar-H), 7.72 - 7.90 (m, 3H, Ar-H), 8.69 (d, 2H, *J* = 4.9 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.7, 100.5, 114.8, 115.0, 120.5, 120.9, 121.0, 122.2, 123.0, 123.2, 125.8, 127.6, 129.0, 131.5, 136.4, 148.8, 157.3, 161.2, 170.4; ESI-MS: *m/z* 486 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{22}H_{15}$ O $N_5F_3S_2$ Calcd: 486.0653, found: 486.0664 [*M*+1]$^+$.

N-(pyrimidin-2-yl)-N-((3-(4-(trifluoromethoxy)phenyl)isoxazol-5-yl)methyl)benzo [d]thiazol-2-amine (20b)

White solid, yield 389 mg, 83 %; mp 124 - 126 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2925, 1573, 1494, 1443, 1253, 797; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (s, 2H, -CH_2-N-), 6.37 (s, 1H, Isoxazol-H), 7.01 (t, 1H, *J* = 4.5 Hz, Ar-H), 7.15 - 7.28 (m, 2H, Ar-H), 7.38 (t, 2H, *J* = 8.3 Hz, Ar-H), 7.57 (s, 1H, Ar-H), 7.65 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.77 (t, 2H, *J* = 7.5 Hz, Ar-H), 8.68 (d, 2H, *J* = 4.5 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.2, 101.0, 115.0, 115.4, 120.9, 121.4, 121.4, 122.7, 123.4, 126.0, 128.1, 129.5, 131.9, 136.8, 157.8, 161.6, 170.8; ESI-MS: *m/z* 470 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{22}H_{15}$ O_2 N_5F_3S Calcd: 470.0879, found: 470.0893 [*M*+1]$^+$.

N-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine (20c)

White solid, yield 315 mg, 76 %; mp 180 - 182 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2924, 1611, 1575, 1496, 1437, 1250, 1025, 838; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H, -OCH_3), 6.11 (s, 2H, -CH_2-N-), 6.31 (s, 1H, Isoxazol-H), 6.83 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.99 (t, 1H, *J* = 4.7 Hz, Ar-H), 7.25 (s, 1H, Ar-H), 7.34 - 7.41 (m, 1H, Ar-H), 7.62 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.77 (t, 2H, *J* = 8.7 Hz, Ar-H), 8.67 (d, 2H, *J* = 4.7 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.7, 55.2, 100.2, 114.0, 114.7, 120.5, 120.9, 123.1, 125.7, 128.0, 129.9, 148.8, 157.3, 160.7, 169.4; ESI-MS: *m/z* 416 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{22}H_{17}O_2N_5NaS$ Calcd: 438.0979, found: 438.0995 [*M*+Na]$^+$.

N-(pyrimidin-2-yl)-N-((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methyl)benzo [d]thiazol-2-amine (20d)

White solid, yield 427 mg, 90 %; mp 208 - 210 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2932, 1585, 1494, 1447, 1369, 1244, 1127, 841; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 3H, -OCH_3), 3.86 (s, 6H, -OCH_3), 6.13 (s, 2H, -CH_2-N-), 6.29 (s, 1H, Isoxazol-H), 6.90 (s, 2H, Ar-H), 7.00 (t, 1H, *J* = 4.9 Hz, Ar-H), 7.24 (t, 2H, *J* = 7.9 Hz, Ar-H), 7.39 (t, 1H, *J* = 7.9 Hz, Ar-H), 7.73 - 7.82 (m, 1H, Ar-H), 8.68 (d, 2H, *J* = 4.9 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.9, 56.2, 60.8, 100.2, 104.0, 114.8, 120.5, 120.9, 123.1, 124.4, 125.7, 133.9, 139.4, 148.8, 153.4, 157.1, 157.3, 160.0, 162.2, 169.9; ESI-MS: *m/z* 476 [*M*+1]$^+$; HRMS (ESI *m/z*) for $C_{24}H_{21}O_4N_5NaS$ Calcd: 498.1190, found: 498.1206 [*M*+Na]$^+$.

N-((3-(4-bromophenyl)isoxazol-5-yl)methyl)-N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine (20e)

White solid, yield 287 mg, 62 %; mp 212 - 214 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 3056, 1567, 1492, 1440, 1251, 1066, 800; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.12 (s, 2H, Ar-H), 6.35 (s, 1H,

Isoxazol-H), 7.00 (t, 1H, *J* = 4.5 Hz, Ar-H), 7.22 - 7.28 (m, 2H, Ar-H), 7.38 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.48 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.60 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.77 (t, 1H, *J* = 7.5 Hz, Ar-H), 8.68 (d, 2H, *J* = 4.5 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.8, 100.2, 114.0, 114.7, 120.5, 120.9, 123.1, 125.7, 128.1, 130.0, 157.3, 160.7, 169.4; ESI-MS: *m/z* 464 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{21}H_{15}Br\ N_5OS$ Calcd: 464.0137, found: 464.0155 $[M+1]^+$.

N-((3-(2-fluorophenyl)isoxazol-5-yl)methyl)-N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine (20f)

White solid, yield 253 mg, 63 %; mp 176 - 178 ^{0}C; IR (KBr, ν_{max} cm^{-1}): 2923, 1575, 1496, 1437, 1251, 1098, 810; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.12 (s, 2H, -CH_2-N-), 6.57 (s, 1H, Isoxazol-H), 6.96 - 7.39 (m, 5H, Ar-H), 7.47 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.64 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.85 (s, 1H, Ar-H), 7.93 (t, 1H, *J* = 7.55 Hz, Ar-H), 8.67 (d, 2H, *J* = 4.7 Hz, Pyrimidine-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.6, 103.1, 103.2, 114.9, 115.9, 116.0, 116.3, 122.2, 123.0, 124.4, 129.0, 131.3, 131.4, 135.8, 147.8, 156.9, 157.3, 160.2, 169.2; ESI-MS: *m/z* 404 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{21}H_{15}\ F\ N_5OS$ Calcd: 404.0954, found: 404.0976 $[M+1]^+$.

(B) Biology

Cell Culture and maintenance

The required cell lines i.e., colon cancer (Colo205), human leukemic monocyte lymphoma (U937), Breast cancer (MCF7) and lung cancer (A549) were obtained from the American Type Culture Collection (Manassas, VA, USA) and were grown in the specific growth medium (RPMI or DMEM) supplemented with 10% fetal bovine serum and antibiotics (100ug/ml penicillin, 100 µg/ml streptomycin sulphate) at 37 °C and 5% CO_2. Cells were trypsinized when

subconfluent from T25 flasks/60 mm dishes and seeded either to T25 or T75 flasks or 96 well plates depending on the assay to be performed.

In vitro cytotoxicity evaluation by MTT assay

MTT assay (Vybrant® MTT Cell Proliferation Assay Kit (V-13154)) was performed to assess the cytotoxicity (/growth inhibition) of the test compounds on the chosen cancer cell lines. Cells were plated into 96 well microtitre plates at $10x10^3$cells/well and allowed to adhere overnight. Following the incubation period, test compounds were added to each of the wells at concentrations of 0, 2, 4, 8 and16 μM and incubated for 24 h. After 24 h, the media was replaced with 100μl of fresh media followed by addition of 10μl of 12mM MTT reagent/well and incubated for 2 hrs in dark at 37°C according to the manufacturer's instructions. Finally, the reaction was terminated by addition of 100μl of SDS-HCL. Readings were taken at 570 nm using Multimode Varioskan Flash (Thermo Fisher Scientifics) with media as a blank. The IC_{50} values were obtained from the results of triplicate determinations of atleast three independent experiments.

Cell Cycle Analysis

Cells (~2×10^5 cells) were seeded in 60 mm dish and allowed to grow for 24h. For treatment with the test compounds, various concentrations (0, 2, 4, 8, 16 μM) of the compounds were added to the culture media, and cells were incubated for an additional 24h. Attached cells were harvested with Trypsin-EDTA and washed twice with Phosphate Buffered Saline (PBS). Cells were fixed by adding 1ml of ice cold 70% ethanol dropwise with vortexing followed by incubation at 4°C overnight. Fixed cells were pelleted by centrifugation at 2,000 rpm for 2 min, washed with PBS and repelleted. The cells were resuspended in PBS containing 40 μg/ml PI, 0.1 mg/ml RNase and

0.1% Triton X-100 in dark for 30 minutes at 37°C.The DNA content of 20,000 events was measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit 4.3 Software.

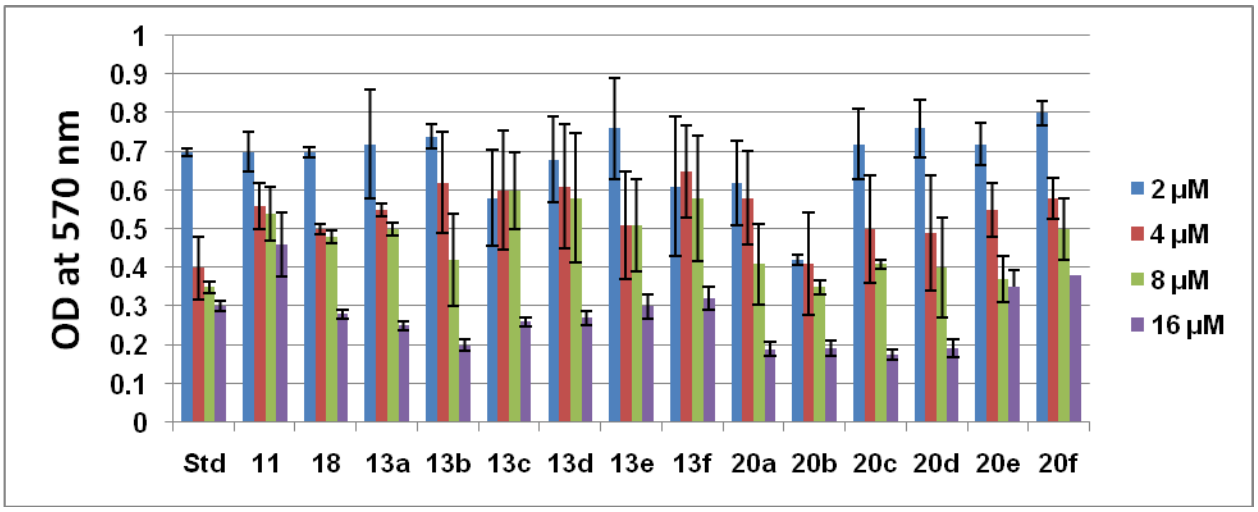
Protein Extraction and Western Blot Analysis

For analysis of protein levels by western blotting, the Colo205 cells were treated with the compounds at the respective IC_{50} concentrations and the total cell lysates were collected at 24h post treatment. Cells were scraped into media and collected by centrifugation, rinsed with PBS and recentrifuged. Cell pellets were resuspended in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) containing 100 μg/ml PMSF, 5 μg/ml Aprotinin, 5 μg/ml leupeptin, 5 μg/ml pepstatin and 100 μg/ml NaF. The protein in the supernatant was quantified by Bradford method (Biorad) using multimode varioskan instrument (Thermo-Fischer Scientifics). Fifty micrograms of protein per lane was electrophoresed in 10% SDS-polyacrylamide gel. After electrophoresis, the protein in the gel was transferred onto polyvinylidine difluoride (PVDF) membrane (GE Healthcare). Blocking of the membranes was performed using 2% BSA in TBS + 0.1% Tween20 (TBST) at room temperature for 2h, followed by primary antibody treatment. Following primary antibodies were used: Cdk1, (Millipore, 2μg/mL); cyclin B1, (Abbiotec, 1:100-1:500); chk2, (Cell signaling, 1:1000); Bax, (Santa Cruz, 1:100-1:1000); Bcl-2 (Abbiotec, 1:50-1:200); Cytochrome C (Imgenex, 0.05-0.5 μg/ml) Apaf1(N-term),(Abbiotec, 1:200-1:500); Caspase 9,(Millipore, 1:1,000 to 1:2,000); Caspase 3(pro and Active), (Imgenex, 1-5 μg/ml); p38 MAPK (Cell Signaling); NFκB(p65), (Abbiotec, 1:200-1:1000); p53, (Abbiotec, 1:1000-1:2000); β-actin, (Imgenex, 0.25-1 μg/ml). Subsequent to primary antibody treatment, the membrane was washed with TBST for 5 min (3 times). After washing, the membranes were incubated with corresponding horseradish

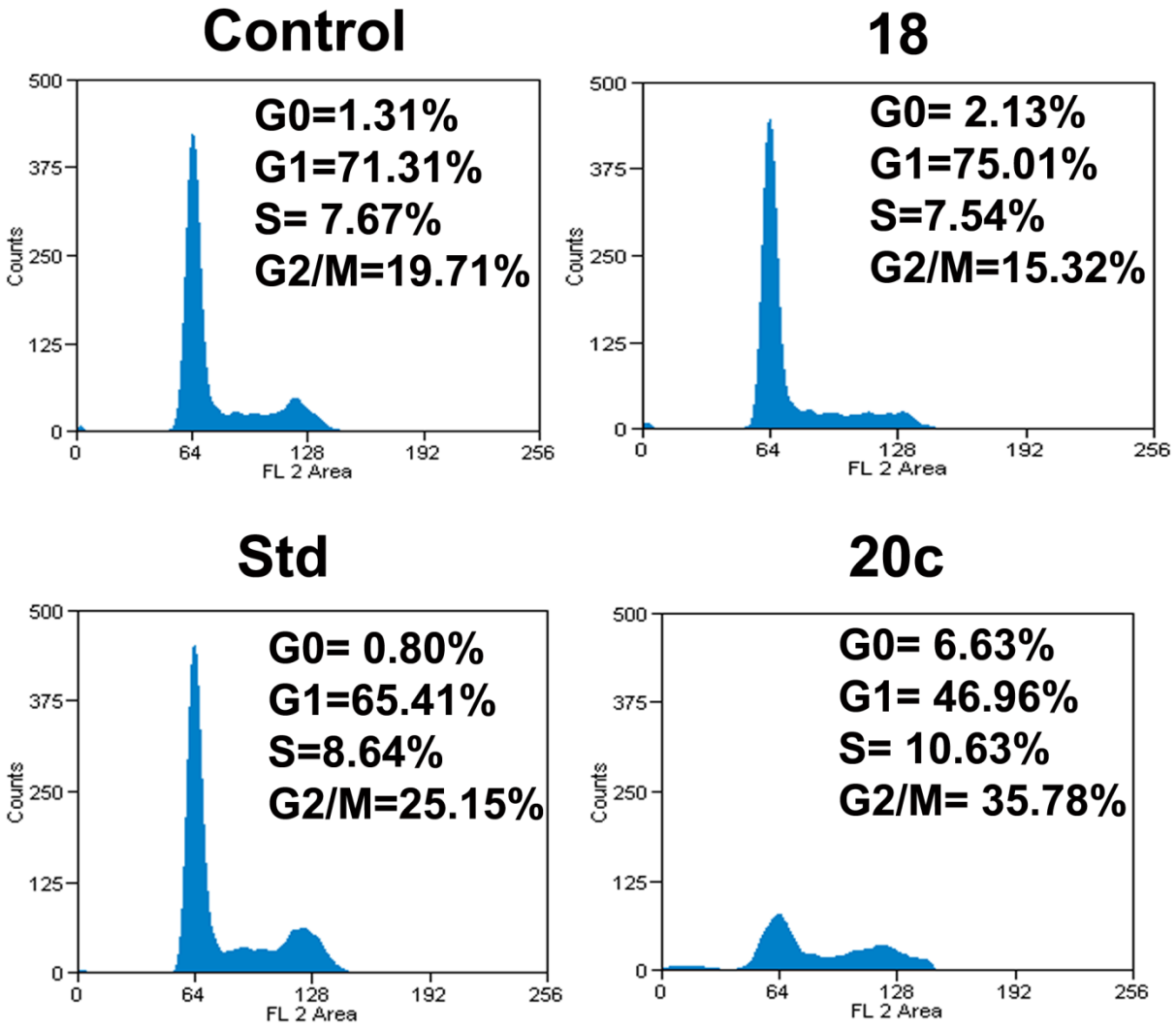
peroxidase-labeled secondary antibody (1:2,000) at room temperature for 1h. Membranes were washed with TBST (3 times for 15 min) and the blots were visualized using chemiluminescence reagent (Thermo Fischer Scientifics Ltd.) and developed on X-ray films by developer and fixed with fixer solution.

Alkaline comet assay (Single cell gel electrophoresis)

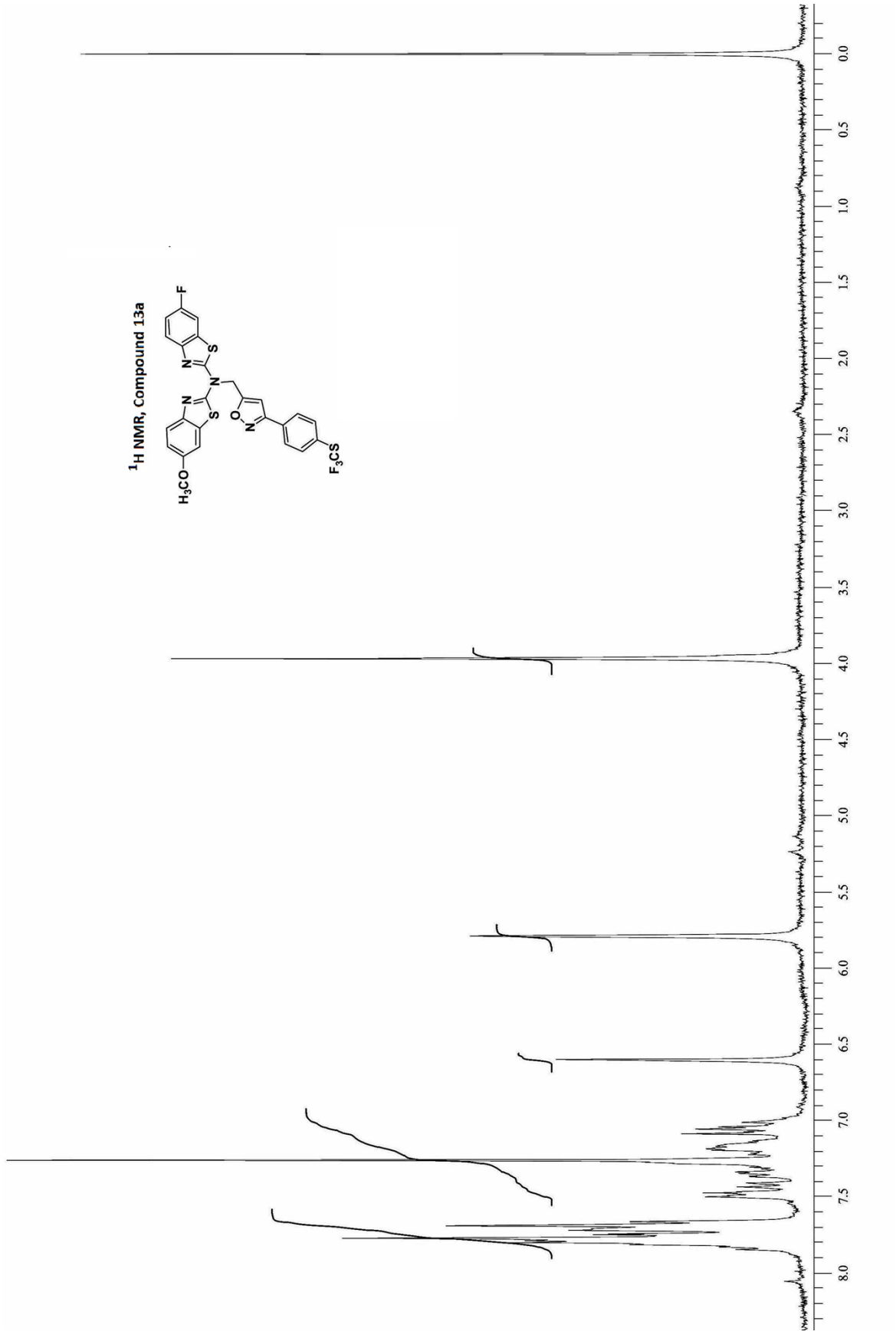
Alkaline comet assay was performed to evaluate DNA damage of Colo-205 cells. A freshly prepared suspension of control and treated Colo-205 cells (~20,000cells) in 0.7% low melting point agarose was embedded onto fully frosted microscope slides pre-coated with 1% normal melting agarose. The cells were then lysed for 2 h at 4°C in a buffer containing 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. For lysis, the microscopic slides were placed in an electrophoresis unit for 40 min, which allows the DNA to unwind in the presence of an alkaline electrophoretic buffer consisting of 300 mM NaOH, 1 mM EDTA, pH > 13. Electrophoresis was conducted at a temperature of 4°C in an electric field strength of 0.74 V/cm (300 mA). The slides were stained with 1X SYBR Green I (Lonza, Cat #50512) and covered with cover slips. All the steps described above were conducted under dim light or in the dark to prevent additional damage to the cells and/or DNA. The Comet images were analyzed with Comet Score software for tail length and atleast 50nuclei or Comets were analysed for each sample.



Supplementary Figure1. Cell viability determined by MTT assay after the treatment of the Colon cancer (Colo205) cell line with various compounds (**13a-f** and **20a-f**) at 2,4,8 and16 µM concentration at a cell density of 10,000cells/ well. Control cells treated with DMSO had O.D=0.8.



Supplementary Figure 2. FACS analysis of cell cycle distribution of Colo-205 cells after treatment with compounds **18, Std (Etoposide),** and **20c** at respective IC_{50} concentrations for 24 h.

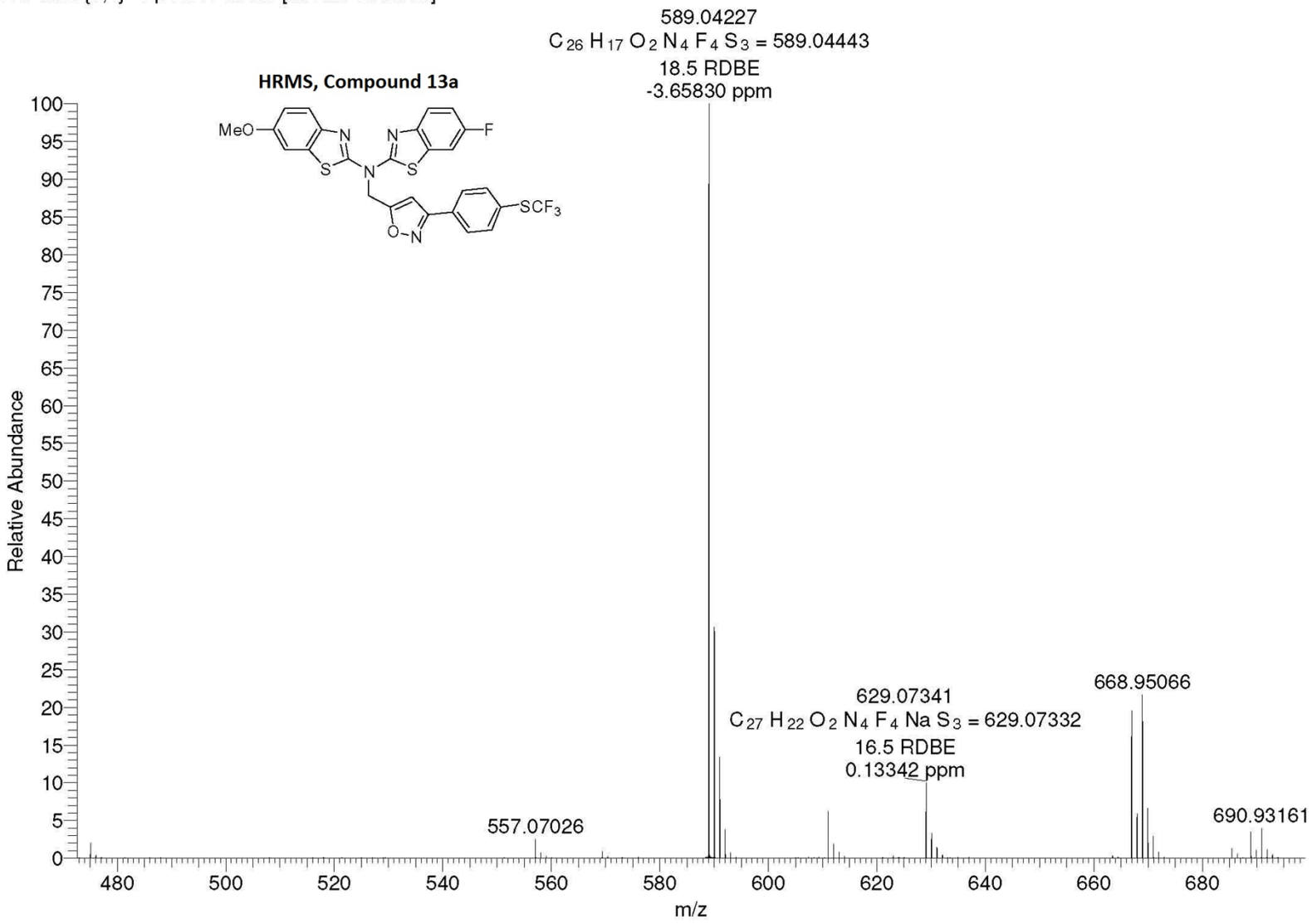

^{1}H NMR, Compound 13a
F
S
N
N
S
N
O
N
H_3CO
F_3CS
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0

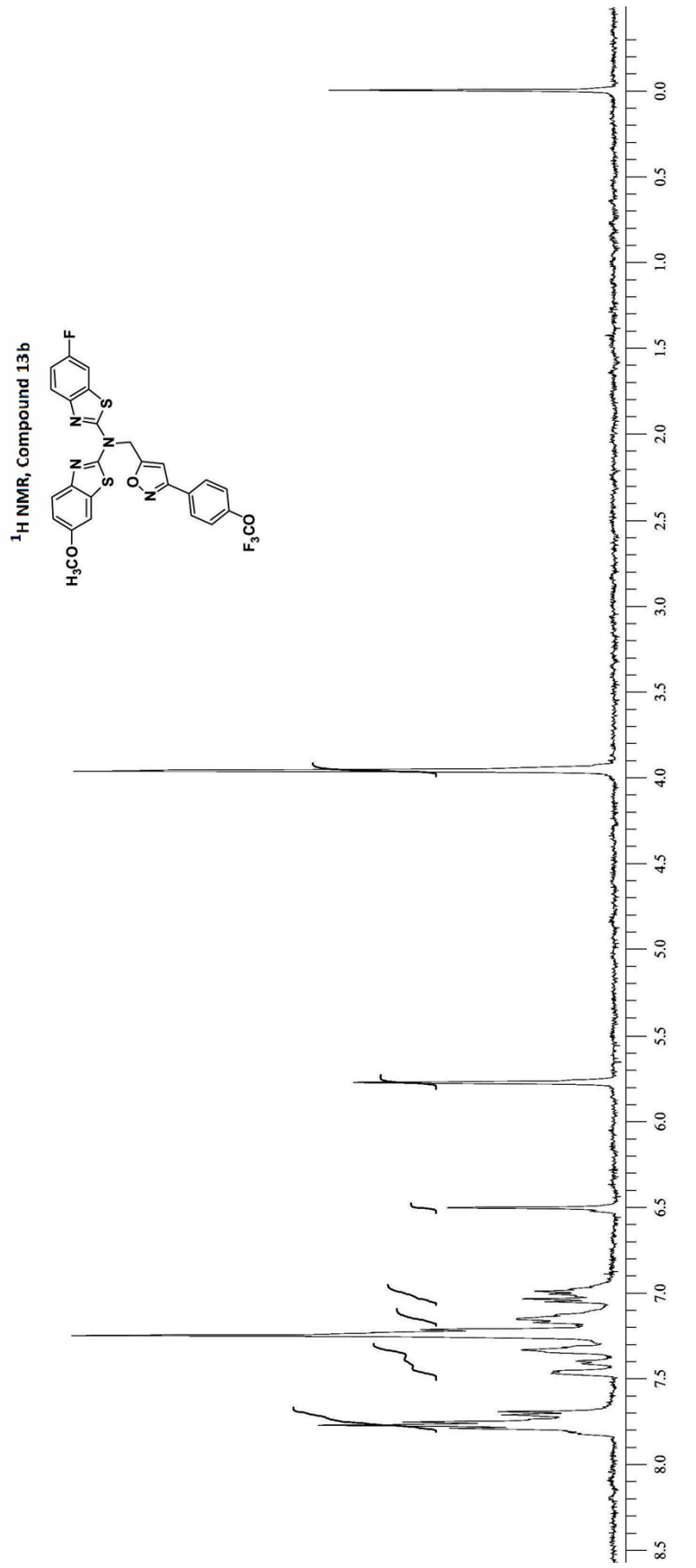
46.960
56.361
101.495
104.409
107.237
107.597
109.485
114.367
115.054
118.836
120.265
121.761
127.730
129.677
131.024
133.162
136.456
144.006
145.998
156.750
158.923
160.344
161.567
167.895
13C NMR, Compound 13a
F
S
N
N
S
O
N
O
F3CS

TULSIRAM
6/22/2012 5:54:55 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-SCF3 #24-55 RT: 0.10-0.20 AV: 32 NL: 3.55E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



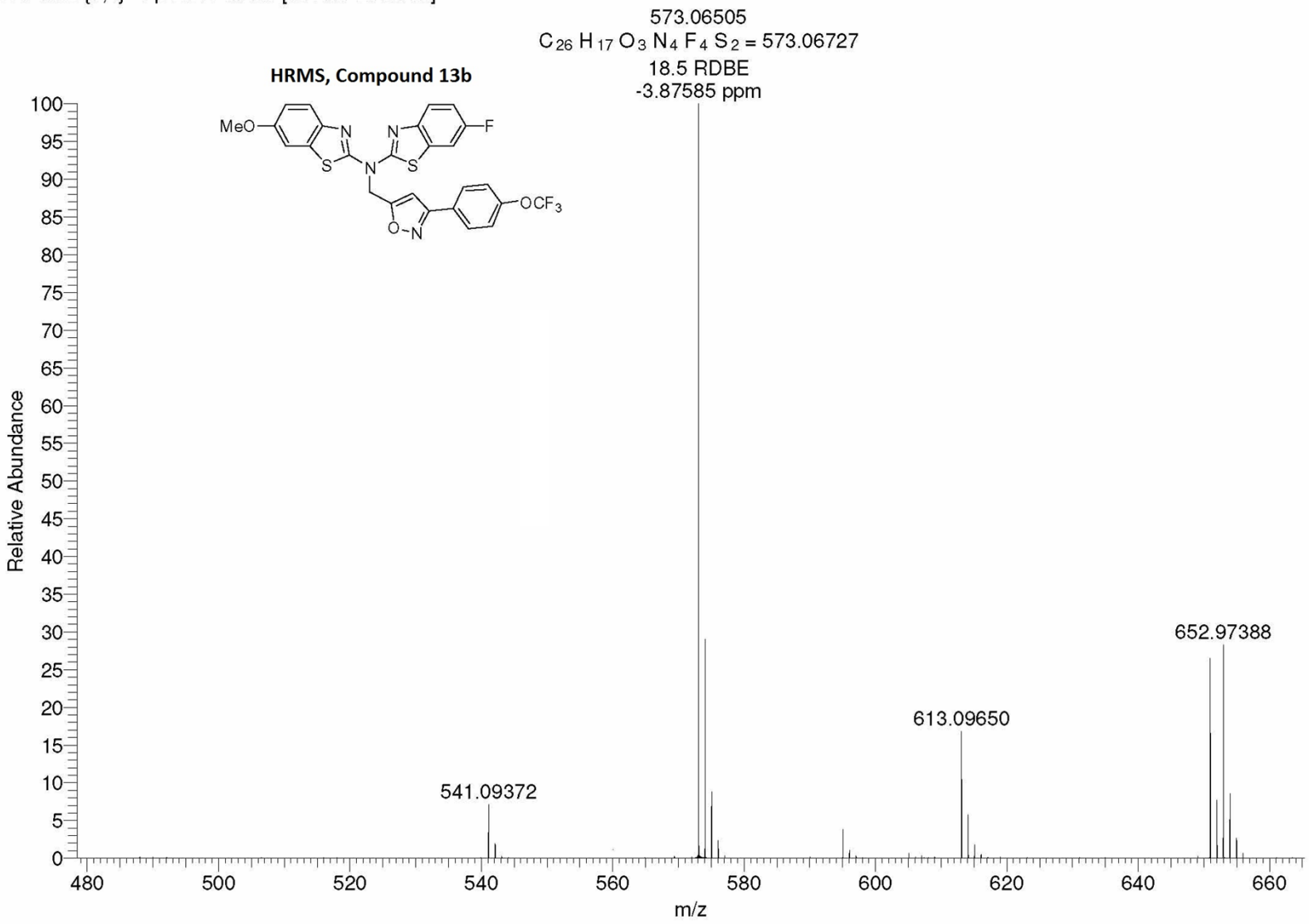

^{1}H NMR, Compound 13b
F
S
N
N
S
N
O
N
H_3CO
F_3CO
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

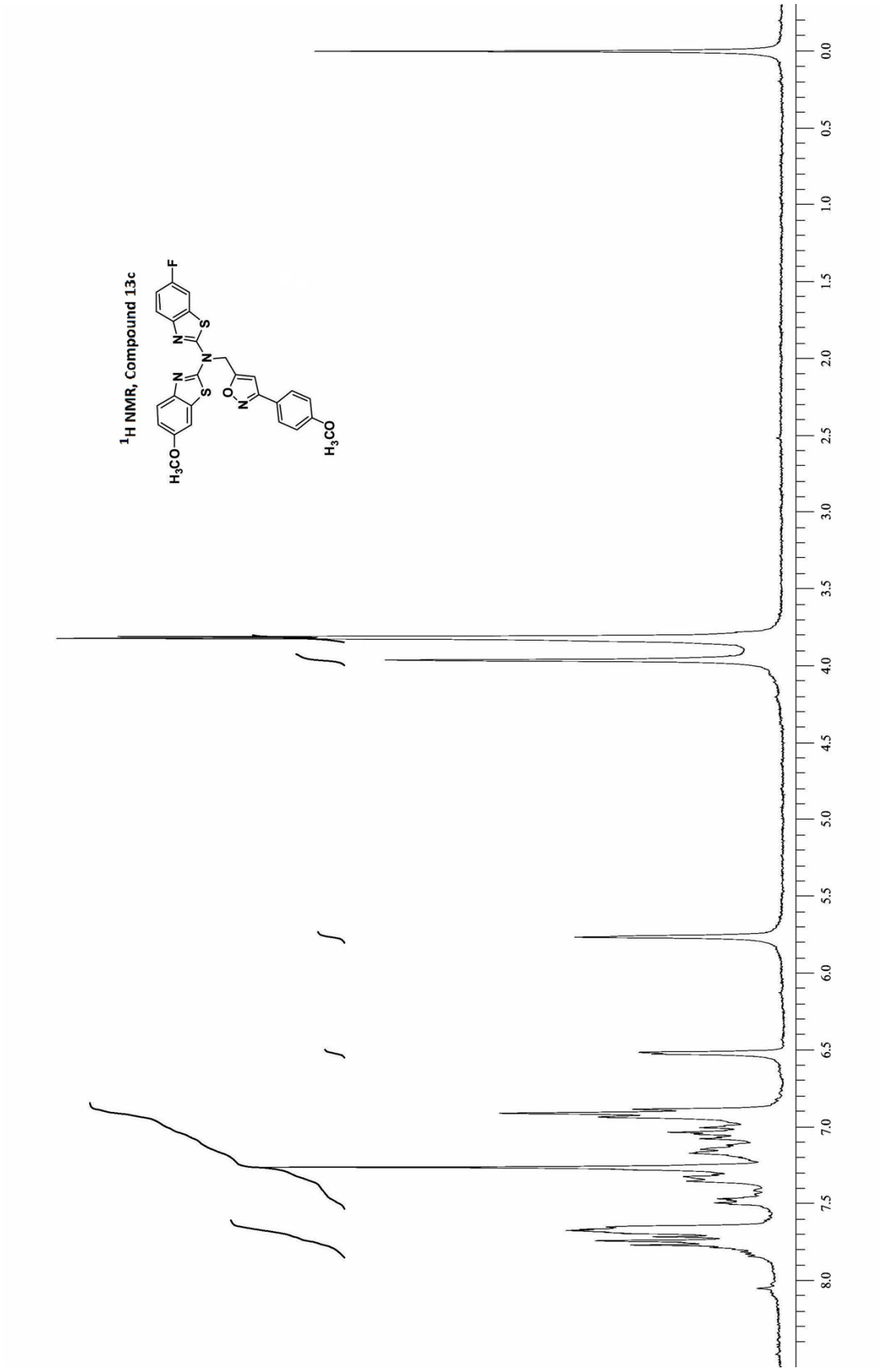
^{13}C NMR, Compound 13b
F
S
N
N
S
O
N
O
F3CO
46.897
55.790
101.491
104.290
104.588
107.221
107.474
109.314
110.567
111.286
114.342
115.001
120.157
121.699
127.699
130.297
130.879
136.490
143.947
145.930
156.636
158.591
161.545
166.942
173.013

TULSIRAM
6/22/2012 5:49:39 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-OCF3 #25-56 RT: 0.10-0.20 AV: 32 NL: 5.79E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]




^{1}H NMR, Compound 13c
F
N
S
N
N
S
O
N
H$_3$CO
H$_3$CO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

13C NMR, Compound 13c
46.804
55.633
56.771
101.331
104.465
107.026
107.379
109.425
109.752
110.536
111.336
114.063
114.791
119.752
121.511
126.404
128.004
132.943
152.214
157.341
160.904
162.222
166.678

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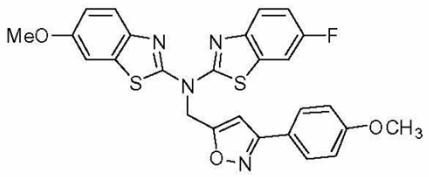
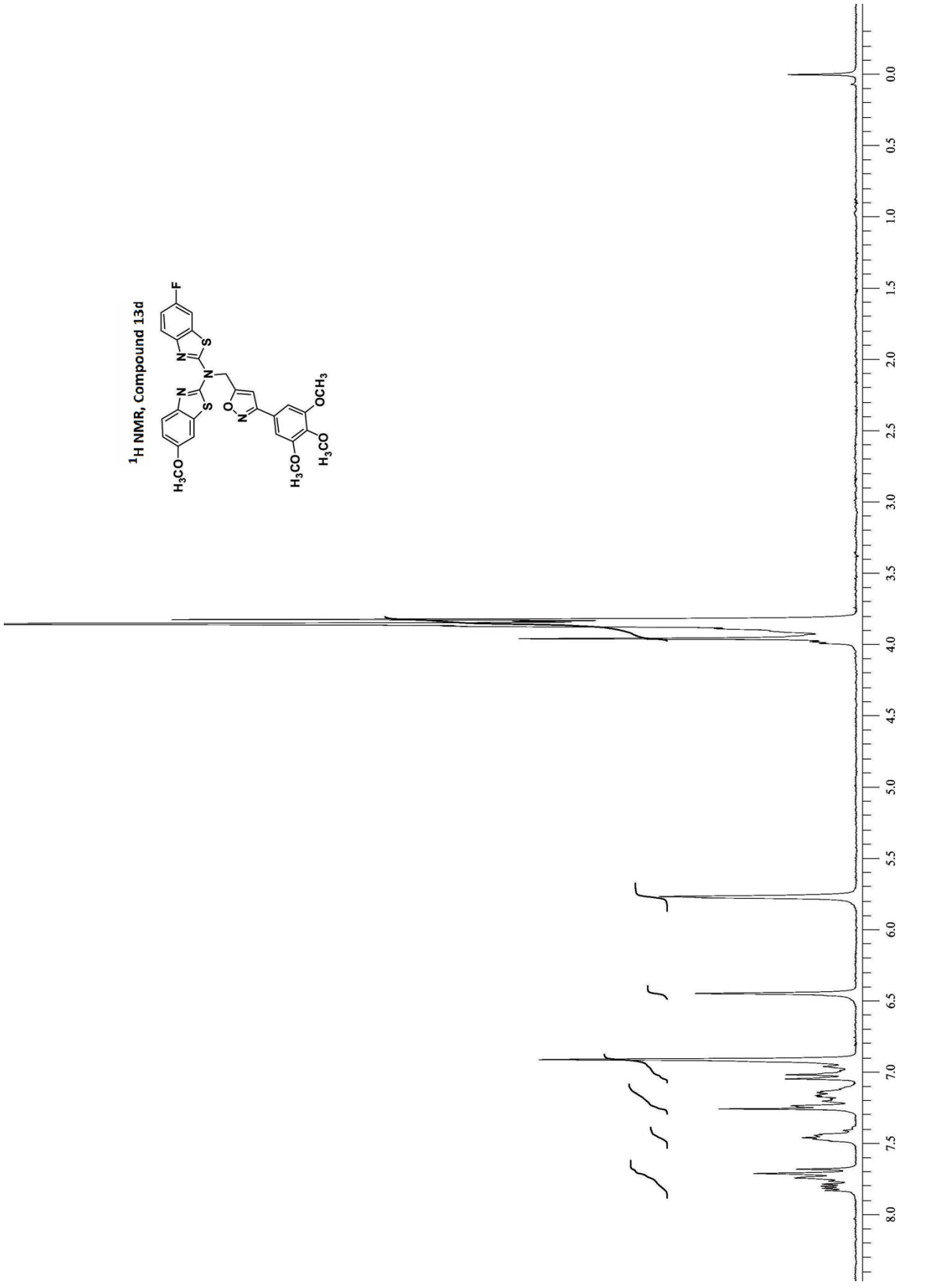
INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-OCH3 #42-65 RT: 0.16-0.23 AV: 24 NL: 1.19E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

519.09370
$C_{26}H_{20}O_3N_4FS_2$ = 519.09554
18.5 RDBE
-3.53078 ppm

HRMS, Compound 13c



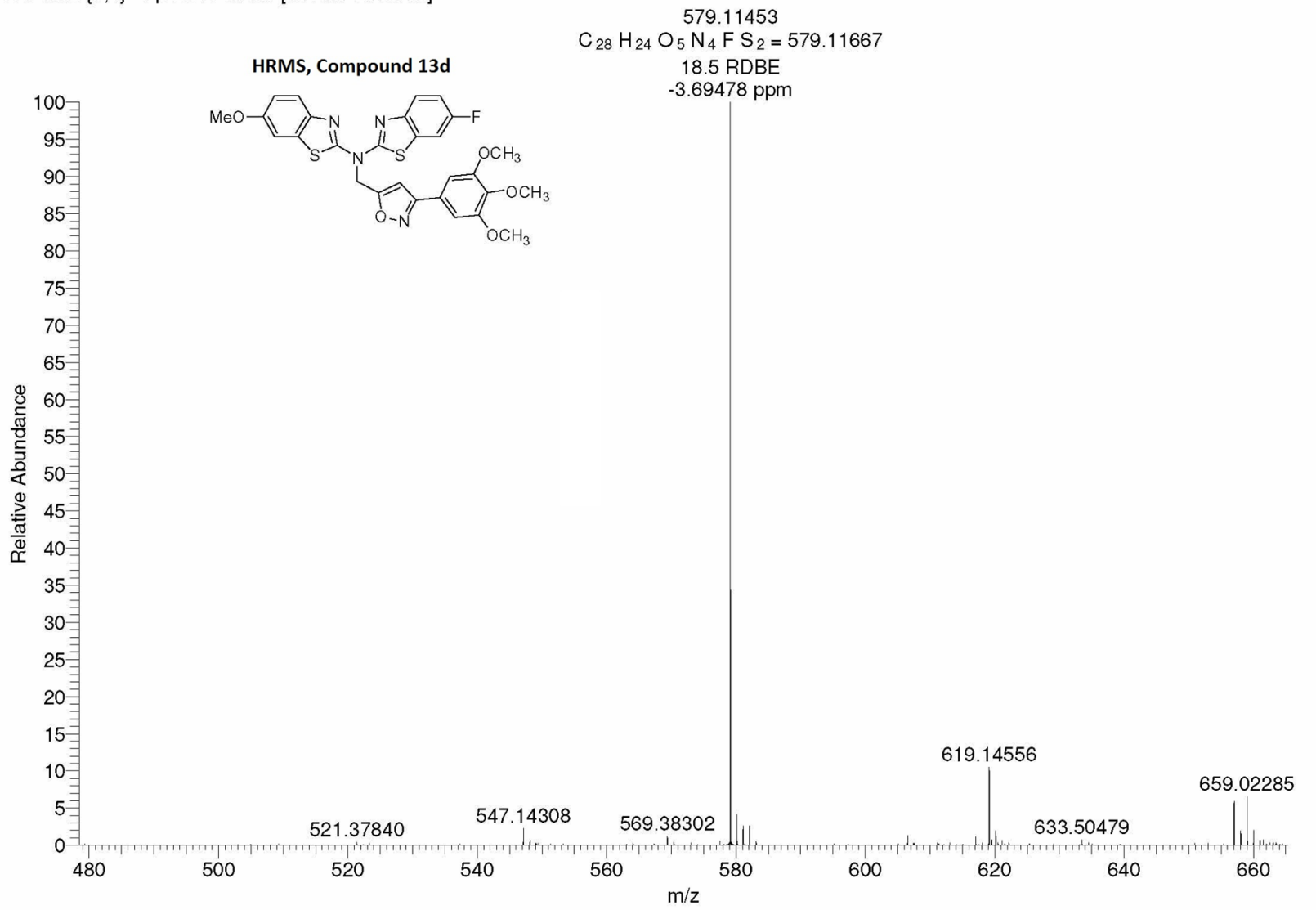

^{1}H NMR, Compound 13d
F
S
N
N
S
N
O
H_3CO
H_3CO
H_3CO
OCH_3
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0

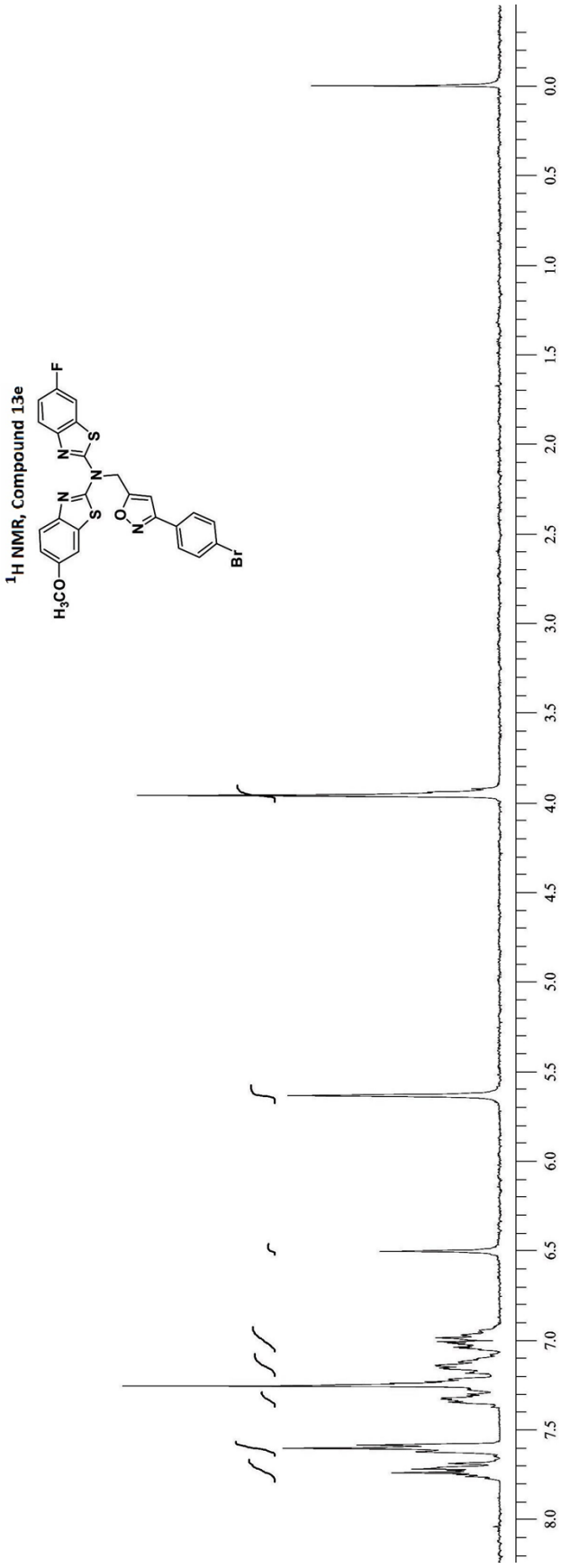
47.303
55.962
56.844
59.943
101.144
104.103
105.080
108.381
112.048
114.229
114.972
120.273
121.082
123.400
132.804
139.081
143.206
145.545
153.157
156.102
156.815
159.118
160.702
162.021
167.328
13C NMR, Compound 13d
OCH3
OCH3
OCH3
F
S
N
N
S
N
O
O
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

TULSIRAM
6/22/2012 5:52:17 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-3OME #25-56 RT: 0.10-0.20 AV: 32 NL: 1.92E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



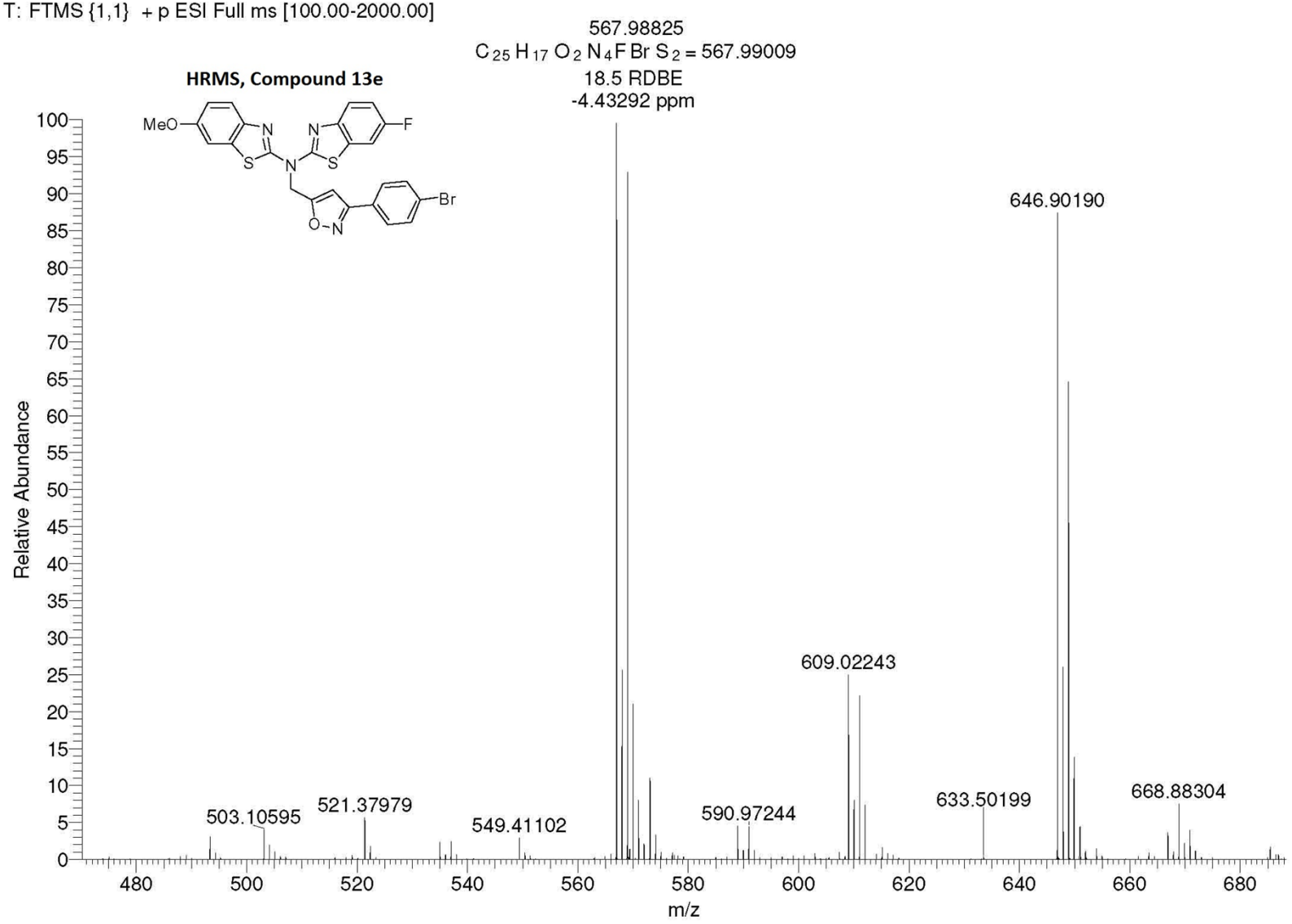

^{1}H NMR, Compound 13e
F
S
N
N
S
N
O
N
Br
H_3CO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

^{13}C NMR, Compound 13e
F
Br
N
S
O
46.815
56.356
100.656
103.615
104.592
107.543
107.893
111.560
114.483
119.784
120.593
122.911
132.316
138.593
142.717
145.057
152.669
155.613
160.213
161.532
166.607
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

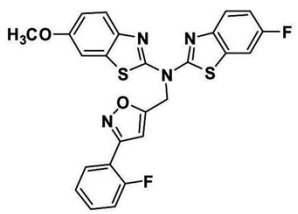
TULSIRAM
6/22/2012 5:46:59 PM

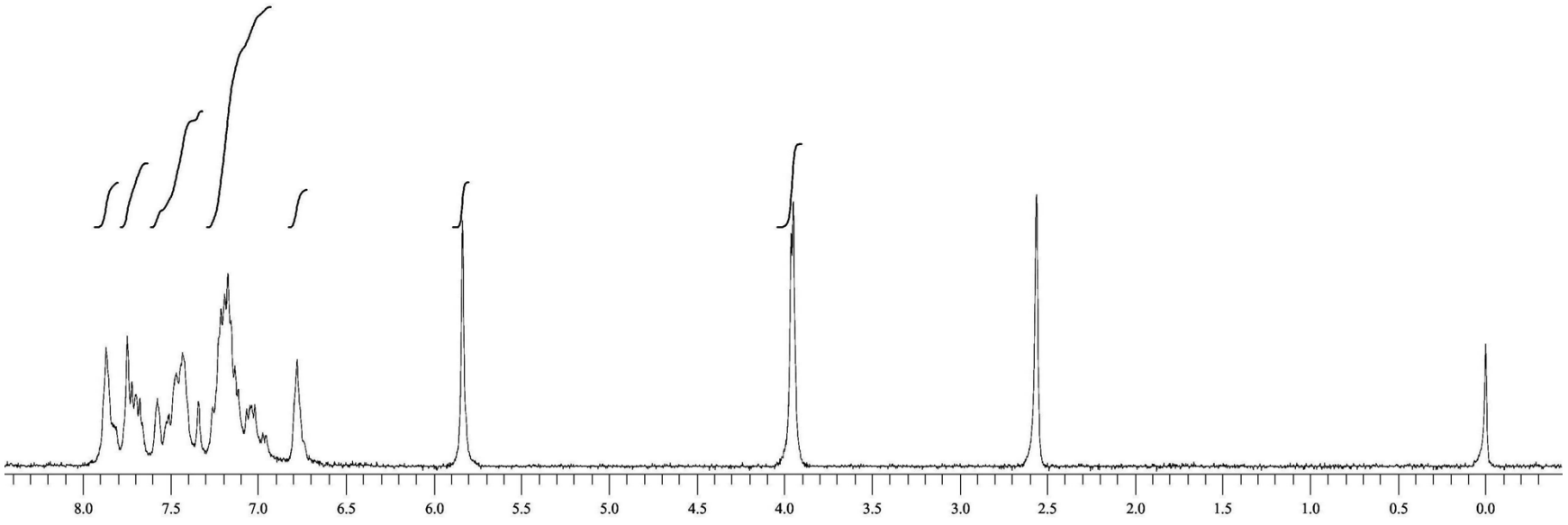
INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-BR #75-81 RT: 0.27-0.29 AV: 7 NL: 4.27E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



^{1}H NMR, Compound 13f





^{13}C NMR, Compound 13f
47.319
57.413
103.615
103.735
104.712
105.010
107.643
107.895
111.708
114.763
115.423
119.781
120.579
122.121
128.120
130.718
131.300
136.912
144.368
146.352
156.921
161.967
173.435

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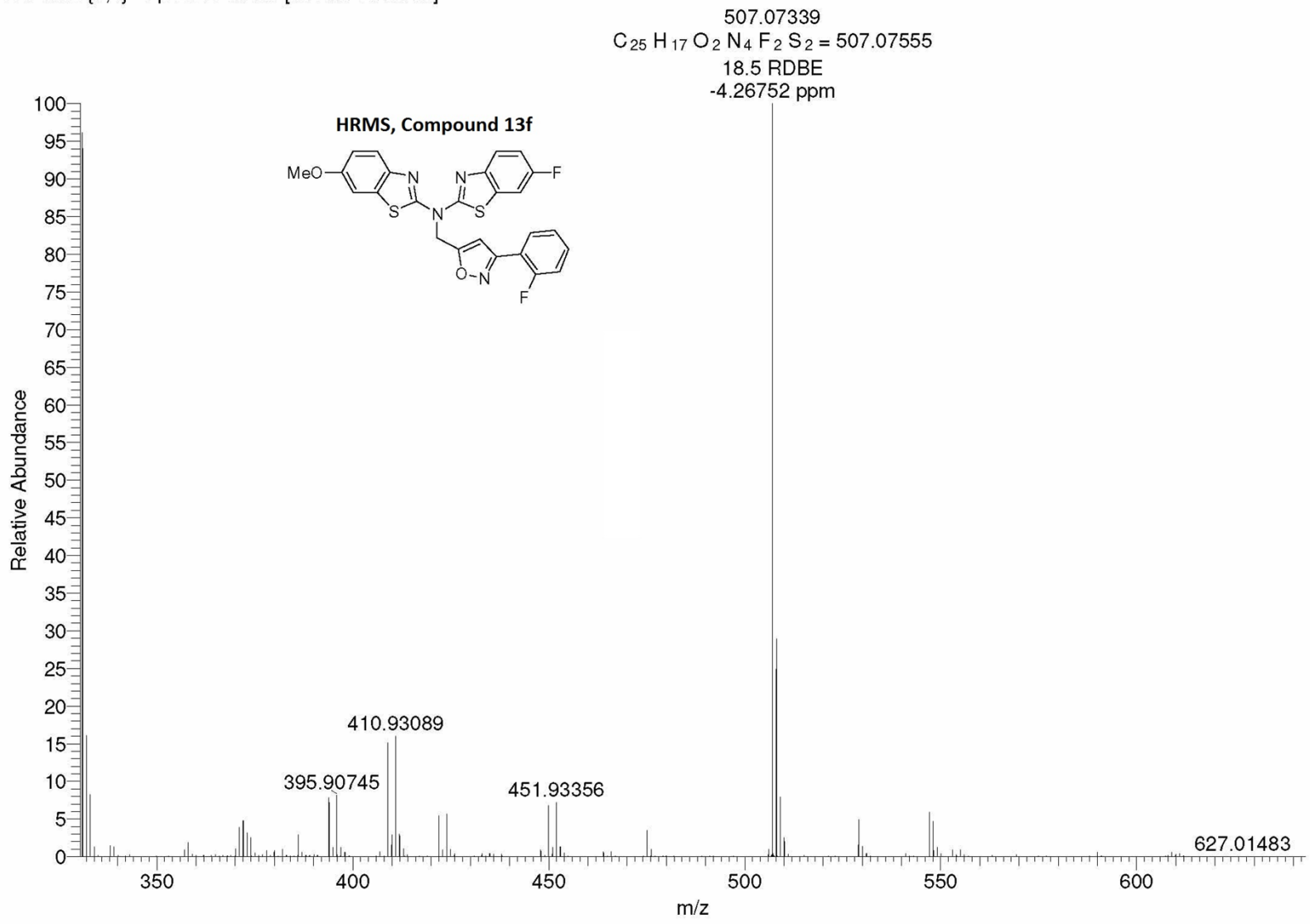
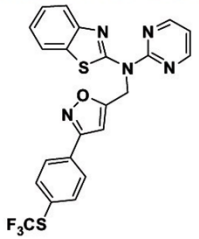
6/22/2012 5:41:45 PM

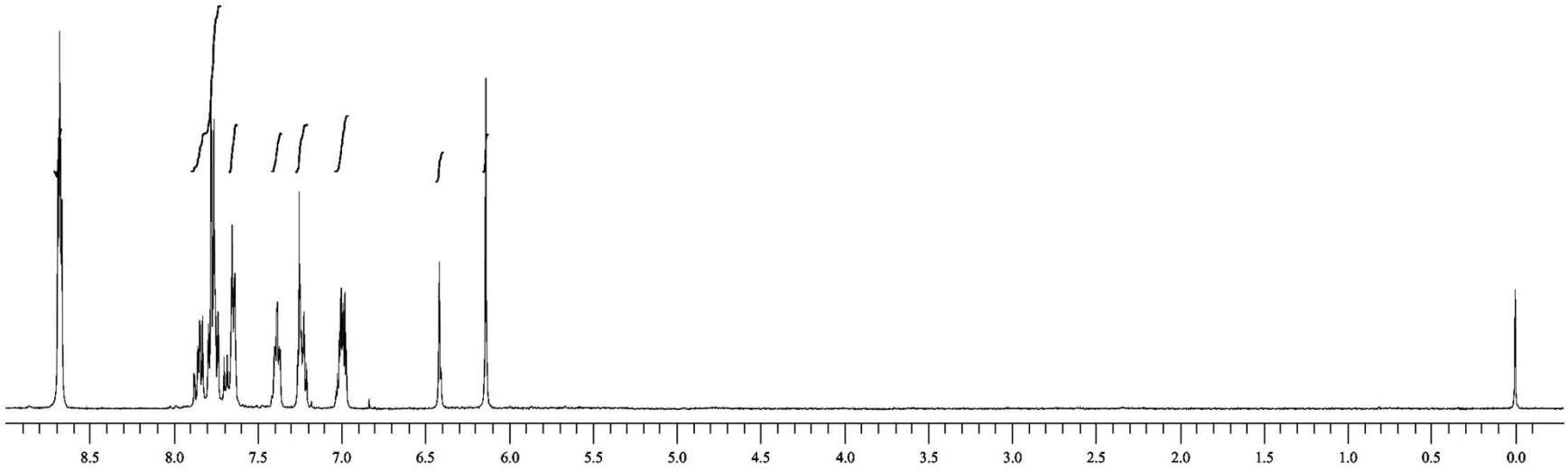
INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

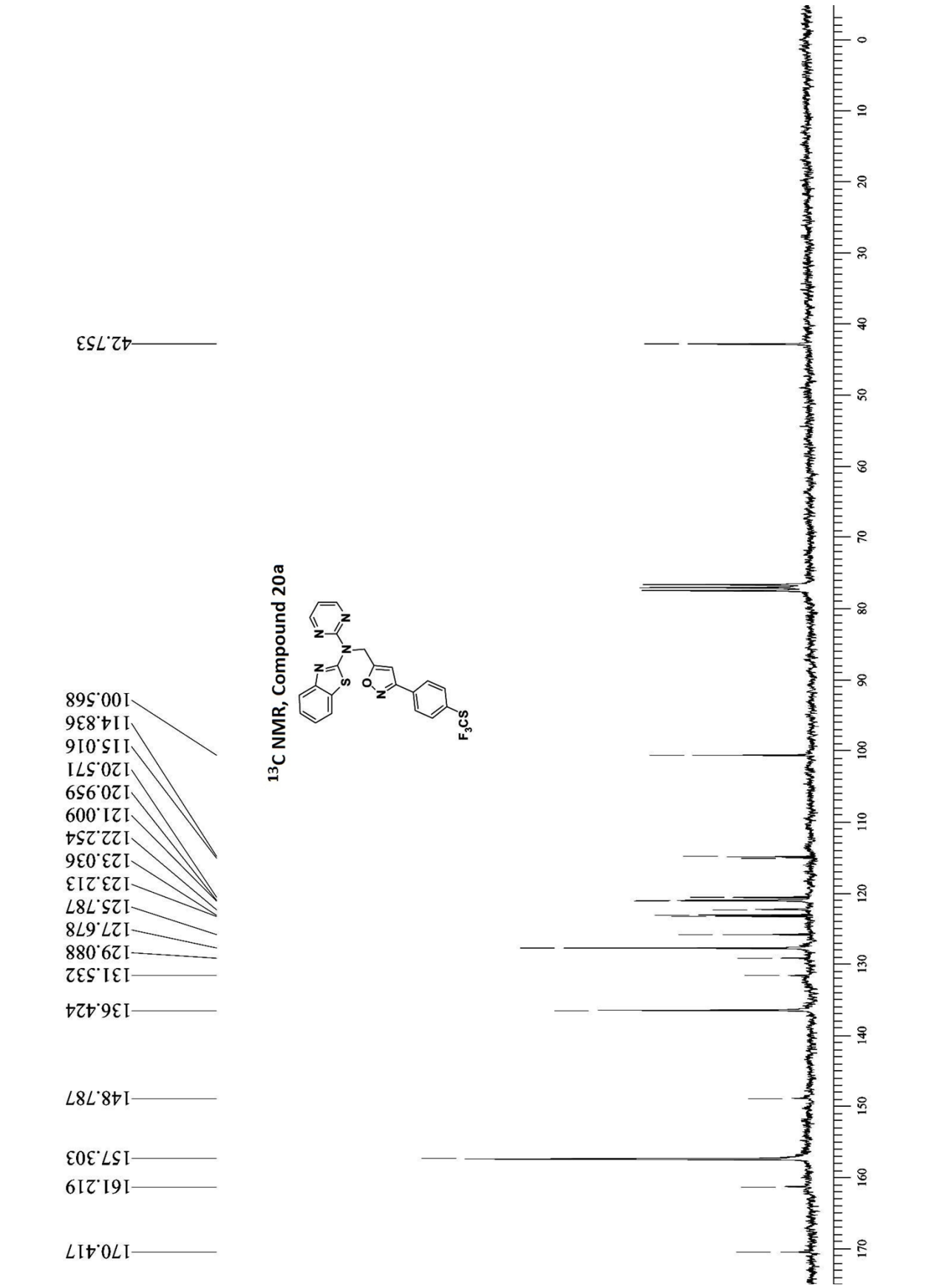
RMK-OX-F #17-65 RT: 0.08-0.24 AV: 49 NL: 3.60E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



^{1}H NMR, Compound 20a






^{13}C NMR, Compound 20a
F3CS
42.753
100.568
114.836
115.016
120.571
120.959
121.009
122.254
123.036
123.213
125.787
127.678
129.088
131.532
136.424
148.787
157.303
161.219
170.417

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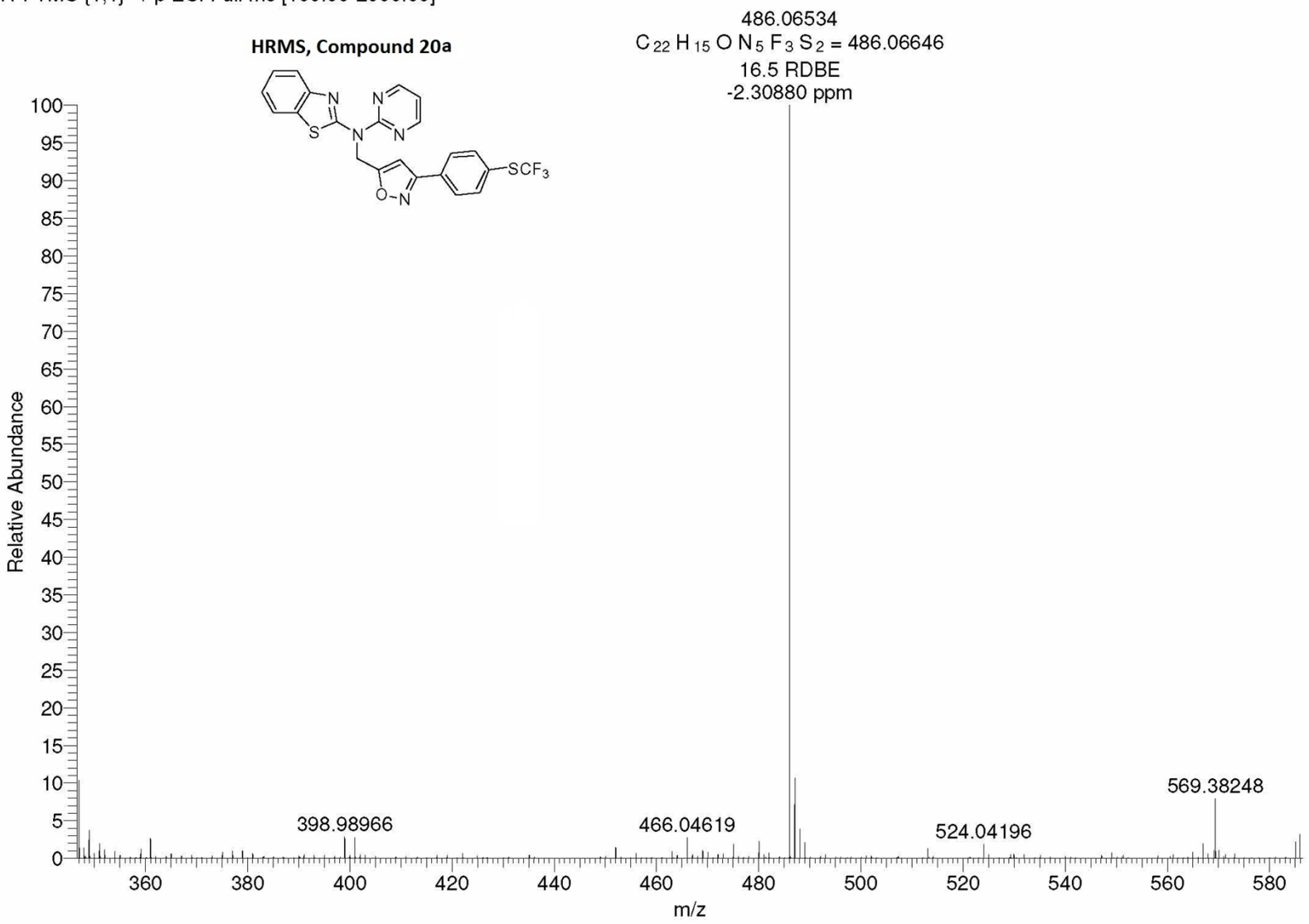
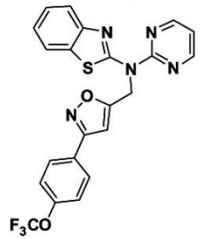
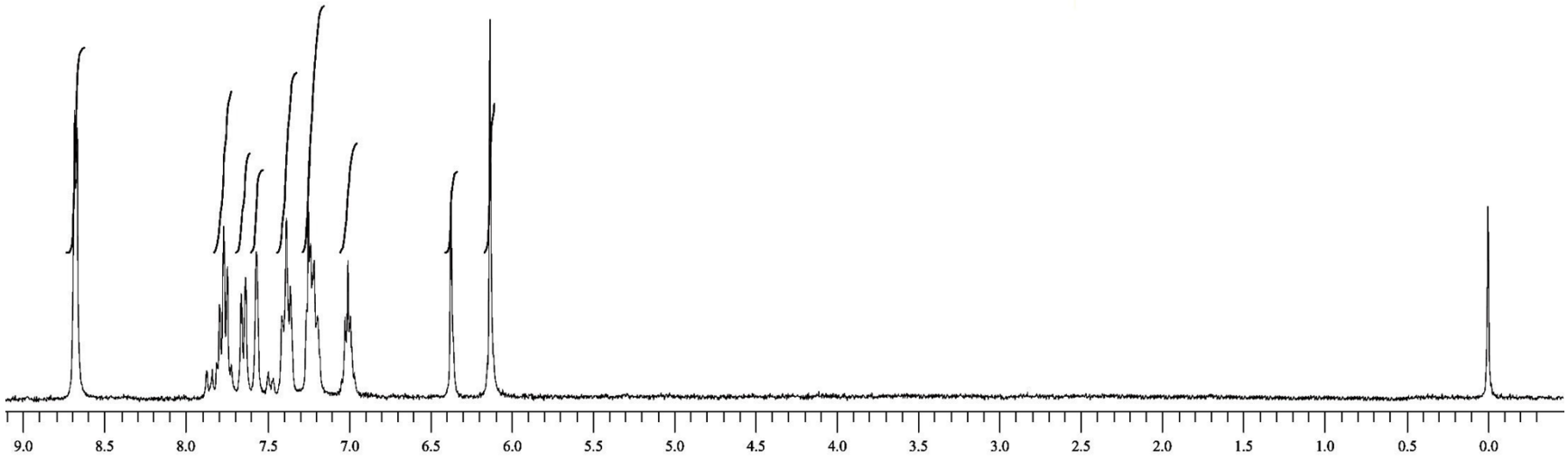
INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-PSCF3 #37-59 RT: 0.14-0.22 AV: 23 NL: 4.68E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



^{1}H NMR, Compound 20b





43.174
100.990
114.971
115.437
120.886
121.381
121.431
122.676
123.458
126.085
128.100
129.510
131.954
136.846
157.784
161.641
170.839
^{13}C NMR, Compound 20b
F3CO

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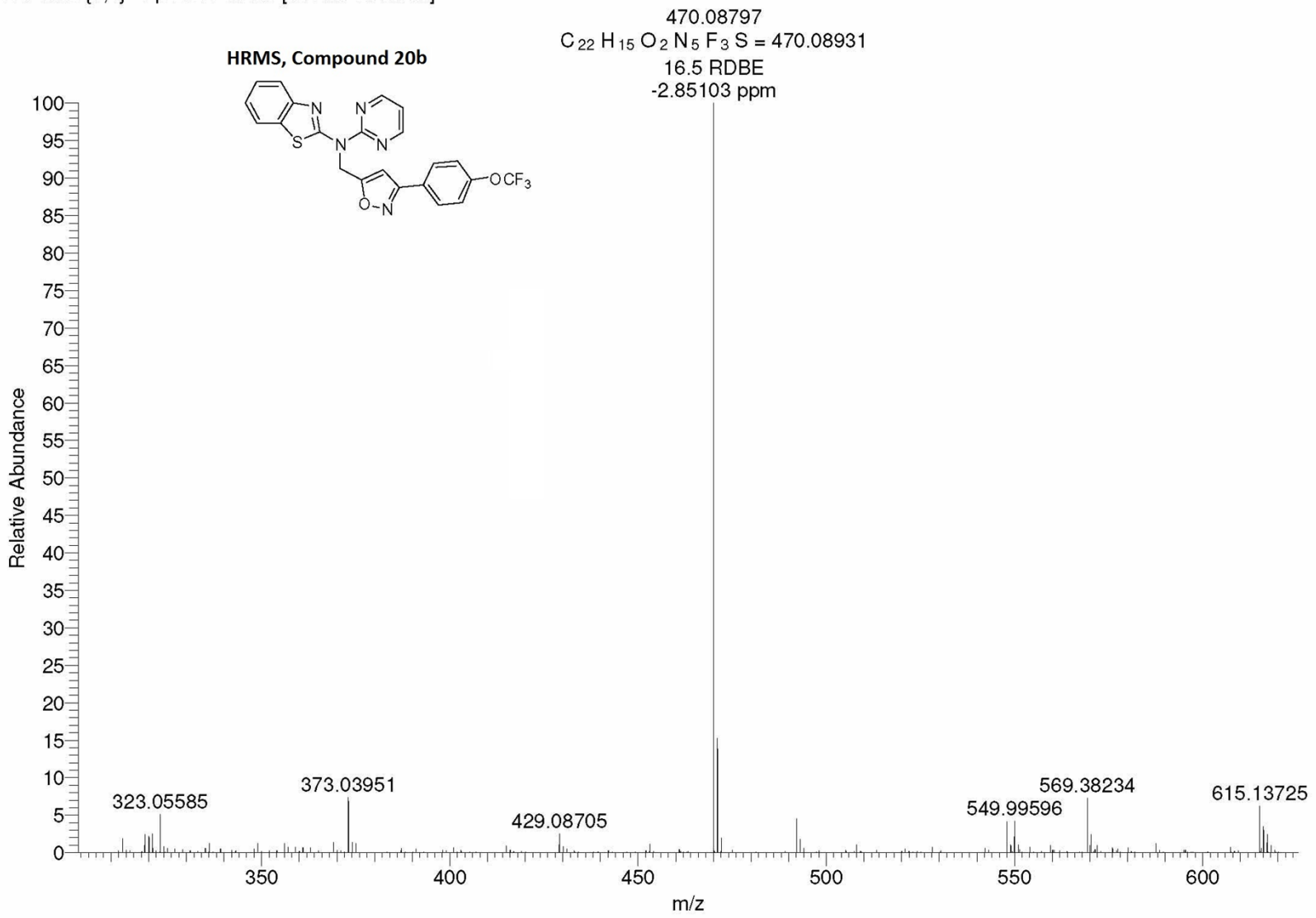
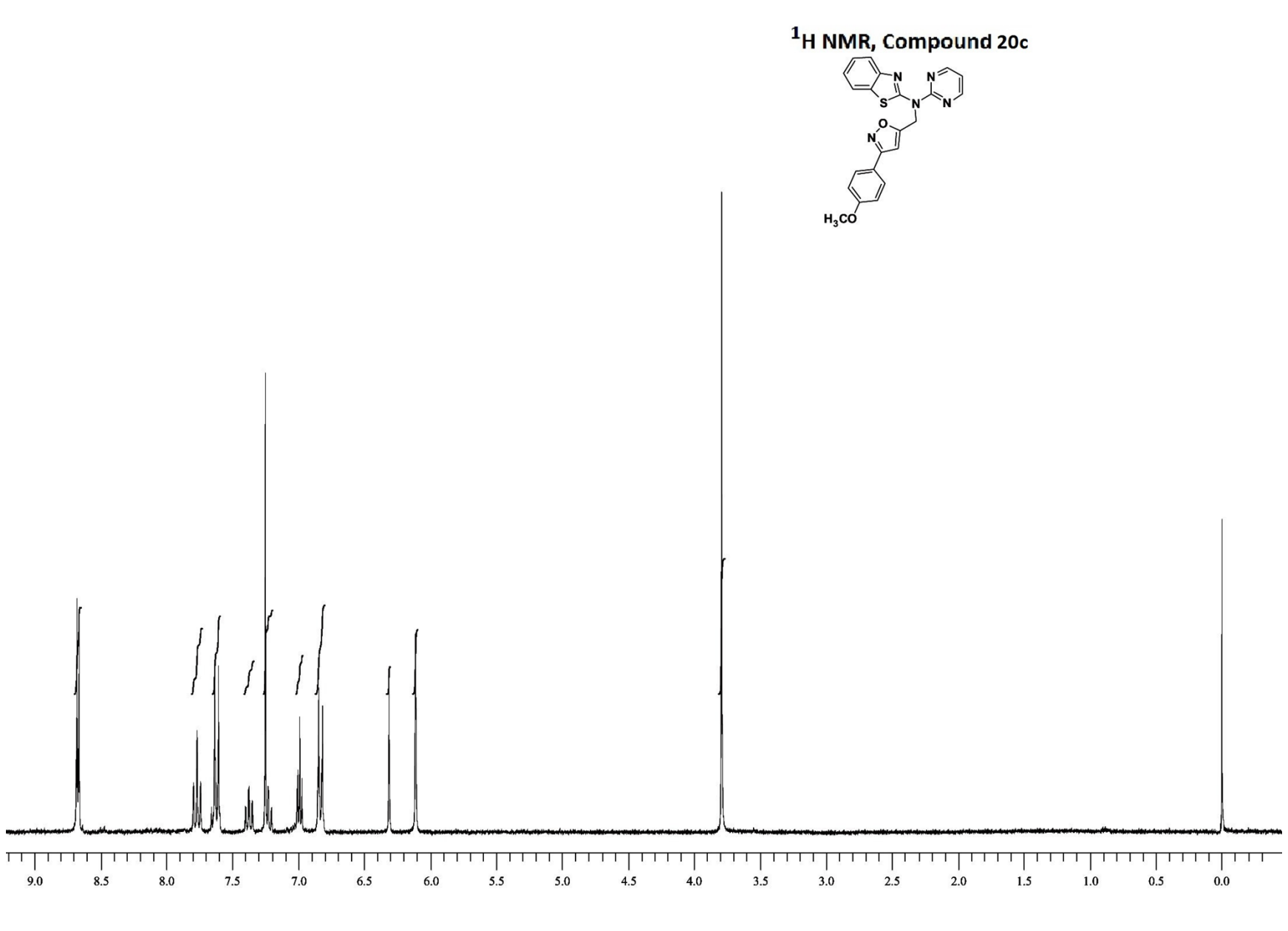
6/22/2012 6:08:05 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-POCF3 #28-47 RT: 0.11-0.18 AV: 20 NL: 9.99E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



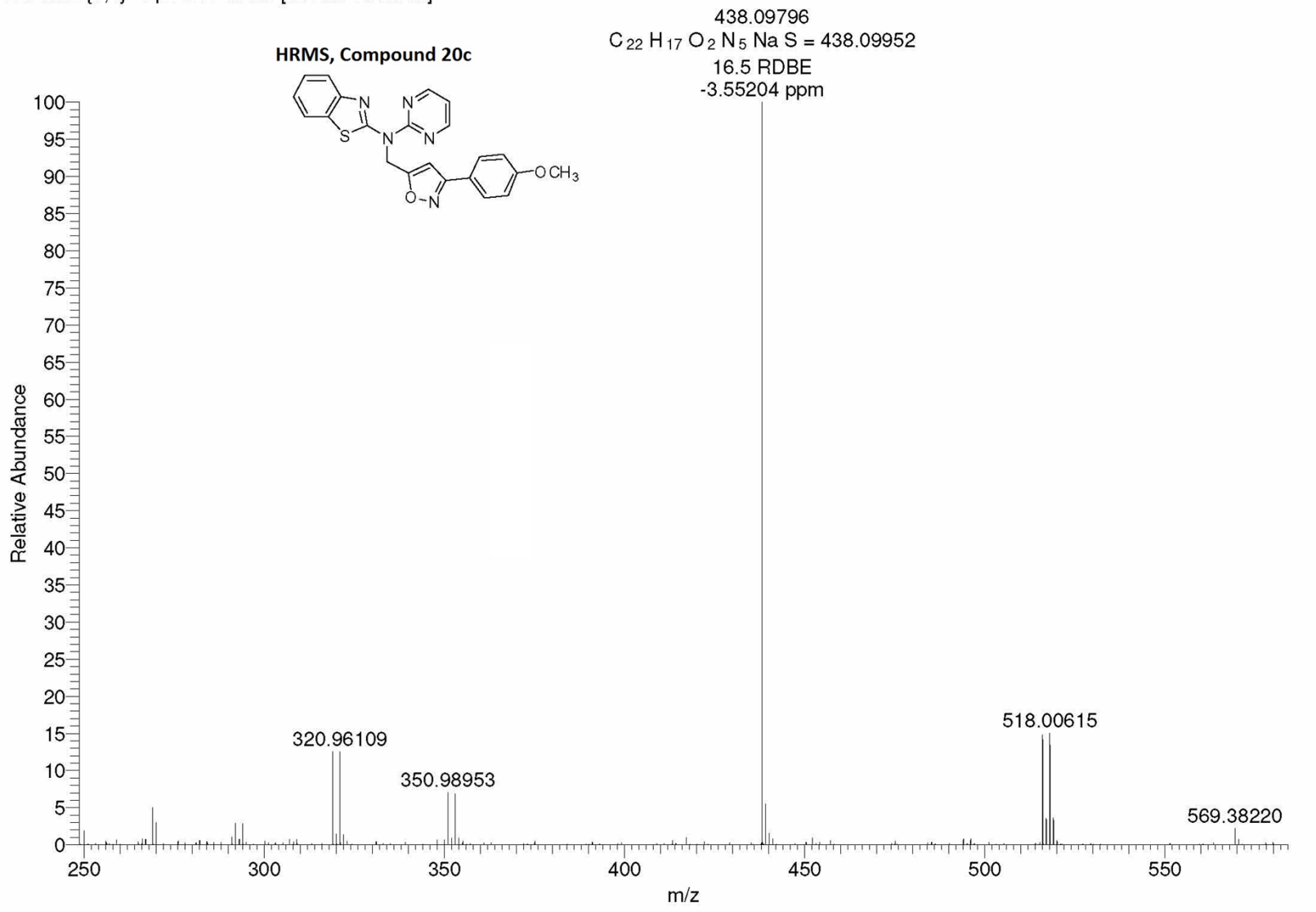

^{1}H NMR, Compound 20c
N
S
N
N
N
O
N
H_3CO
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

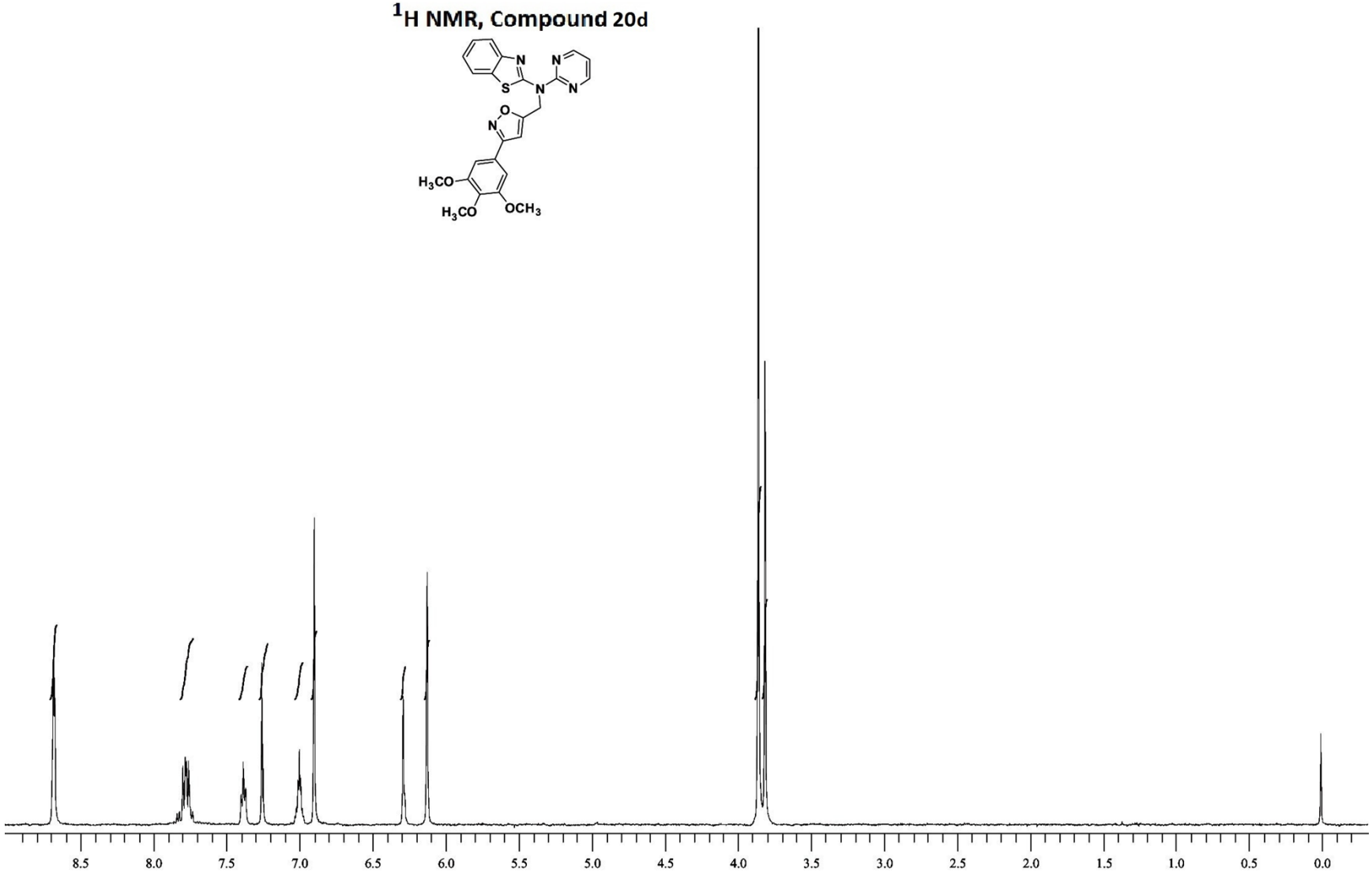
^{13}C NMR, Compound 20c
OCH3
N
S
O
42.763
55.215
100.221
114.040
114.736
120.523
120.935
123.117
125.728
128.081
129.947
148.800
157.288
160.731
169.429
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

TULSIRAM
6/22/2012 5:57:33 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-POCH3 #24-55 RT: 0.10-0.20 AV: 32 NL: 1.54E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



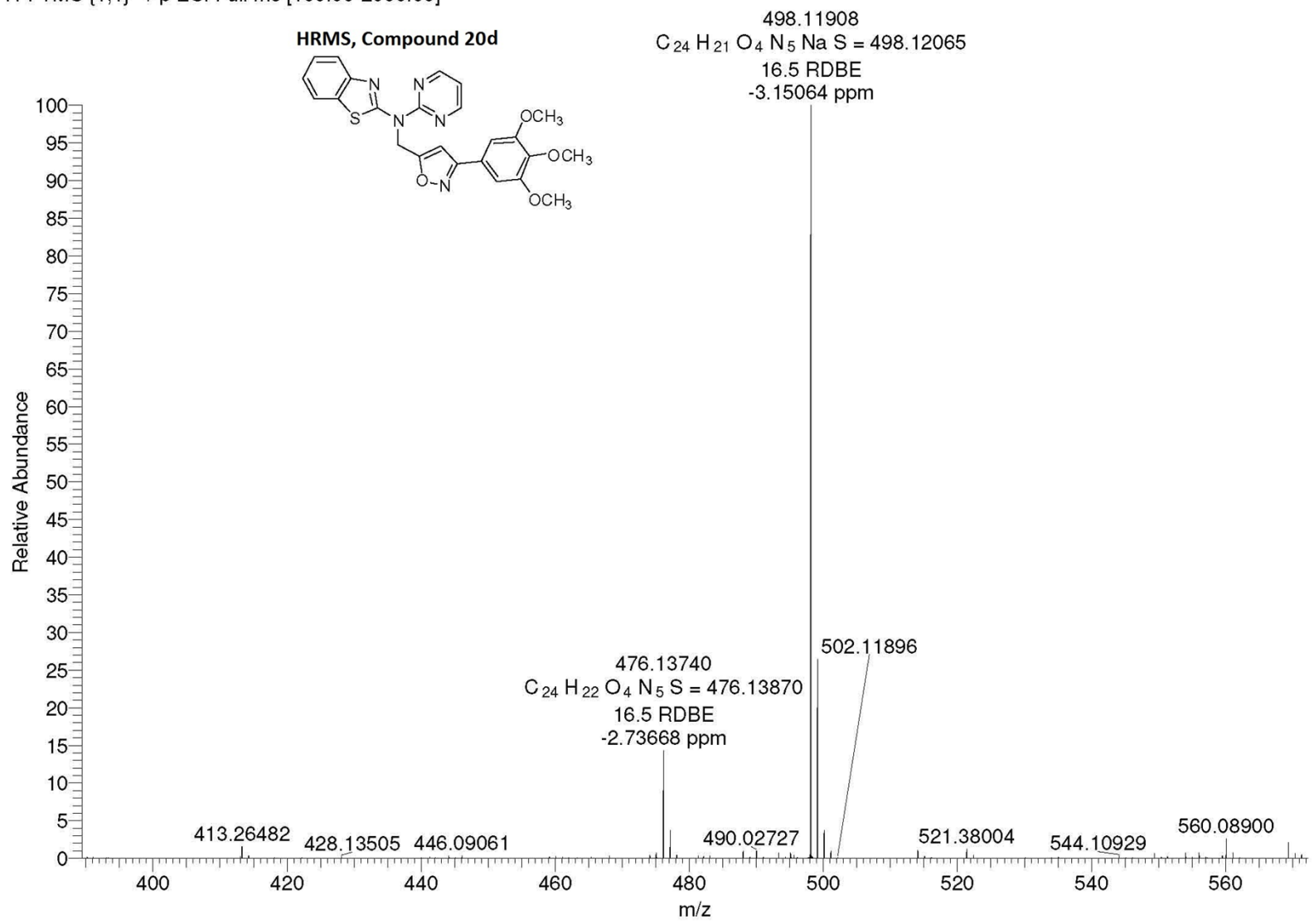

1H NMR, Compound 20d
N
S
N
N
N
O
H3CO
H3CO
OCH3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

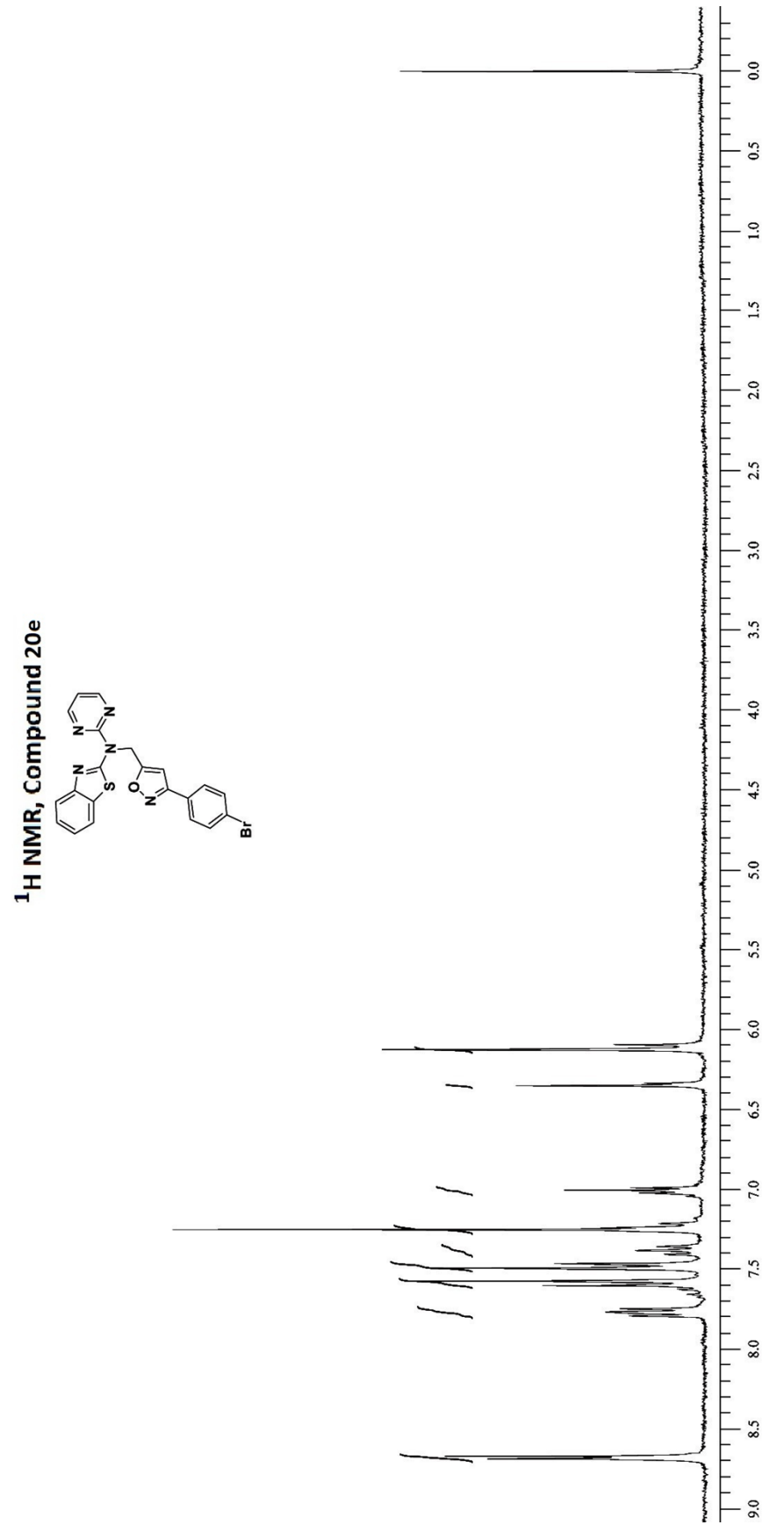
42.935
56.204
60.830
100.197
104.059
114.784
120.526
120.949
123.157
124.450
125.753
133.941
139.429
148.833
153.398
157.324
159.978
162.211
169.894
13C NMR, Compound 20d
OCH3
OCH3
OCH3
N
O
N
N
N
S
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

TULSIRAM
6/22/2012 6:02:49 PM

INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

RMK-OX-P3OME #39-60 RT: 0.15-0.22 AV: 22 NL: 1.23E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]




^{1}H NMR, Compound 20e
N
N
N
S
N
O
Br
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

42.792
100.250
114.069
114.765
120.552
120.964
123.146
125.757
128.110
129.977
157.317
160.760
169.458
13C NMR, Compound 20e
Br
N
N
N
S
N
O
N
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

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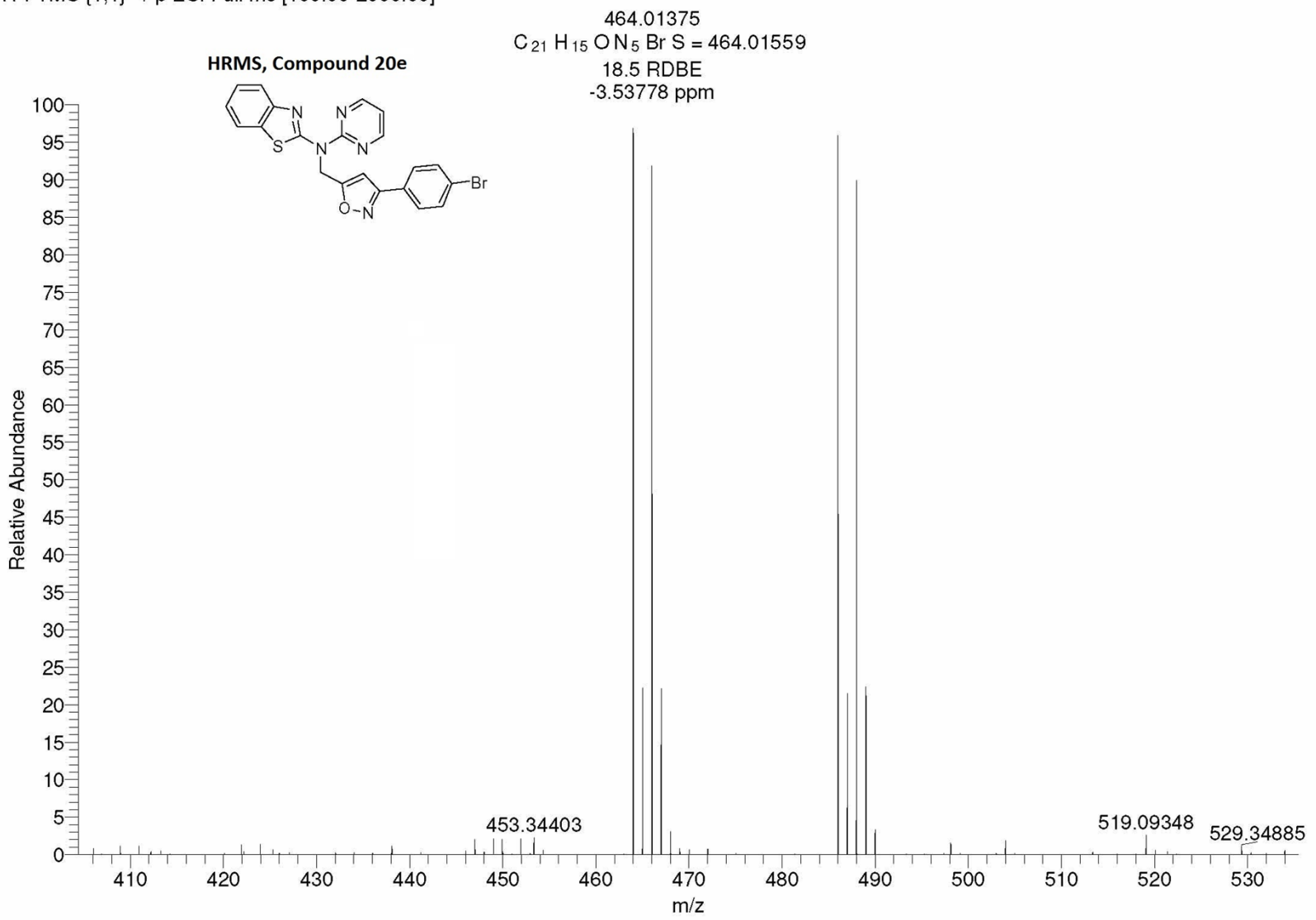
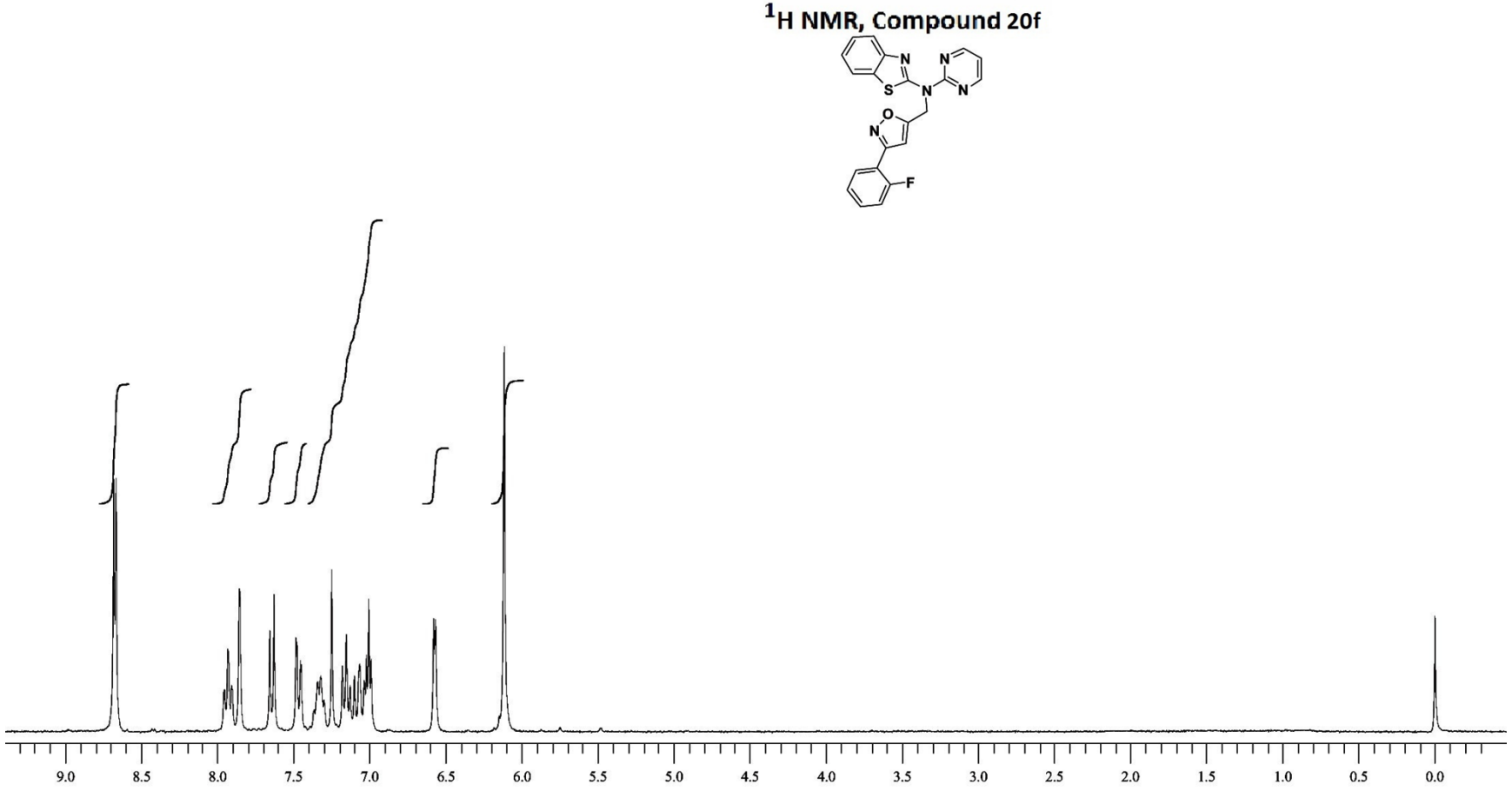
RMK-OX-PBR #25-56 RT: 0.10-0.20 AV: 32 NL: 2.45E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]




1H NMR, Compound 20f
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

42.601
103.126
103.220
114.906
115.889
116.052
116.328
122.182
122.970
124.405
129.041
131.352
131.463
135.805
147.814
156.931
157.311
160.218
169.173
^{13}C NMR, Compound 20f
F
N
O
S

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INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
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RMK-OX-PF #24-55 RT: 0.10-0.20 AV: 32 NL: 1.54E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]