Structural Optimization of Indole Derivatives Acting at Colchicine Binding Site as Potential Anticancer Agents

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Table of Contents

1. **Experimental Section** **2**
2. **Structures of known colchicine inhibitors: Figure S1** **9**
3. **Molecular Modeling: Figure S2** **10**
4. **Quantitative data of cell cycle analysis: Figure S3** **14**
5. **Analytical data for 41, 42, and 43 and their derivatives: Figure S4** **16-46**

1. Experimental Section

All chemicals for synthesis were purchased from Sigma-Aldrich Chemical Co., Fisher Scientific (Pittsburgh, PA), Matrix Scientific (Columbia, SC), AK Scientific (Mountain View, CA), Oakwood Products (West Columbia, SC) etc. and used without further purification. Moisture-sensitive reactions were carried under an argon or nitrogen atmosphere. Analytical thin-layer chromatography (TLC) was carried out on pre-coated silica gel (Merck Kieselgel 60 F_{254} layer thickness 0.25 mm). NMR spectra were obtained on a Bruker Avance III 400 (Billerica, MA) spectrometer. Chemical shifts are reported as parts per million (ppm) relative to TMS in $CDCl_3$. The structure of synthesized compounds was also assigned by ^1H-^1H 2D-COSY NMR analytic method. Flash column chromatography was performed on using silicagel (230-400 mesh, Merck). Mass spectral data was collected on a Bruker Esquire-LC/MS system (Bruker Daltonics, Billerica, MA) equipped with electrospray/ion trap instrument in positive and negative ion modes (ESI source). The purity of the final compounds was analyzed by an Agilent 1100 HPLC system (Santa Clara, CA). HPLC conditions: 45% acetonitrile at flow rate of 1.0 mL/min using a Phenomenex LUNA 5 μ C18 100A column (250 × 4.60 mm) purchased from Phenomenex (Torrance, CA) at ambient temperature. UV detection was set at 340 nm or 245 nm. Purities of the compounds were established by careful integration of areas for all peaks detected and determined as ≥95% for all compounds tested biological study. Log P & molecular weight were calculated by ChemBioOffice registered in UTHSC. M14/LCC6MDR1 melanoma cell line was shared with Dr. Robert Clarke's lab at the Georgetown University, and Taxol resistant prostate cancer cell line PC-3/TxR and DU145/TxR with Dr. Evan T. Keller's lab at the University of Michigan.

General synthetic procedures. Method A for protected indolyl bromides of **27** ~ **32**: A mixture of 2-bromopyridine-3,4-diamine (**26**, 1.0 mmol), protected-indolyl-carbaldehydes (**20**~**25**, 1.1 mmol), and TsOH (0.10 mmol) in 1,4-dioxane (10 mL) or, alternatively, c-H_2SO_4 as a catalytic amount in toluene/DMF (10/1, v/v) in a Dean-Stark apparatus. The solution was heated to reflux overnight. The mixture was concentrated under reduced pressure and poured into EtOAc, which was washed with water and dried over anhydrous $MgSO_4$, concentrated, purified by silica gel chromatography (EtOAc/*n*-hexane) and recrystallized under EtOAc/hexane to give the desired products. **Method B** for protected indolyl imidazopyridines **33** ~ **38**: A mixture of compounds **27** ~ **32** (0.15 mmol), tetrakis(triphenylphosphine) palladium (0) (4.5 μmol), and **8** [(3,4,5-trimethoxyphenyl)boronic acid (0.15 mmol)] in THF/MeOH (1/1 mL) with sodium carbonate (0.3 mmol, 2 *N* in deoxygenated water) were loaded into a vessel with a cap. Reaction vessels were placed in a reactor block in the microwave. A programmable microwave irradiation cycle of 30 min at 300 W and 25 min of fan-cooling was executed (irradiation time, 30 min). The mixture was transferred to a round bottom flask to be concentrated under reduced pressure and poured into EtOAc, which was washed with water and dried over anhydrous $MgSO_4$, concentrated, purified by silica gel chromatography (EtOAc/*n*-hexane) to afford the desired products. **Method C** for compounds **39** ~ **44:** To a solution of protected indoles (**33 ~ 38**) (0.56 mmol) in 10 mL ethanol was added a 10% solution of NaOH (5.68 mmol), and the mixture was refluxed for 20 h. Then, ethanol was evaporated, brine and CH_2Cl_2 were added, and the organic phase extracted with CH_2Cl_2 and then purified by flash column chromatography on silica gel using EtOAc/hexane or CH_2Cl_2/hexane as an eluent to give the target compounds.

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-2-yl)-1*H* -imidazo[4,5-*c*]pyridine (27).

Method A; Yield 32%; Yellow solid; MS (ESI): 450.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0022 [M + H] $^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 12.73 (bs, 1H, NH), 8.20 (d, *J* = 5.6 Hz, 1H), 8.20 (d, *J* = 5.6 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 5.6 Hz, 1H), 7.66 (m, 2H), 7.55 (t, *J* = 8.0 Hz, 2H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 7.36 (t, *J* = 7.4 Hz, 1H).

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-3-yl)-1*H*-imidazo[4,5-*c*]pyridine (**28**).

Method A; Yield 51%; Light yellow solid; MS (ESI): 450.9 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0026 [M + H] $^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.67 (bs, NH), 8.80 (bs, 1H), 8.61 (m, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 5.6 Hz, 1H) 8.08 (d, *J* = 8.0 Hz, 2H), 8.03 (m, 1H), 7.75 (t, *J* = 7.0 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.50 -7.49 (m, 2H).

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-4-yl)-1*H*-imidazo[4,5-*c*]pyridine (**29).**

Method A; Yield 50%; Light yellow solid; MS (ESI): 450.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0023 [M + H] $^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 12.67 (bs, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 5.6 Hz, 1H), 8.07-8.04 (m, 2H), 8.04-8.01 (m, 2H), 7.97 (d, *J* = 3.6 Hz, 1H), 7.71 (m, 1H), 7.61-7.55 (m, 4H), 7.53 (t, *J* = 8.0 Hz, 1H).

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-5-yl)-1*H*-imidazo[4,5-*c*]pyridine (**30).**

Method A; Yield 51%; Light brown solid; MS (ESI): 450.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0021 [M + H] $^+$;^{1}H NMR (acetone-d_6, 400 MHz) δ 12.69 (bs, 1H), 8.51 (s, 1H), 8.27 (dd, *J* = 1.6, 8.8 Hz, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 8.11-8.06 (m, 3H), 7.85 (d, *J* = 3.4 Hz, 1H), 7.70 (m, 1H), 7.61 (m, 2H), 7.56 (d, *J* = 5.2 Hz, 1H), 6.96 (d, *J* = 3.4 Hz, 1H).

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-6-yl)-1*H*-imidazo[4,5-*c*]pyridine (**31).**

Method A; Yield 50%; Light yellow solid; MS (ESI): 450.8 [M – H] $^-$, LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0023 [M + H] $^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.90 (s, 1H), 8.25 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.13 (d, *J* = 5.2 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 2H), 7.88 (d, *J* = 3.6 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.61-7.56 (m, 3H), 6.92 (d, *J* = 3.6 Hz, 1H).

4-Bromo-2-(1-(phenylsulfonyl)-1*H*-indol-7-yl)-1*H*-imidazo[4,5-*c*]pyridine (32).

Method A; Yield 56%; Light yellow solid; MS (ESI): 450.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{20}H_{13}BrN_4O_2S$: 453.0021. Found: 453.0019 [M + H] $^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.45 (bs, NH, 1H), 7.96 (d, *J* = 5.2 Hz, 1H), 7.59 (d, *J* = 5.2 Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 3.6 Hz, 1H), 7.41 (m, 1H), 7.28-7.20 (m, 4H), 6.67 (d, *J* = 3.6 Hz, 1H).

2-(1-(Phenylsulfonyl)-1*H*-indol-2-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (33).

Method B; Yield 60%; Yellow solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M - H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1547 [M + H] $^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.50 (d, *J* = 5.6 Hz, 1H), 8.42 (bs, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.71-7.60 (m, 3H), 7.56-7.42 (m, 3H), 7.42 (s, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 3.94 (s, 6H), 3.84 (s, 3H).

2-(1-(Phenylsulfonyl)-1*H*-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (34).

Method B; Yield 65%; Yellow solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1555 [M + H] $^+$;^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.48 (bs, NH), 8.82 (s, 1H), 8.77 (d, *J* = 7.6 Hz, 1H), 8.45 (d, *J* = 5.6 Hz, 1H), 8.39

(s, 2H), 8.09 (d, *J* = 8.0 Hz, 2H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 5.6 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 4.18 (s, 6H), 3.78 (s, 3H).

2-(1-(Phenylsulfonyl)-1*H*-indol-4-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (35).

Method B; Yield 82%; Yellow solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1552 [M + H] $^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.43 (d, *J* = 4.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 2H), (bs, NH), 8.82 (s, 1H), 8.77 (d, *J* = 7.6 Hz, 1H), 8.45 (d, *J* = 5.6 Hz, 1H), 8.39 (s, 2H), 8.09 (d, *J* = 8.0 Hz, 2H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 5.6 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 4.18 (s, 6H), 3.78 (s, 3H).

2-(1-(Phenylsulfonyl)-1*H*-indol-5-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (36).

Method B; Yield; 57%; Pistachio green solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1545 [M + H]$^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.37 (d, *J* = 5.2 Hz, 1H), 8.27 (s, 1H), 8.11-8.06 (m, 2H), 7.94 (bs, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 3.0 Hz, 1H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.33 (bs, 1H), 6.66 (d, *J* = 3.0 Hz, 1H), 3.92 (s, 6H), 3.89 (s, 3H).

2-(1-(Phenylsulfonyl)-1*H*-indol-6-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (37).

Method B; Yield; 65%; Yellow solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1550 [M + H] $^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91 (s, 1H), 8.40 (d, *J* = 5.6 Hz, 1H), 8.22 (bs, 2H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J*

= 7.6 Hz, 2H), 7.61 (d, *J* = 3.6 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.34 (m, 1H), 6.68 (d, *J* = 3.6 Hz, 1H), 4.10 (s, 6H), 3.88 (s, 3H).

2-(1-(Phenylsulfonyl)-1*H*-indol-7-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (38).

Method B; Yield; 56%; Yellow solid; MS (ESI): 398.1 [M - ($PhSO_2$)] $^-$; 538.8 [M – H] $^-$; LCMS (ESI) *m/z* calcd for $C_{29}H_{24}N_4O_5S$: 541.1546. Found: 541.1558 [M + H] $^+$; MS (ESI): 538.8 [M – H] $^-$; 541.2 [M + H] $^+$; 563.2 [M + Na] $^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (bs, NH, 1H), 8.10 (bs, 1H), 7.67-7.61 (m, 4H), 7.55 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.48-7.44 (m, 4H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.10 (bs, 2H), 6.78 (d, *J* = 3.6 Hz, 1H), 3.96 (s, 6H), 3.94 (s, 3H).

2-(1*H*-Indol-2-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (39).

Method C; Yield 71%; Yellowish solid; MS (ESI): 399.1 [M – H] $^-$; 401.1 [M + H] $^+$; LCMS (ESI) *m/z* calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1616 [M + H] $^+$; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.32 (d, *J* = 5.6 Hz, 1H), 7.66 (s, 2H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 5.6 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.24 (s, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 3.96 (s, 6H), 3.85 (s, 3H); HPLC: t_R 21.02 min, purity = 97.50%.

2-(1*H*-Indol-3-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (40).

Method C; Yield 71%; Yellowish solid; MS (ESI): 399.1 [M – H] $^-$; 401.3 [M + H] $^+$; LCMS (ESI) *m/z* calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1613 [M + H] $^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 12.02 (bs, 1H, NH), 10.90 (bs, 1H, NH), 8.85 (d, *J* = 6.4 Hz, 1H), 8.61 (s, 2H), 8.34 (d, *J* = 5.2 Hz, 1H), 8.27 (s, 2H), 7.56 (m, 2H), 7.39 (d, *J* = 5.2 Hz, 1H), 7.30-7.24 (m, 2H),4.02 (s, 6H), 3.88 (s, 3H); HPLC: t_R 14.04 min, purity > 99%.

2-(1*H*-Indol-4-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (41).

Method C; Yield 75%; Yellowish solid; MS (ESI): 399.1 $[M - H]^-$; 401.3 $[M + H]^+$; LCMS (ESI) *m/z* calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1614 $[M + H]^+$; ^1H NMR (acetone-d_6, 400 MHz) δ 11.5 (bs, 1H), 9.8 (bs, 1H), 7.73 (s, 2H), 7.56 (d, *J* = 5.2 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 1H), 6.94-6.68 (m, 3H), 6.63 (d, *J* = 5.2 Hz, 1H), 6.45 (t, *J* = 8.0 Hz, 1H), 3.18 (s, 6H), 2.99 (s, 3H); HPLC: t_R 12.26 min, purity 99.06%.

2-(1*H*-Indol-5-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (42, 5-IIP).

Method C; Yield 68%; Pistachio green solid; MS (ESI): 399.4 $[M - H]^-$; 401.5 $[M + H]^+$; LCMS (ESI) m/z calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1613 $[M + H]^+$; ^1H NMR (acetone-d_6, 400 MHz) δ 12.5 (bs, 1H), 10.7 (bs, 1H), 8.54 (s, 2H), 8.57 (s, 1H), 8.54 (bs, 2H), 8.37 (d, *J* = 5.2 Hz, 1H), 8.18 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.46-7.45 (m, 2H), 4.01 (s, 6H), 3.83 (s, 3H); HPLC: t_R 11.75 min, purity = 96.10%.

2-(1*H*-Indol-6-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (43, 6-IIP).

Method C; Yield 71%; Yellowish solid; MS (ESI): 399.1 $[M - H]^-$; 401.4 $[M + H]^+$; LCMS (ESI) *m/z* calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1614 $[M + H]^+$; ^1H NMR (acetone-d_6, 400 MHz) δ 12.4 (bs, 1H), 10.7 (bs, 1H), 8.54 (s, 2H), 8.50 (s, 1H), 8.37 (d, *J* = 5.2 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.52 (s, 1H), 7.45 (d, *J* = 5.2 Hz, 1H), 6.59 (s, 1H), 4.00 (s, 6H), 3.83 (s, 3H); HPLC: t_R 12.26 min, purity = 97.44%.

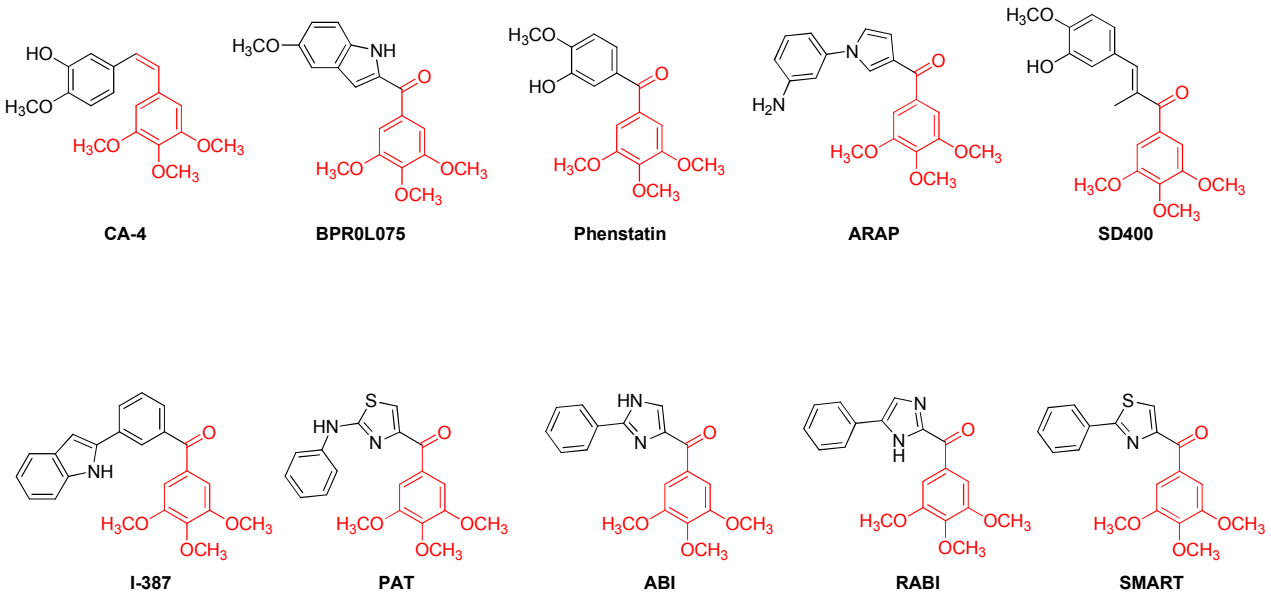
2-(1*H*-Indol-7-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (44, 7-IIP).

Method C; Yield 58%; Light yellowish solid; MS (ESI): 399.1 $[M - H]^-$; 401.3 $[M + H]^+$; LCMS (ESI) *m/z* calcd for $C_{23}H_{20}N_4O_3$: 401.1614. Found: 401.1616 $[M + H]^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.1 (bs, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 7.94 (s, 2H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.35 (m, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 6.66 (t, *J* = 2.6 Hz, 1H), 3.97 (s, 6H), 3.96 (s, 3H); HPLC: t_R 13.78 min, purity > 99%.

2-(1*H*-Indol-7-yl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]pyridine (44a).

Yellow solid; MS (ESI): 403.3 $[M + H]^+$; 401.0 $[M - H]^-$; LCMS (ESI) *m/z* calcd for $C_{23}H_{22}N_4O_3$: 403.1770. Found: 403.1772 $[M + H]^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.73 (bs, 1H, N*H*), 8.35 (s, 1H), 8.20 (d, *J* = 5.6 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.36 (dd, *J* = 3.2, 2.4 Hz, 1H), 7.36 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 6.89 (s, 2H), 6.68 (d, *J* = 5.6 Hz, 1H), 6.65 (dd, *J* = 3.2, 2.4 Hz, 1H), 4.53 (bs, 2H, N*H*), 3.87 (s, 3H), 3.54 (s, 6H); HPLC: t_R 16.57 min, purity > 99%.

2. Figure S1. Structures of known colchicine inhibitors



3. Molecular Modeling

Docking study was performed in Schrodinger Molecular Modeling Suite 2014 (Schrodinger Inc., Portland, OR) followeding a similar procedure as described before[17b] using the DAMA colchicine-tubulin complex (Protein Data Bank code: 1SA0) as the template. All the compounds including native ligand were first prepared by LigPrep panel in Schrodinger software to generate at most 32 favored conformations for each compound under physiological pH and temperature. Tubulin receptor grid was generated by taking off the native ligand but reserving the center binding site residue orientations. Then both the tubulin inhibitors investigated in this study and native ligand DAMA colchicine were docked into the same colchicine binding pocket and the output poses were minimized through molecular dynamics calculation.

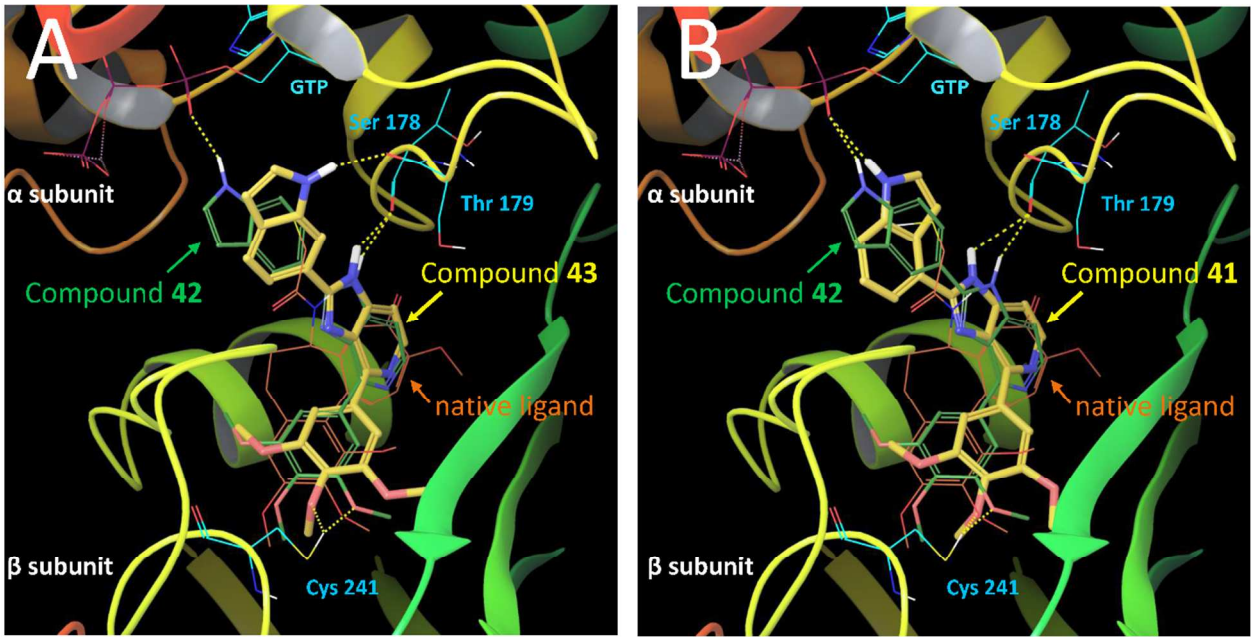


Figure S2. Proposed binding poses for 6-indolyl compound **43** (yellow thick tube in panel A) and 4-indolyl compound **41** (yellow thick tube in panel B) in colchicine binding site of α,β-tubulin (PDB code:1SA0), compared with 5-indolyl compound **42** (green thin tube in panels A and B) and native ligand DAMA-colchicine (orange wire in panels A and B).

We docked **41** and **43** into the colchicine binding site of the DAMA-colchicine-α,β-tubulin complex (Figure 3, PDB code:1SA0) and they generally showed similar binding poses as shown with **42** in our previous study.[17b] The C-ring TMP moiety of all three compounds inserted deeply into the β-subunit and formed hydrogen bonds with residue Cys-241, in a manner similar to the native ligand DAMA-colchicine (Figure 3, panel A and B). The imidazole B-ring moieties of all three compounds overlapped very well and were anchored by hydrogen bonds between imidazole NH groups and α-subunit residue Thr-179. Meanwhile, the A-ring indolyl moiety of all three compounds extended toward the interface between α- and β-subunit. Interestingly, the orientation of A-ring indole moieties varied with the point of attachment of the indole to the imidazole. Unlike the 5-indolyl **42**, in which the indolyl NH group is pointing towards the GTP in α-subunit, **43** (6-indolyl) had its indole moiety oriented in the opposite direction and connected with α-subunit residue Ser-178 through a hydrogen bond (Figure 3, panel A). **41** (4-indolyl) slightly glided out of the β-subunit pocket and its orientation favored a hydrogen bond between the indolyl NH group and the GTP in α-subunit (Figure 3, panel B). In our modeling approach, the docking scores of **41**, **42**, and **43** (-8.18, -8.84 and -8.61, respectively) were comparable to that of native ligand (-9.12), indicating good binding affinity of these analogs with tubulin dimer.

4. Biological Studies

4.1 Cell Culture and Viability Assay of Human Melanoma and Prostate Cancer Cells

All cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Human melanoma cells A375, WM164, M14 and M14/LCC6MDR1 were cultured in DMEM medium (Mediatech, Inc., Manassas, VA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) and 1% antibiotic-antimycotic mixture (Sigma-Aldrich, St. Louis, MO). Human prostate cancer cells PC-3, DU145 and PPC-1 were cultured in RPMI-1640 medium (Mediatech, Inc., Manassas, VA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) and 1% antibiotic-antimycotic mixture (Sigma-Aldrich, St. Louis, MO). The antiproliferation activities of all the compounds were tested with a MTS cell viability assay after 48 h incubation following the same procedure described before.

4.2 *In Vitro* Tubulin Polymerization Assay

HTS-tubulin polymerization assay kit (Cytoskeleton, Denver, CO) was used to determine the changes of tubulin polymerization profile in the presence of our compounds. Briefly, porcine brain tubulin (0.4 mg, >97% pure) was reconstituted in ice-cold 100 μL of general tubulin buffer containing 5 μM or 10 μM of the test compounds and incubated at 37 °C for tubulin polymerization (n = 3). Reaction system with same final concentration of DMSO (1%) was used as assay control. The absorbance of wavelength at 340 nm was kinetically read every 1 min for 60 min by a SYNERGY 4 microplate reader (Bio-Tek Instruments, Winooski, VT).

4.3 Cell Cycle Analysis

Human melanoma A375 cells or human prostate cancer PC-3 cells were cultured in 10 cm tissue culture dishes (n = 3) until the confluence reached 70%. Then the cells were starved to

synchronize in FBS-free medium for 24 h before they were treated by tested compound solution at the concentrations of 10 nM or 50 nM for 24 h in 10% FBS growth medium. Cell pellets were harvested by trypsin and fixed in 70% ethanol overnight at -20 °C. Cellular DNA was stained with 50 μg/mL propidium iodide before they were analyzed in a BD LSR-II cytometer (BD Biosciences, San Jose, CA) with 10,000 cells sorted for each sample. Data were processed using Modfit 2.0 program (Verify Software House, Topsham, ME).

4.4 Liver Microsomal Stability Assay

The *in vitro* metabolic stability of the selected compounds were studied by incubating the test compounds (2 μM) in a total liver microsomal reaction system (1 mg/mL protein in 0.2 M of phosphate buffer solution (pH 7.4), 1.3 mM NADPH, 3.3 mM glucose-6-phosphate, and 0.4 U/mL glucose-6-phosphate dehydrogenase) at 37 °C in a shaking incubator (n =3). Pooled human liver microsomes (HLM) and pooled male CD1 mouse liver microsomes (MLM) were purchased from Xenotech, LLC (Lenexa, KS). Aliquots (100 μL) were sampled from the reaction mixtures at 0, 5, 10, 20, 30, 60, and 90 min after the beginning of the 37 °C incubation. Each of the aliquots were quenched by adding 300 μL ice-cold acetonitrile. Samples were incubated on ice for 20 min then centrifuged at 10,000 g for 15 min at 4 °C, and the supernatant was analyzed by a LC-MS/MS system (AB Sciex API4500). For metabolite identification, the reaction mixture was incubated for 5 h with test compound at the concentration of 50 μM in the same conditions. The quenched samples were filtered through a 0.2 μm membrane then analyzed with a Water Xevo G2-S high resolution mass spectrometer.

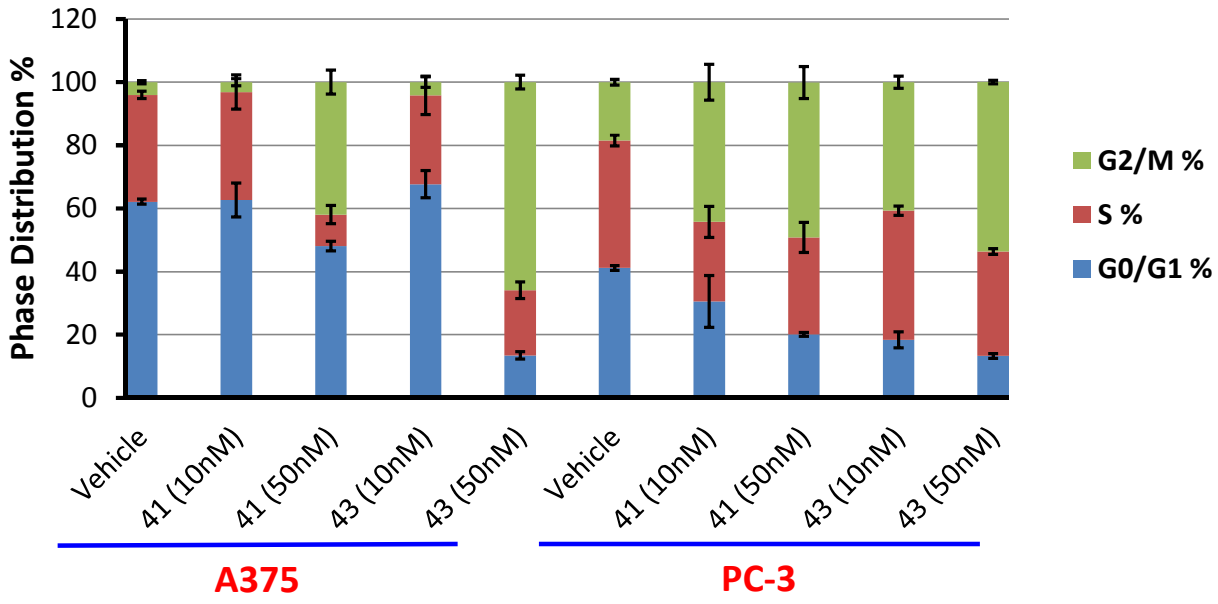
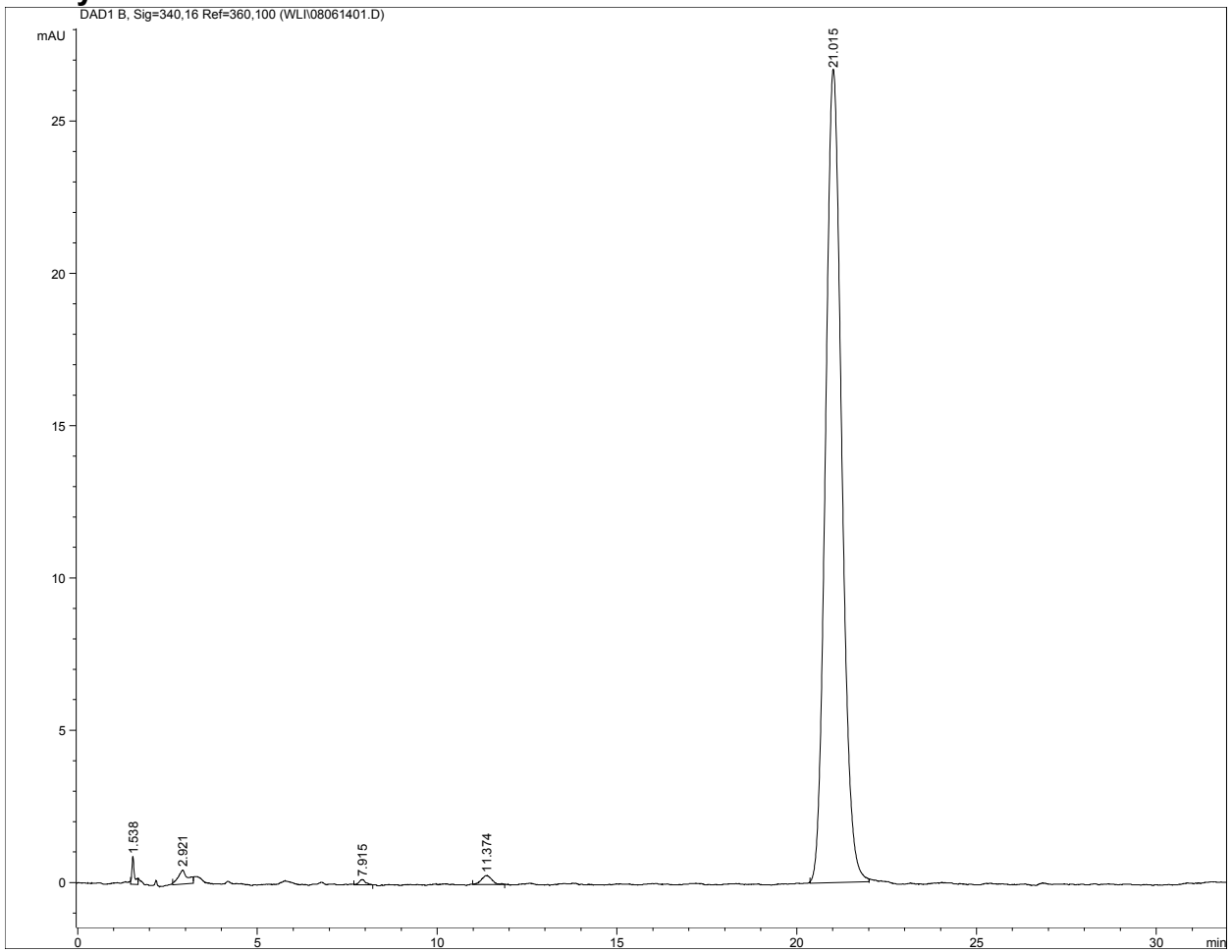


Figure S3. Quantitative data of flow cytometry for 6-indolyl compound **43** and 4-indolyl compound **41** showing effectively inhibited the tubulin polymerization *in vitro*.

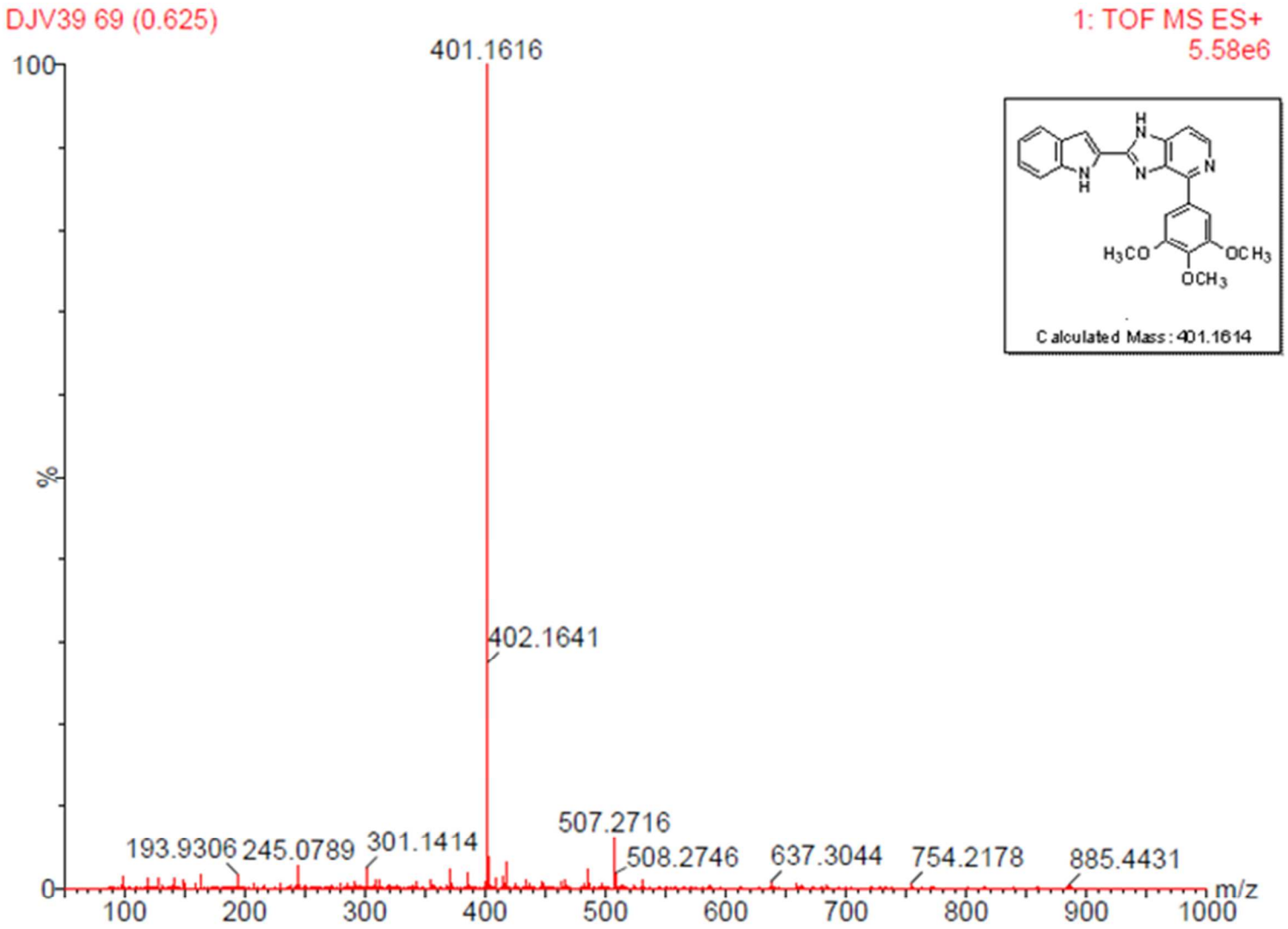
5. **Figure S4.** Analytical data for compounds 41, 42, and 43 and their derivatives.

Compound **39**, 2-IIP: **HPLC** Analysis
Column: Phenomenex LUNA 5μ C18 250*4.6 mm
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 340nm

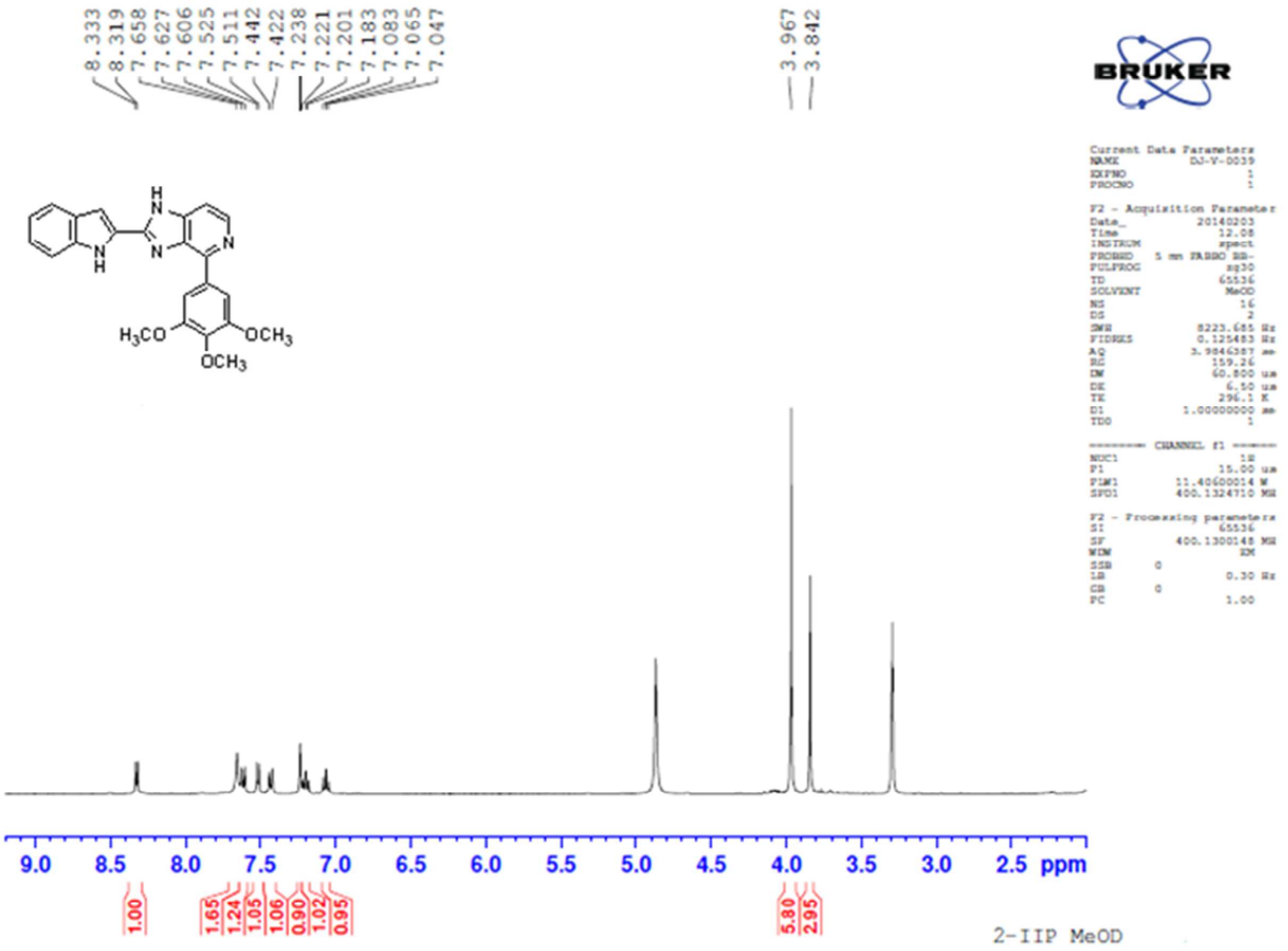
Purity 97.50%



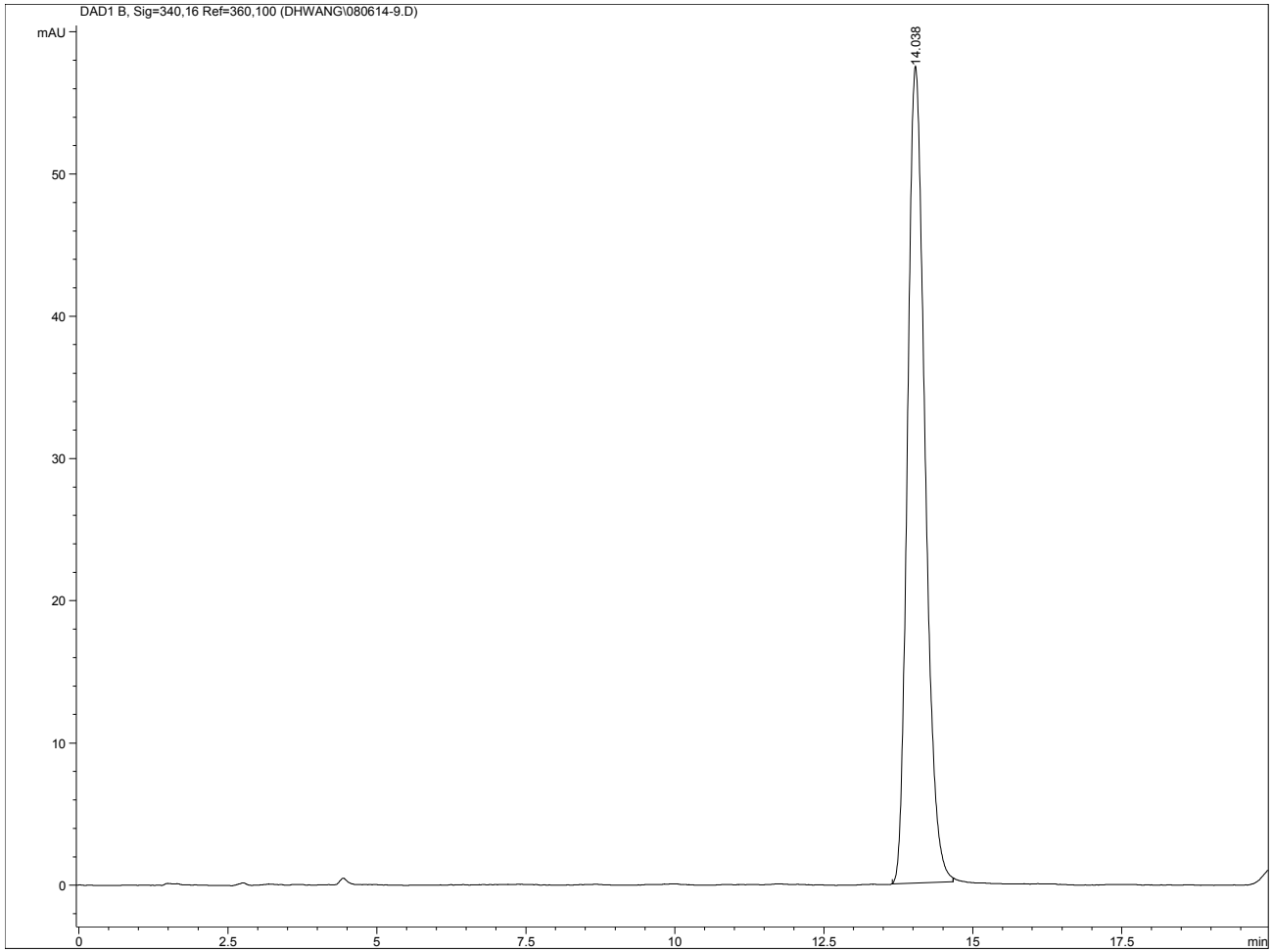
Compound **39**, 2-IIP: **HRMS** Analysis



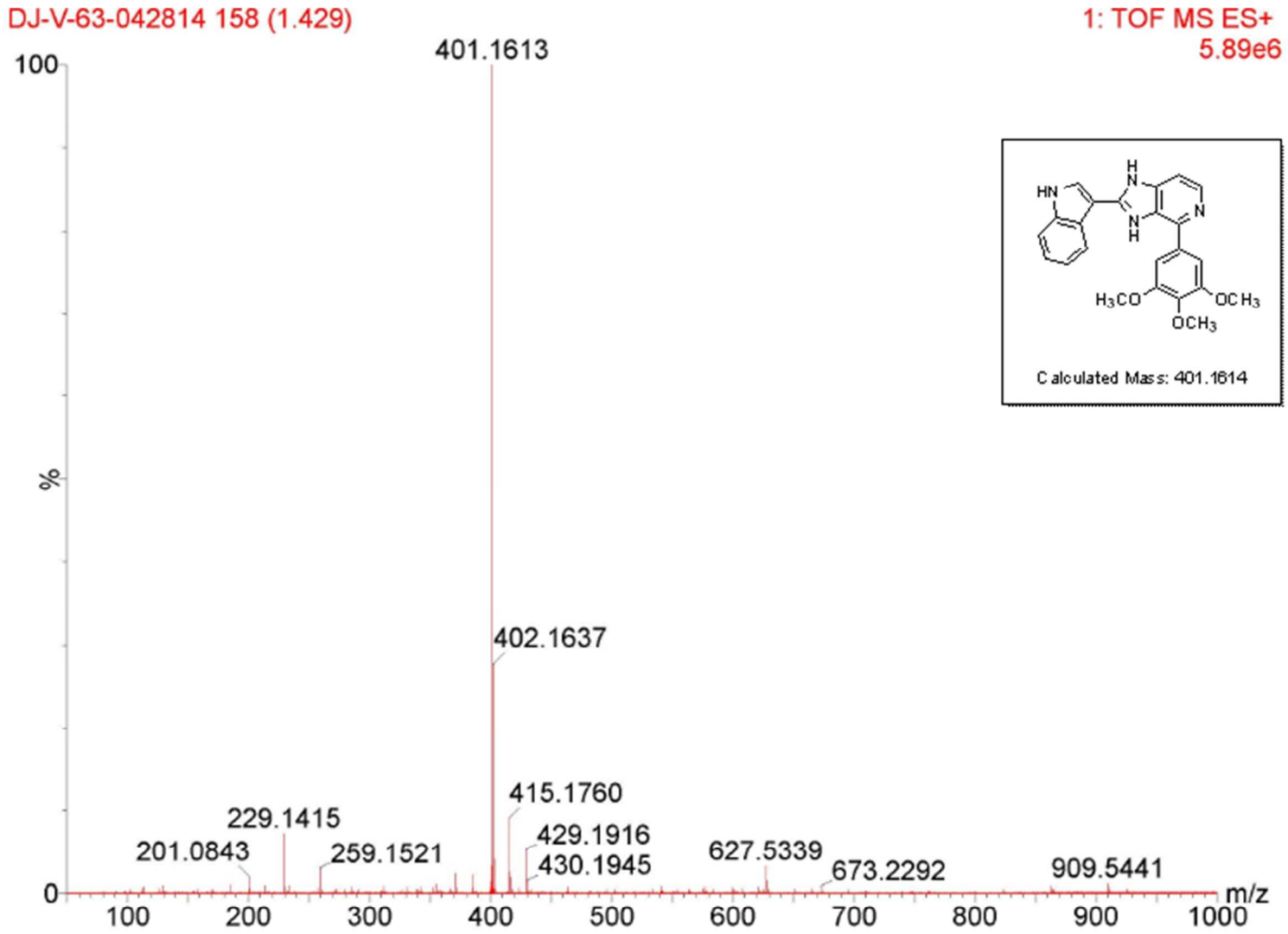
Compound **39**, 2-IIP: **^{1}H NMR** (MeOD-d_4, 400 MHz)



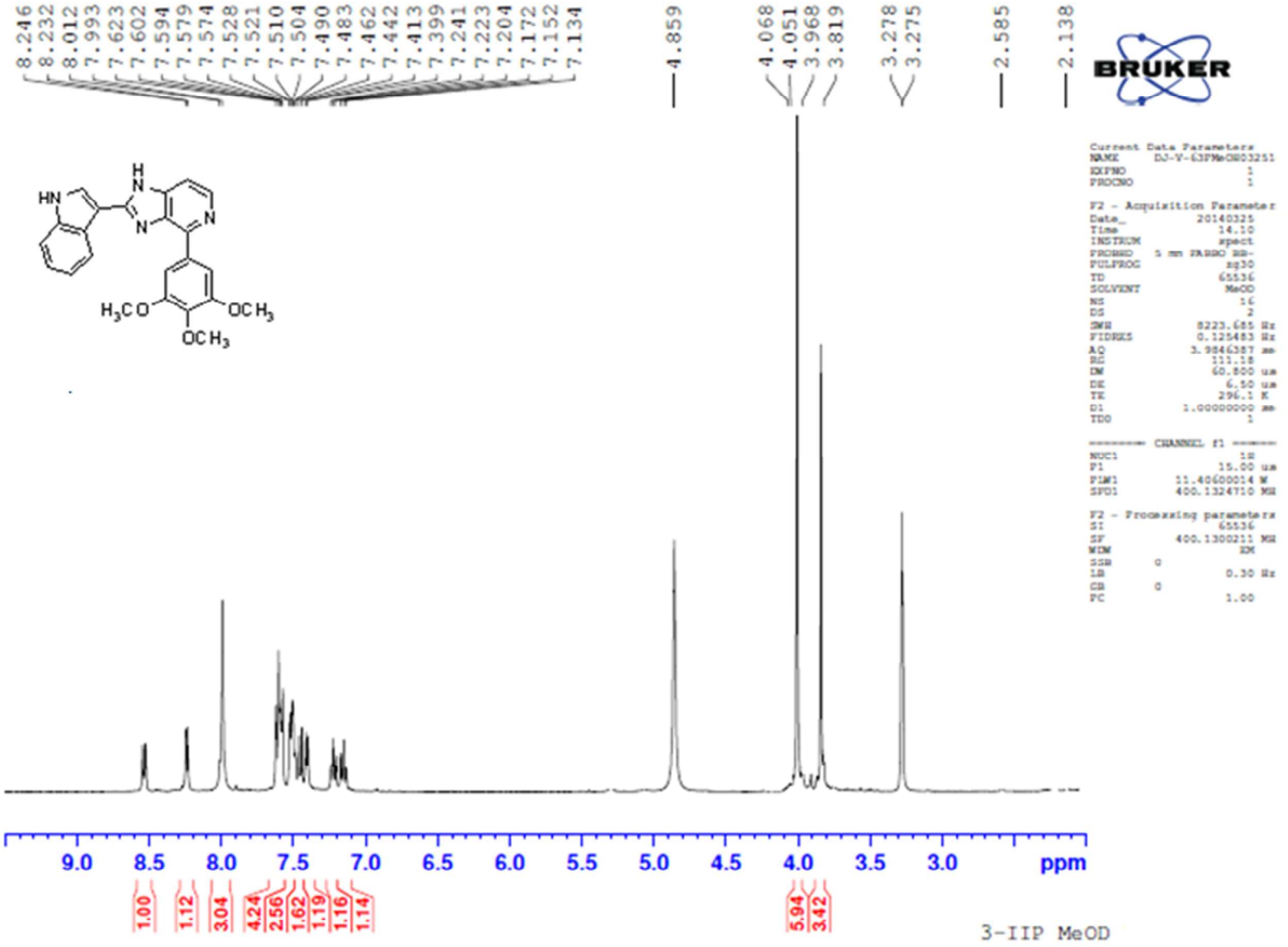
Compound **40**, 3-IIP: **HPLC** Analysis
Column: Phenomenex LUNA 5μ C18
250*4.6 mm
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 340nm
Purity > 99.99



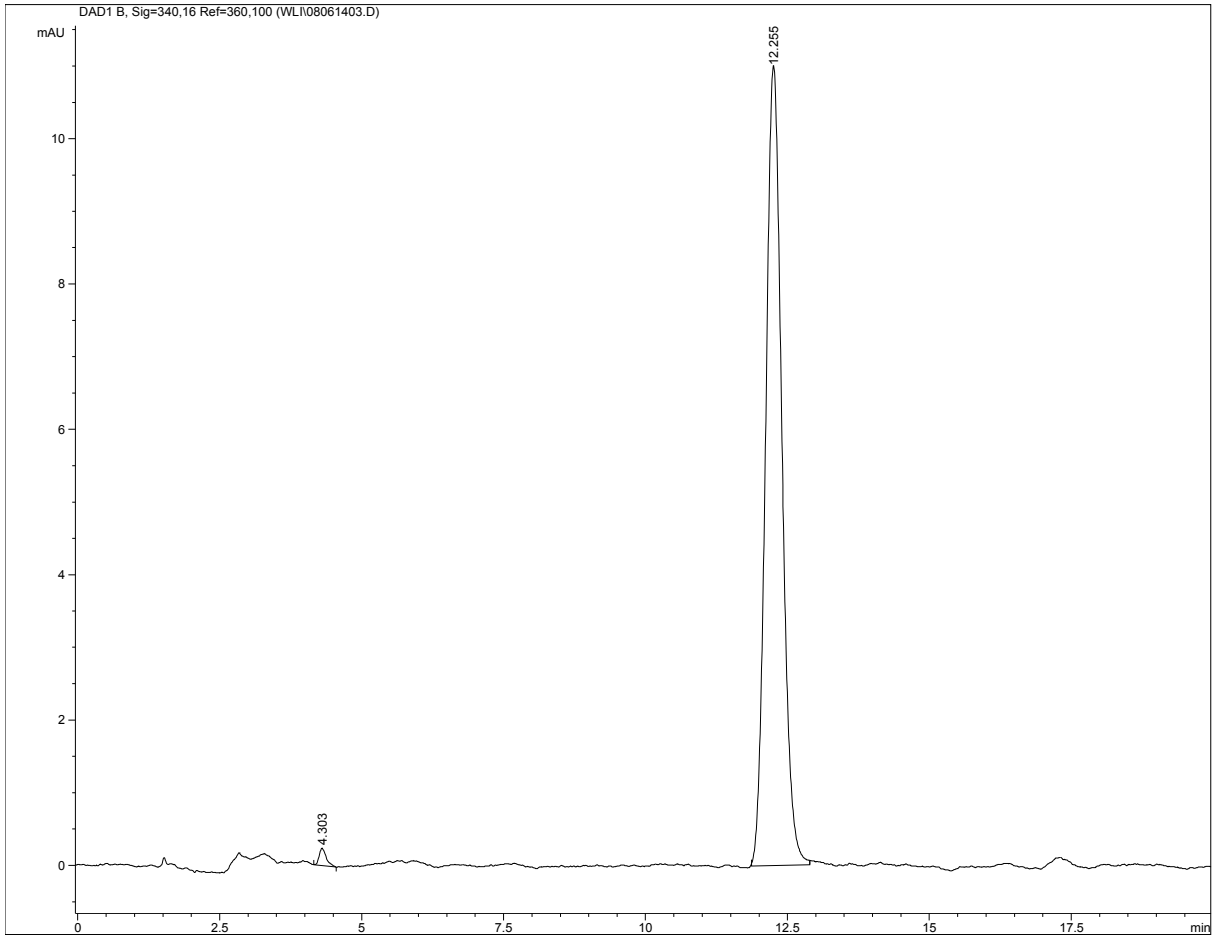
Compound **40**, 3-IIP: **HRMS** Analysis



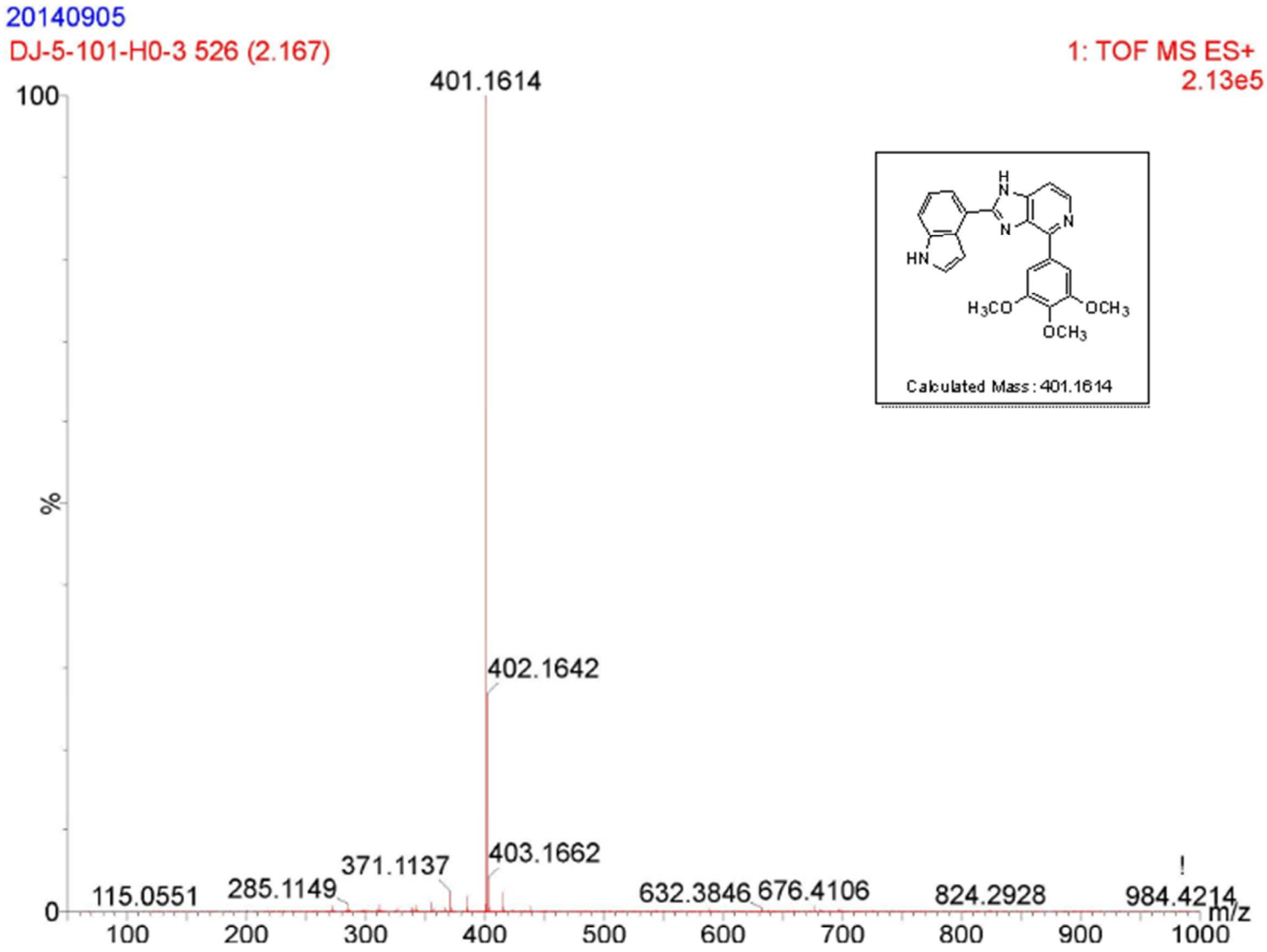
Compound **40**, 3-IIP: **^{1}H NMR** (MeOD-d_4, 400 MHz)



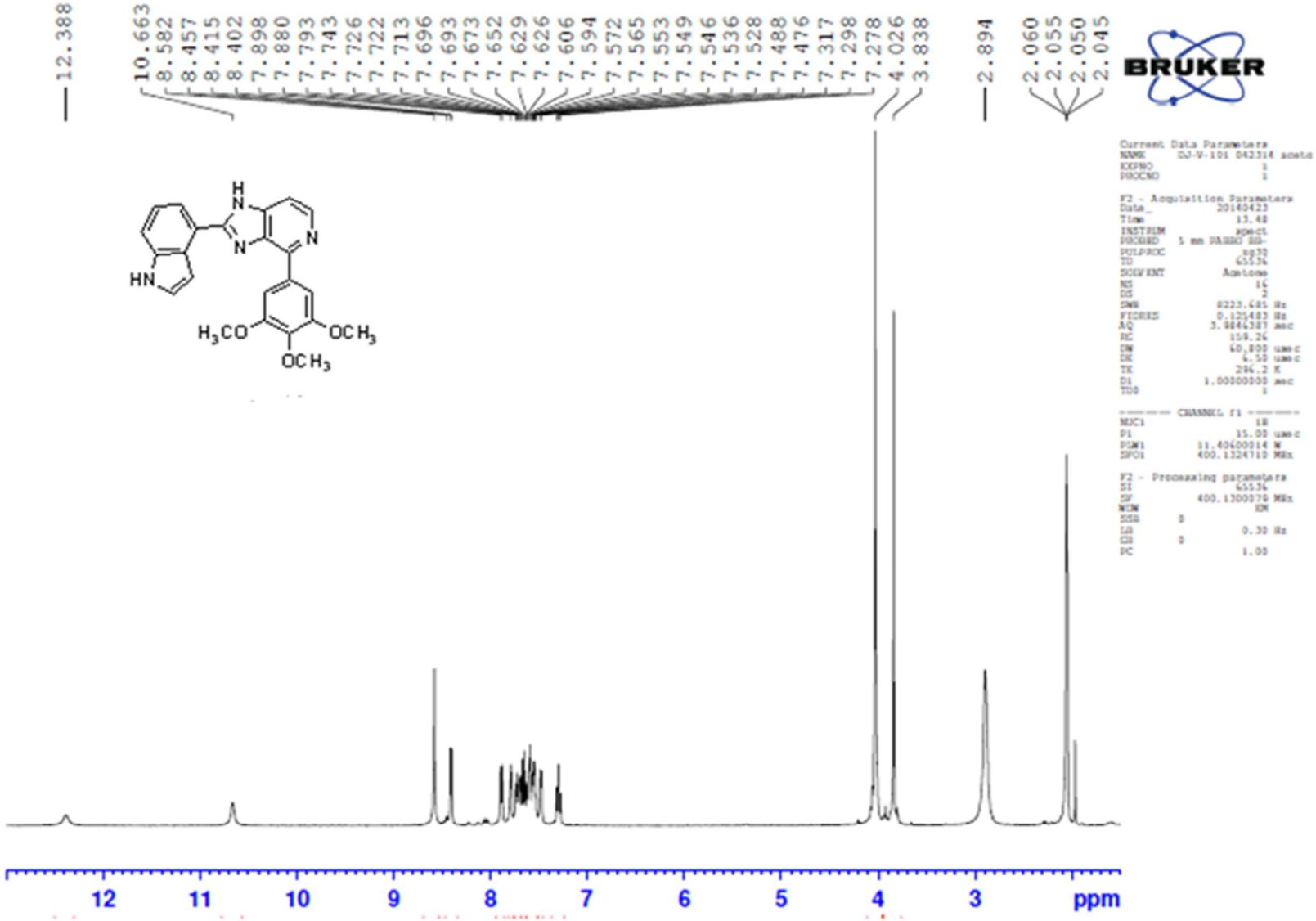
Compound **41**, 4-IIP: **HPLC** Analysis
Column: Phenomenex LUNA 5μ C18
250*4.6 mm
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 340nm
Purity 99.06%



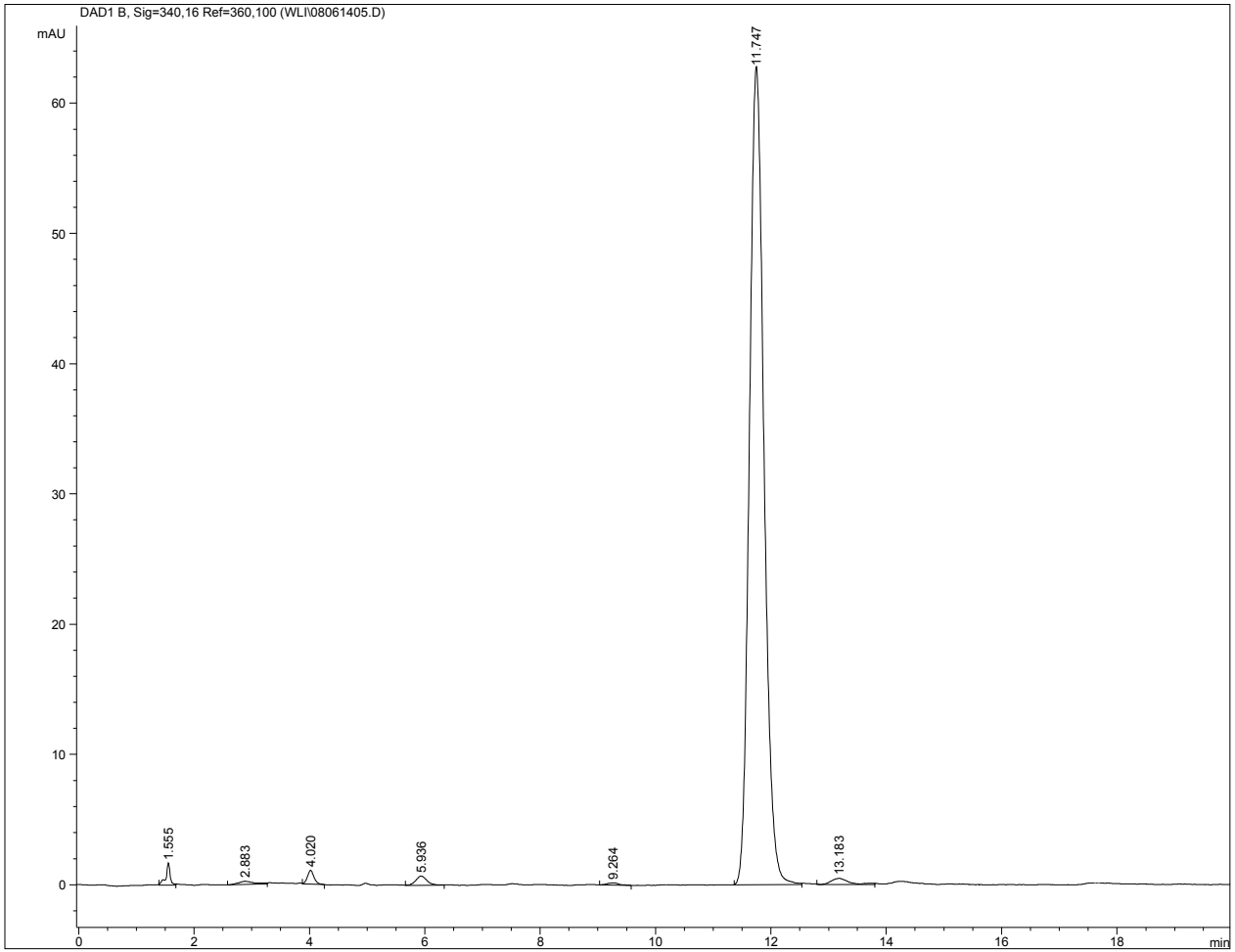
Compound **41**, 4-IIP: **HRMS** Analysis



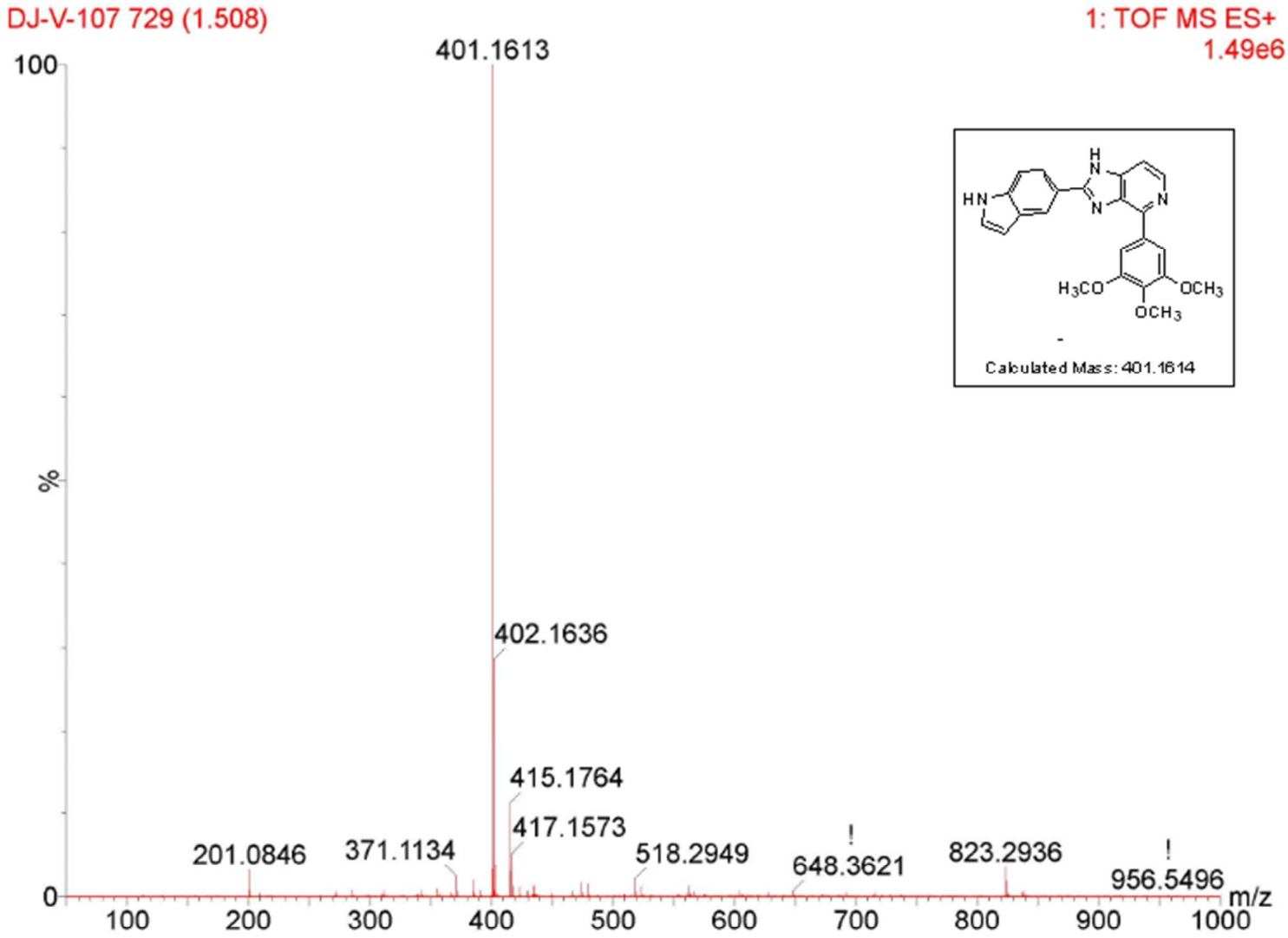
Compound **41**, 4-IIP: **^{1}H NMR** (Acetone d_6, 400 MHz)



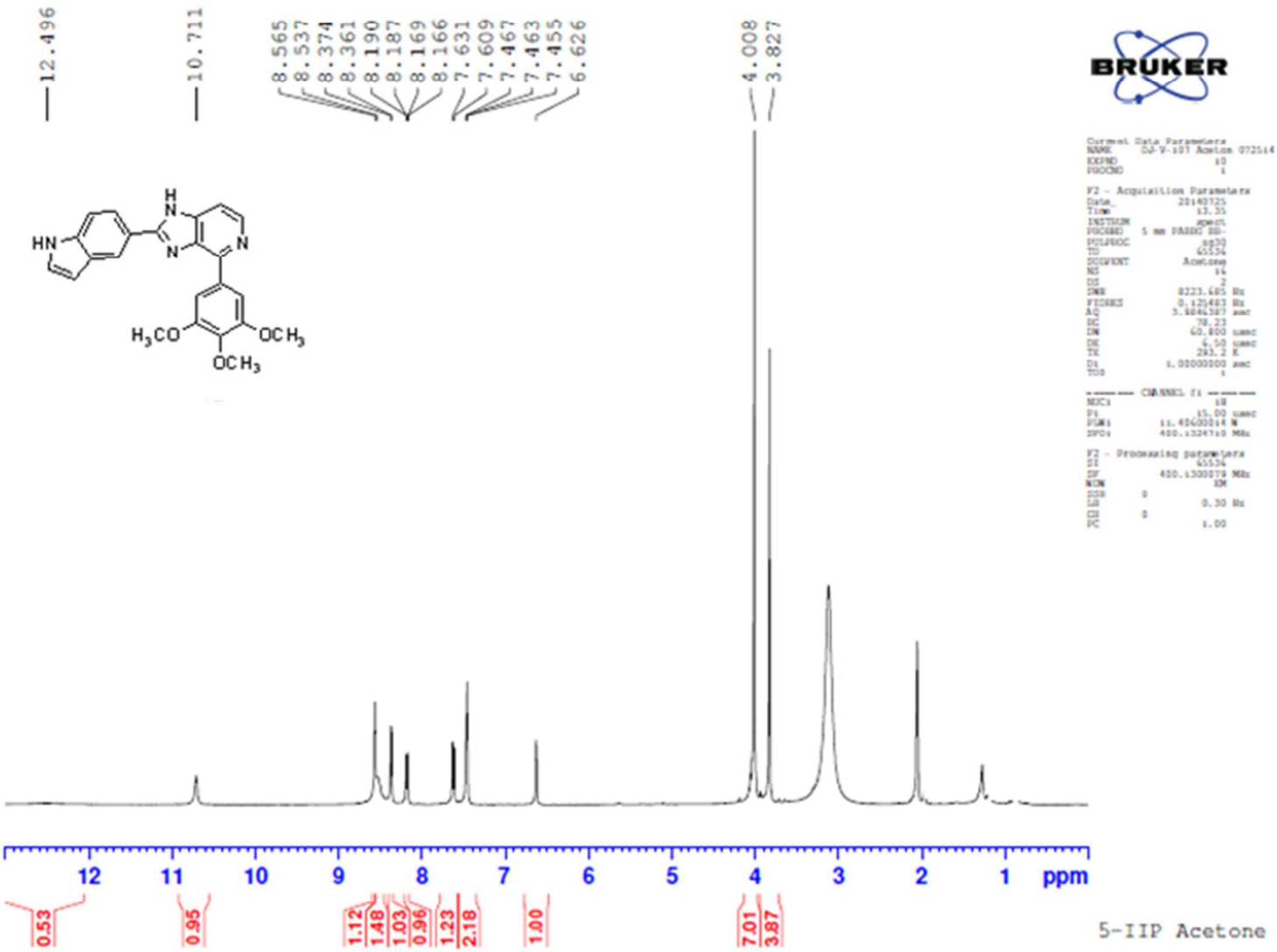
Compound **42**, 5-IIP: **HPLC** Analysis
Column: Phenomenex LUNA 5μ C18
250*4.6 mm
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 340nm
Purity 96.10%



Compound **42**, 5-IIP: **HRMS** Analysis



Compound **42**, 5-IIP: **^{1}H NMR** (Acetone $d_{6,}$ 400 MHz)



Compound **43**, 6-IIP: **HPLC** Analysis
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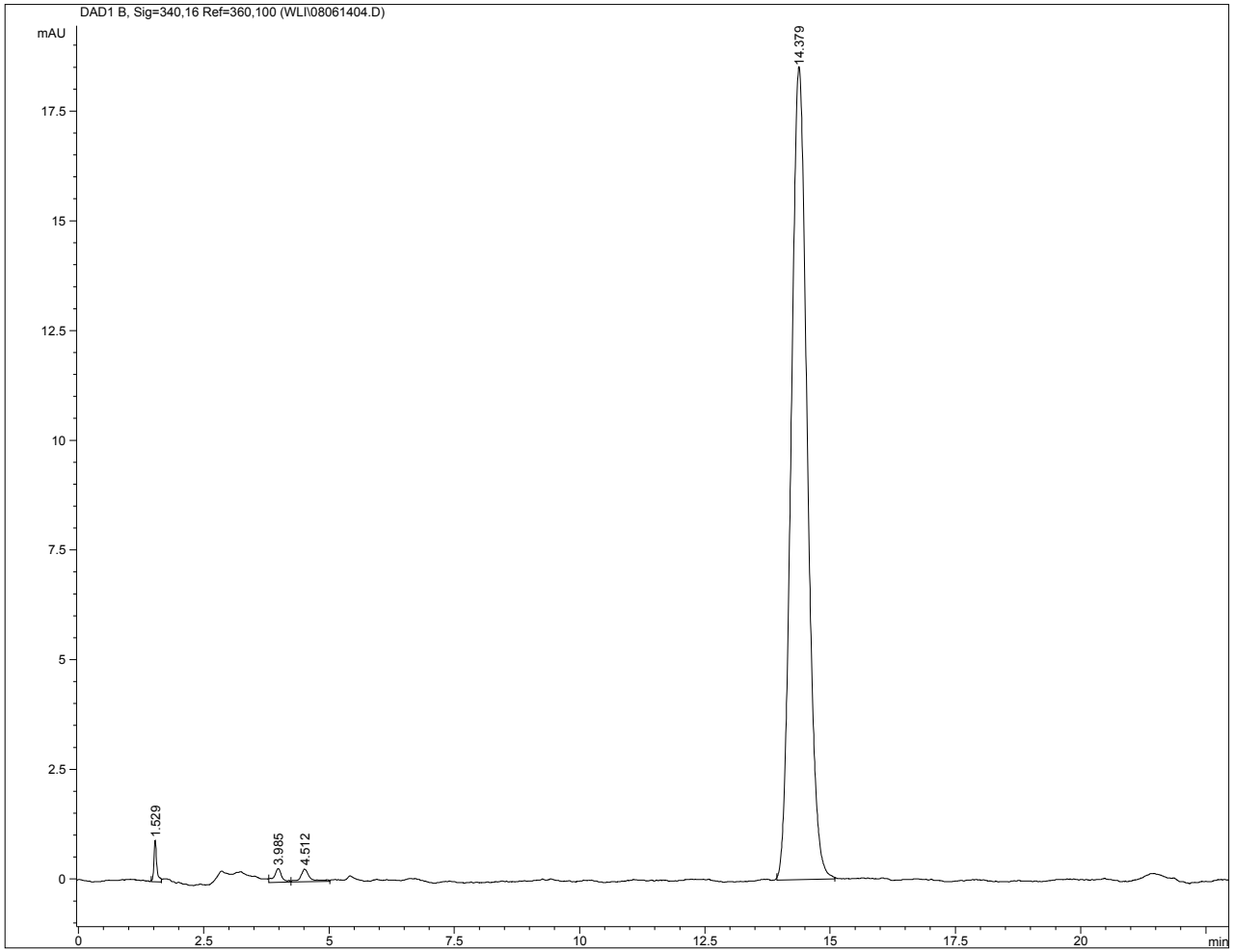
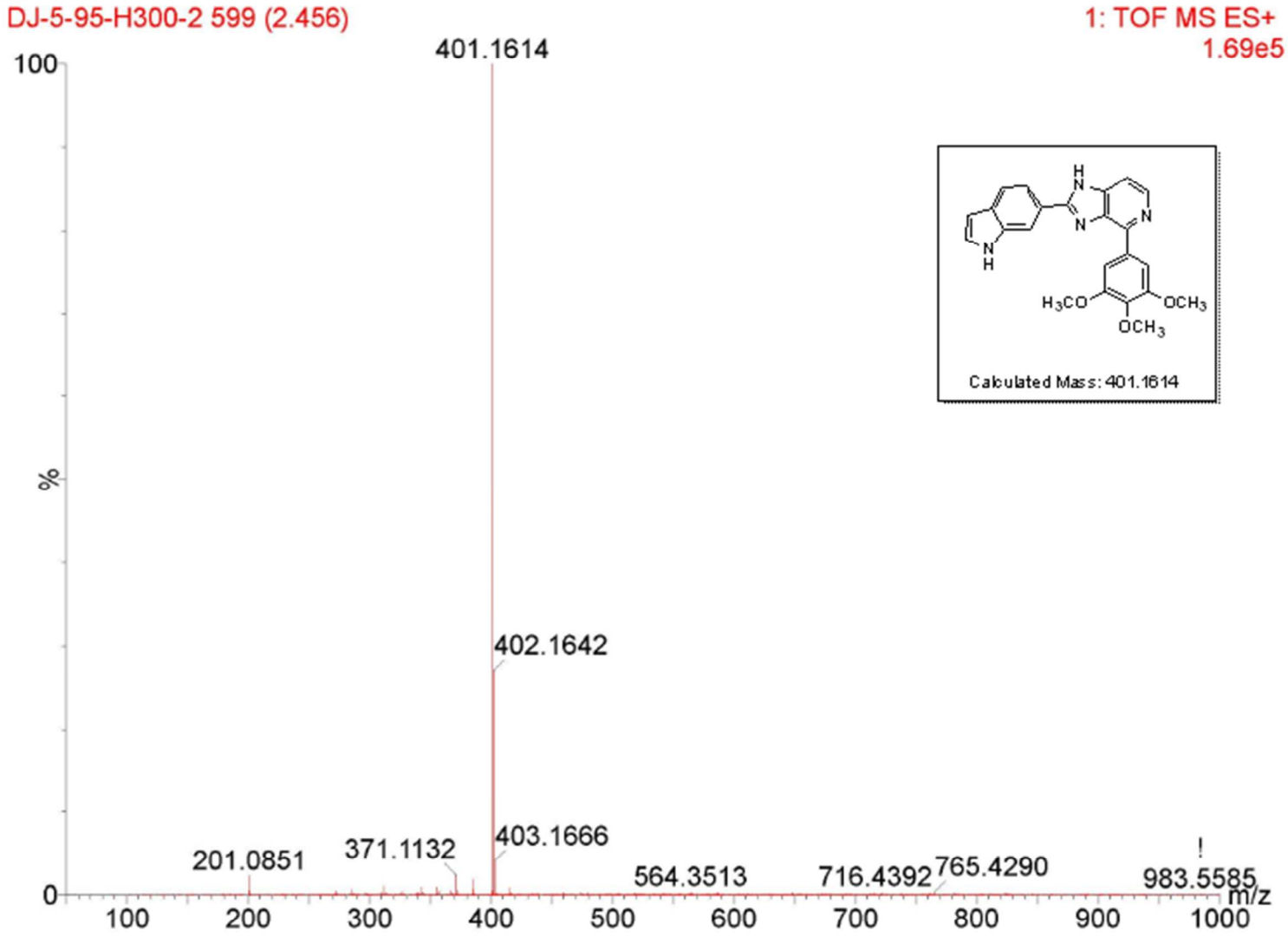
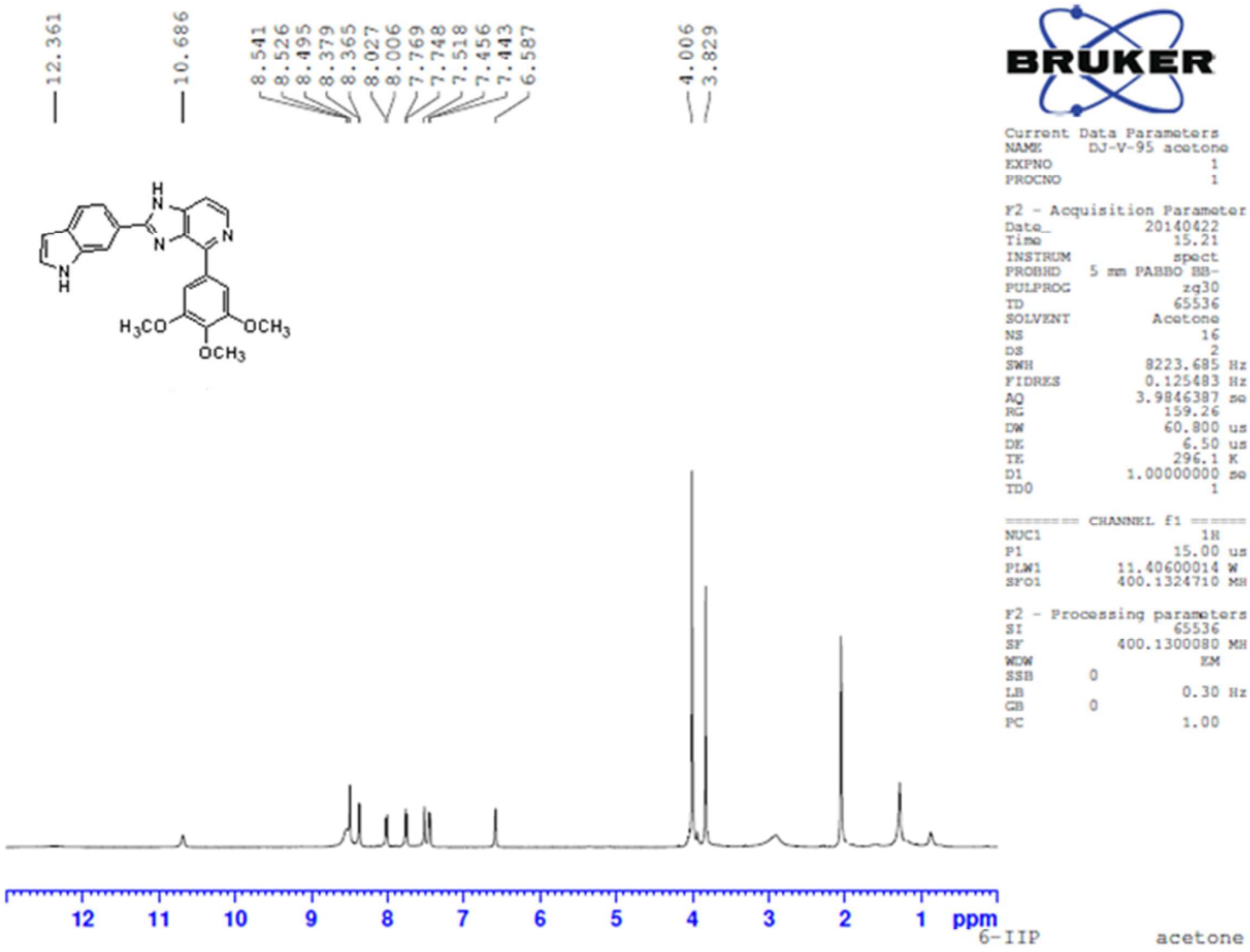
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 340nm
Purity 97.44%



Compound **43**, 6-IIP: **HRMS** Analysis



Compound **43**, 6-IIP: **^{1}H NMR** (Acetone $d_{6,}$ 400 MHz)



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PC                 1.00
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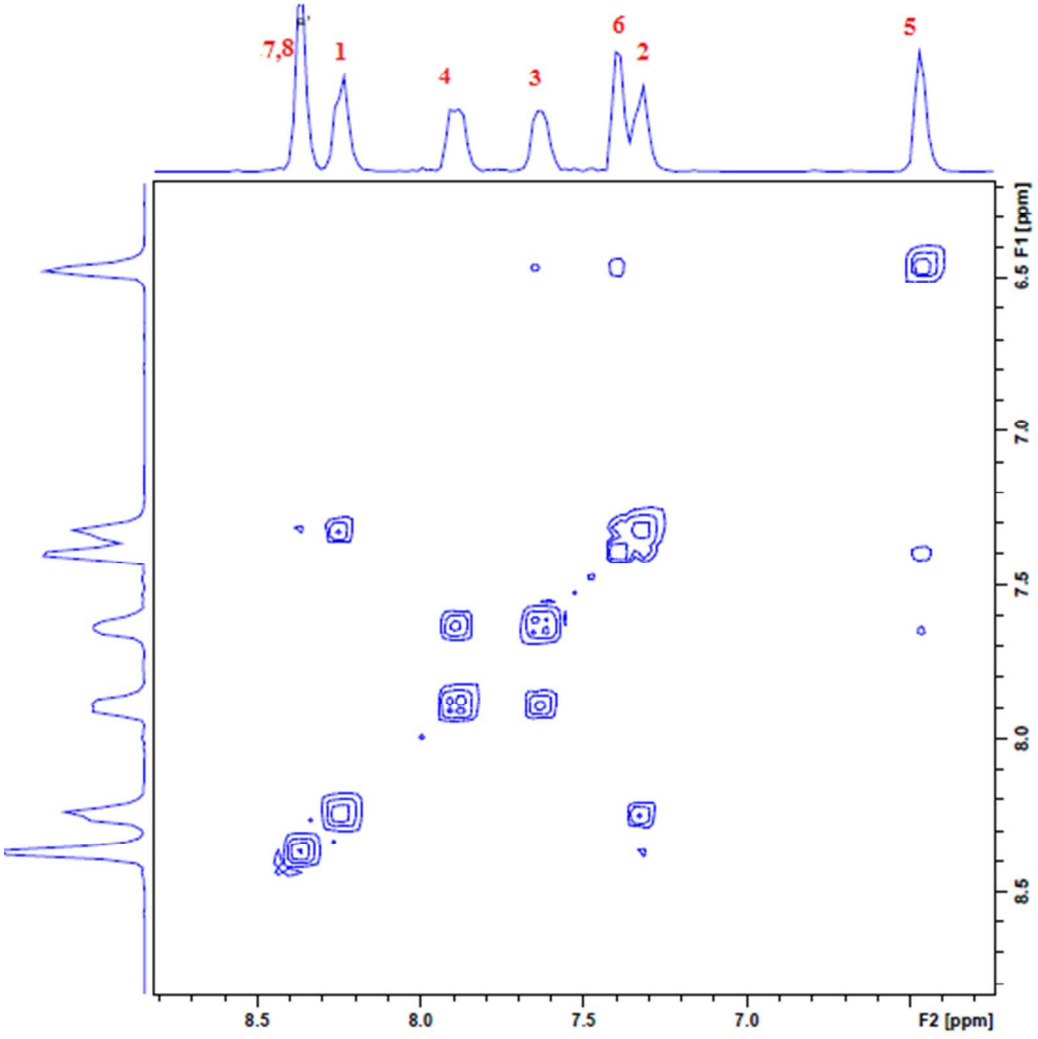
Compound **43**, 6-IIP: **^{1}H-^{1}H Cosy NMR** (Acetone $d_{6,}$ 400 MHz)



"DJ-V-95 aceton

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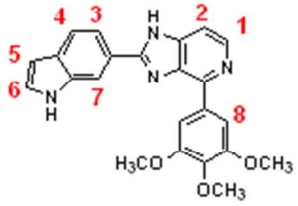

Compound **44**, 7-IIP: **HPLC** Analysis
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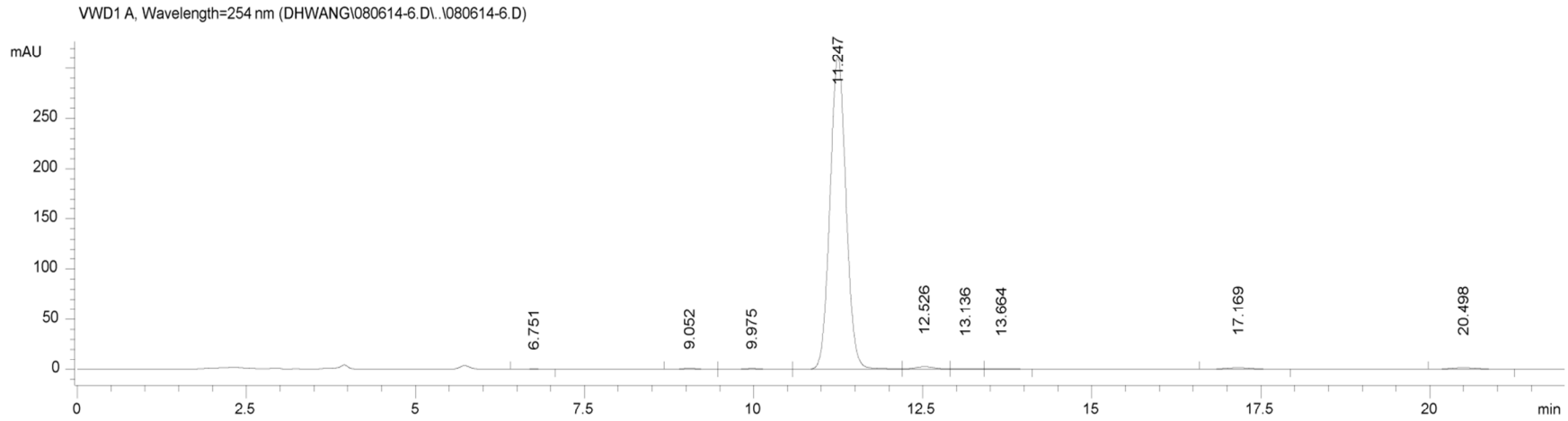
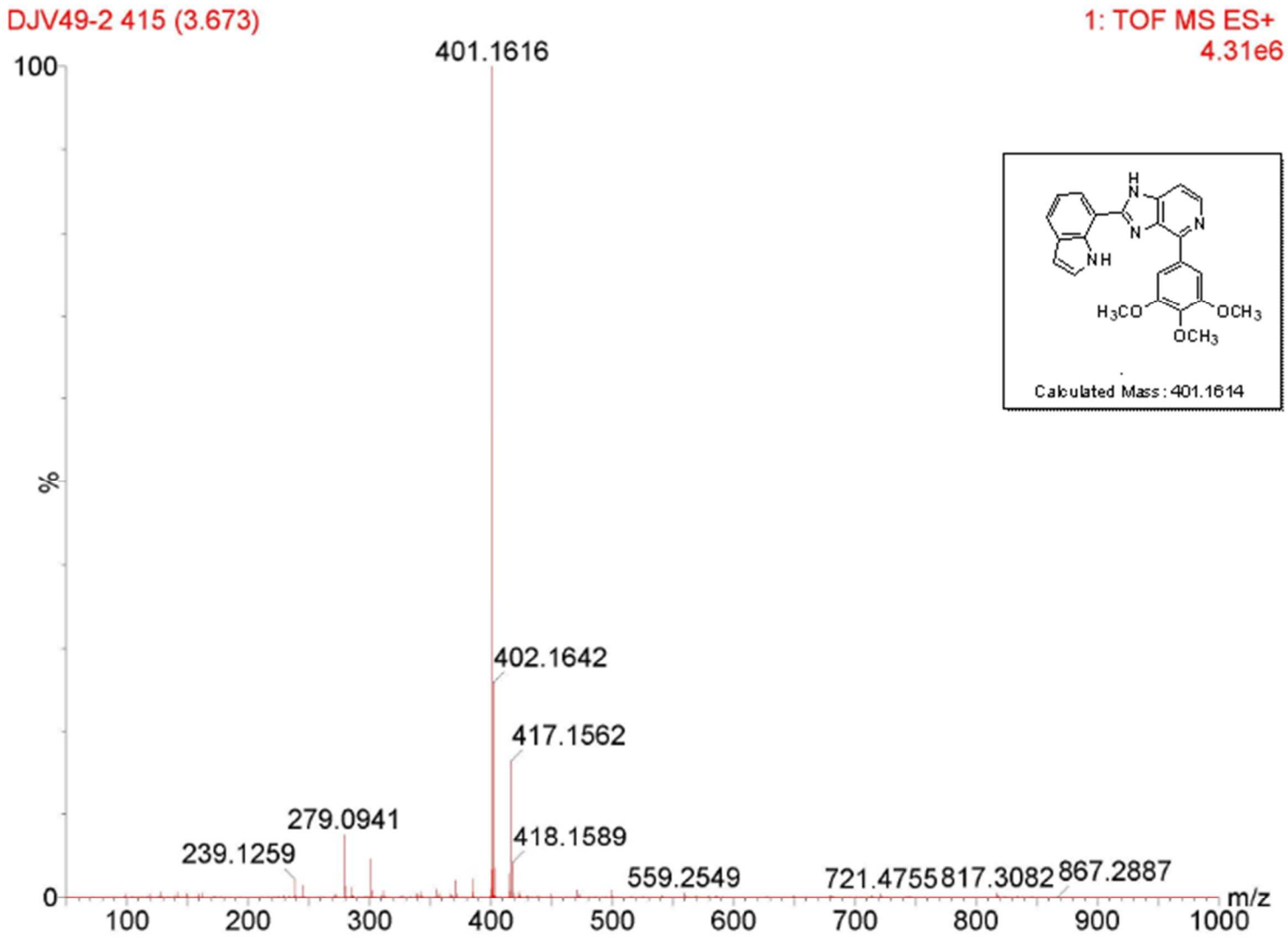
A: 55% water; B: 45% ACN Flow rate: 1.0 mL/min
Detection wave length = 245 nm

Purity >99%



Compound **44**, 7-IIP: **HRMS** Analysis



Compound **44a**: **HPLC** Analysis

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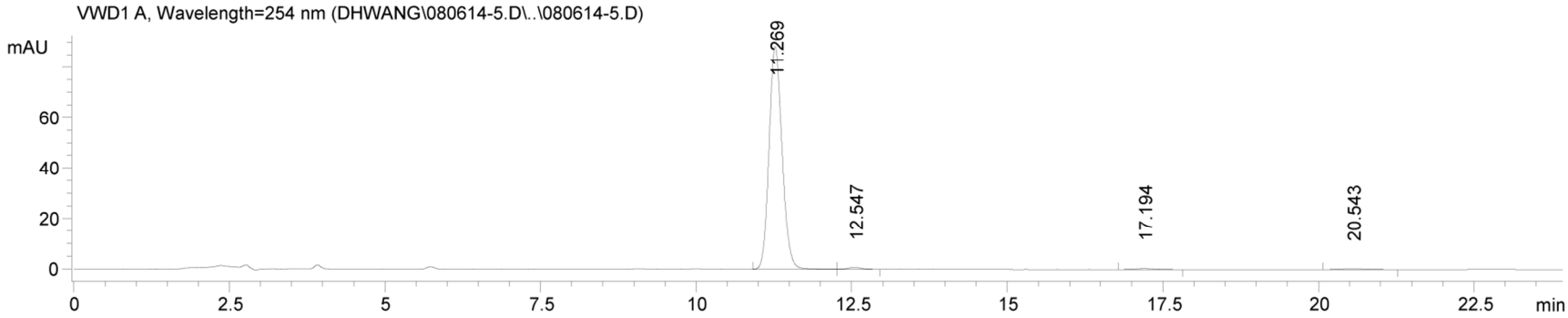
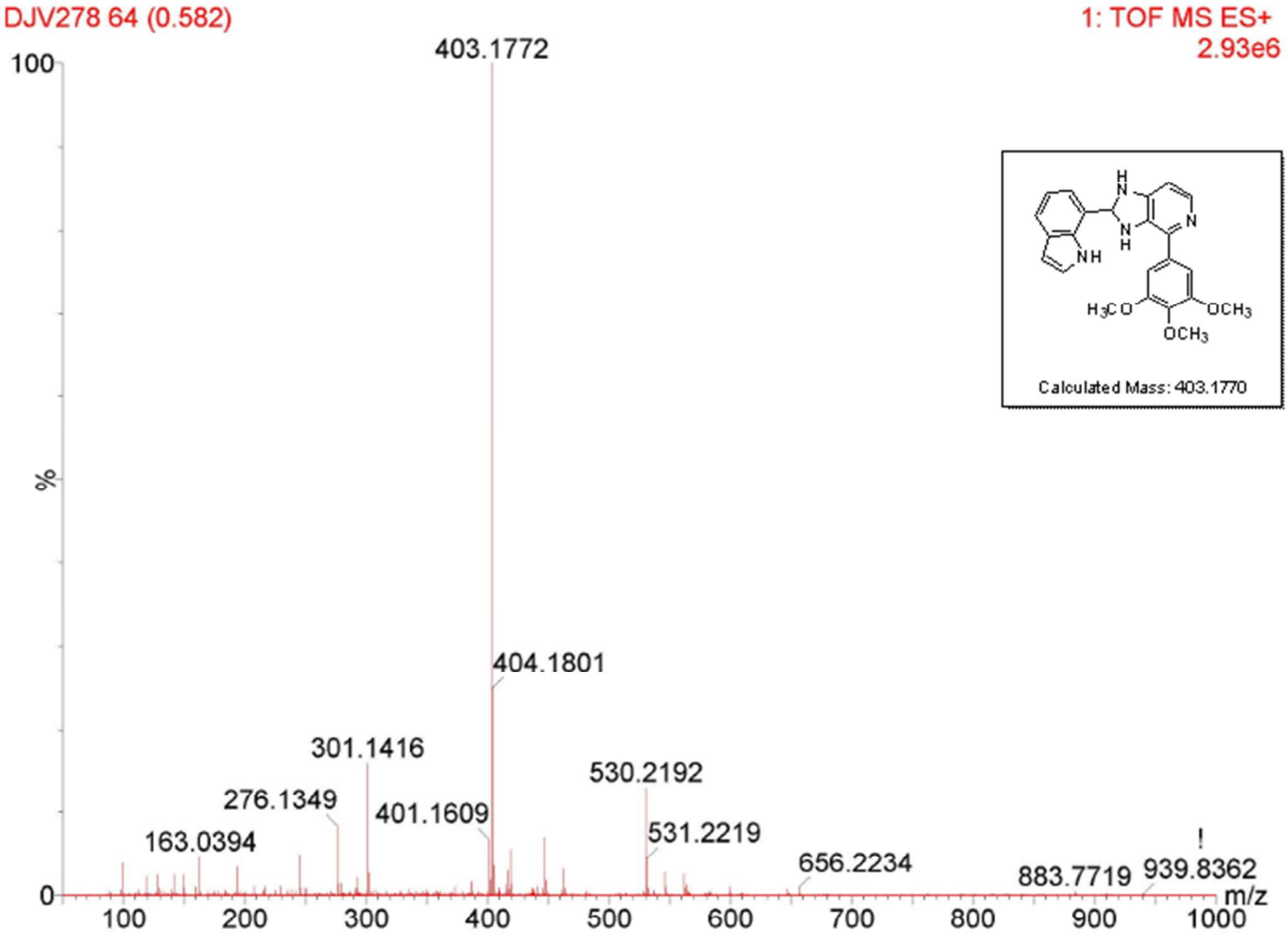
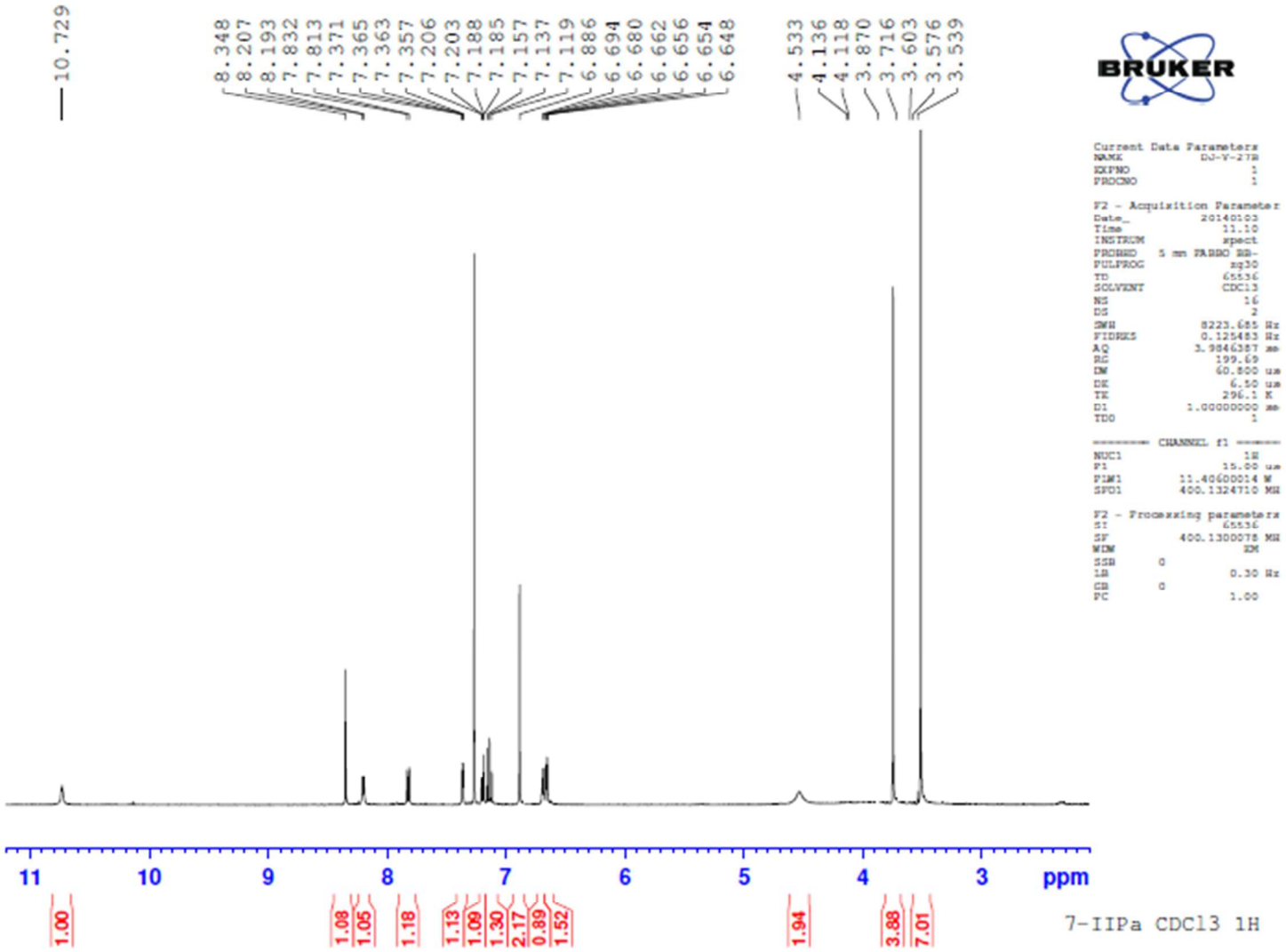
Purity >99%



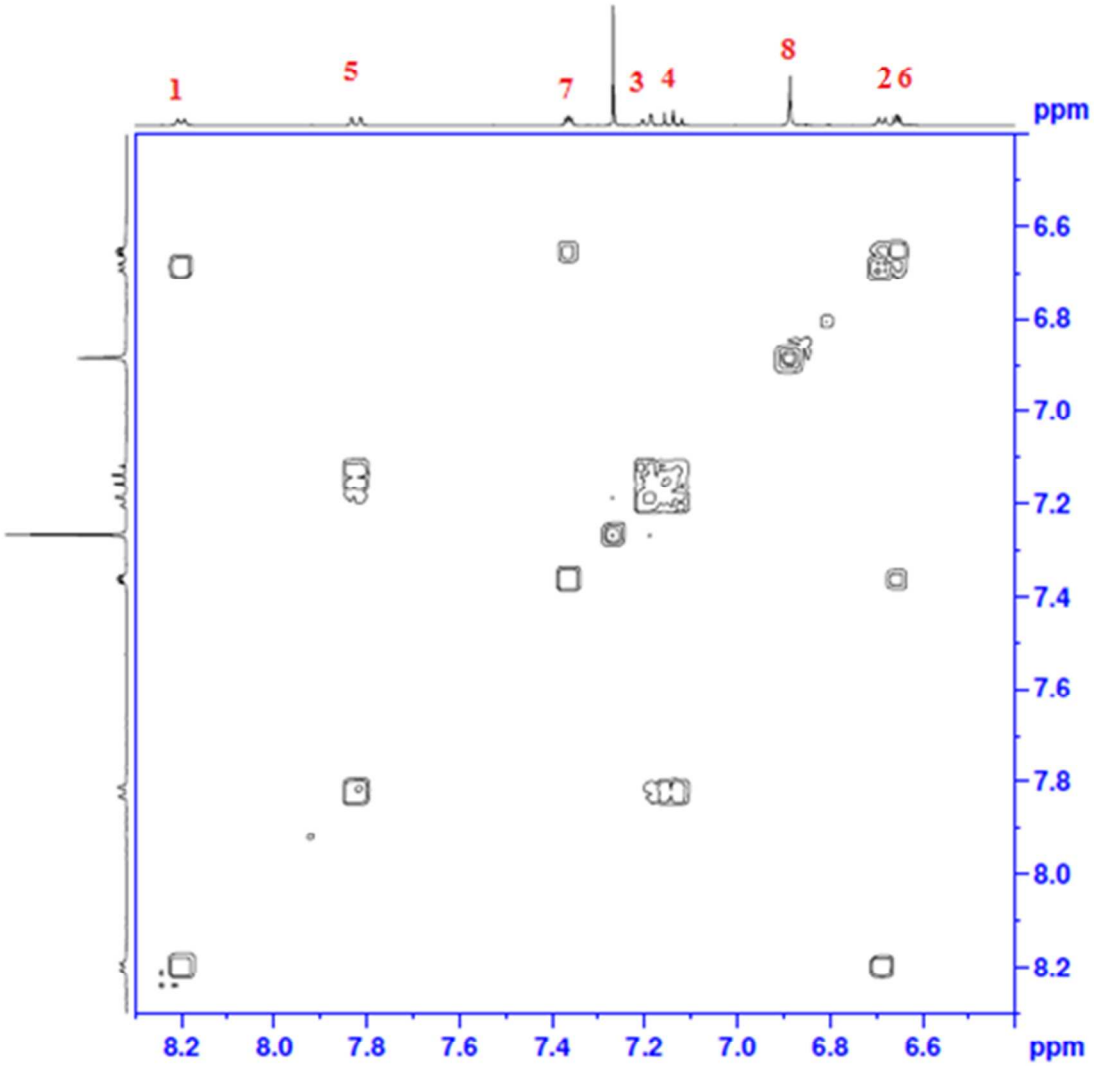
Compound **44a**: **HRMS** Analysis



Compound **44a**: **^{1}H NMR** ($CDCl_3$, 400 MHz)



Compound **44a**: **^{1}H-^{1}H Cosy NMR** ($CDCl_3$, 400 MHz)



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TE                    296.2 K
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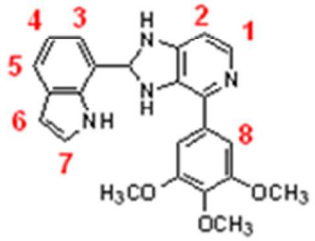
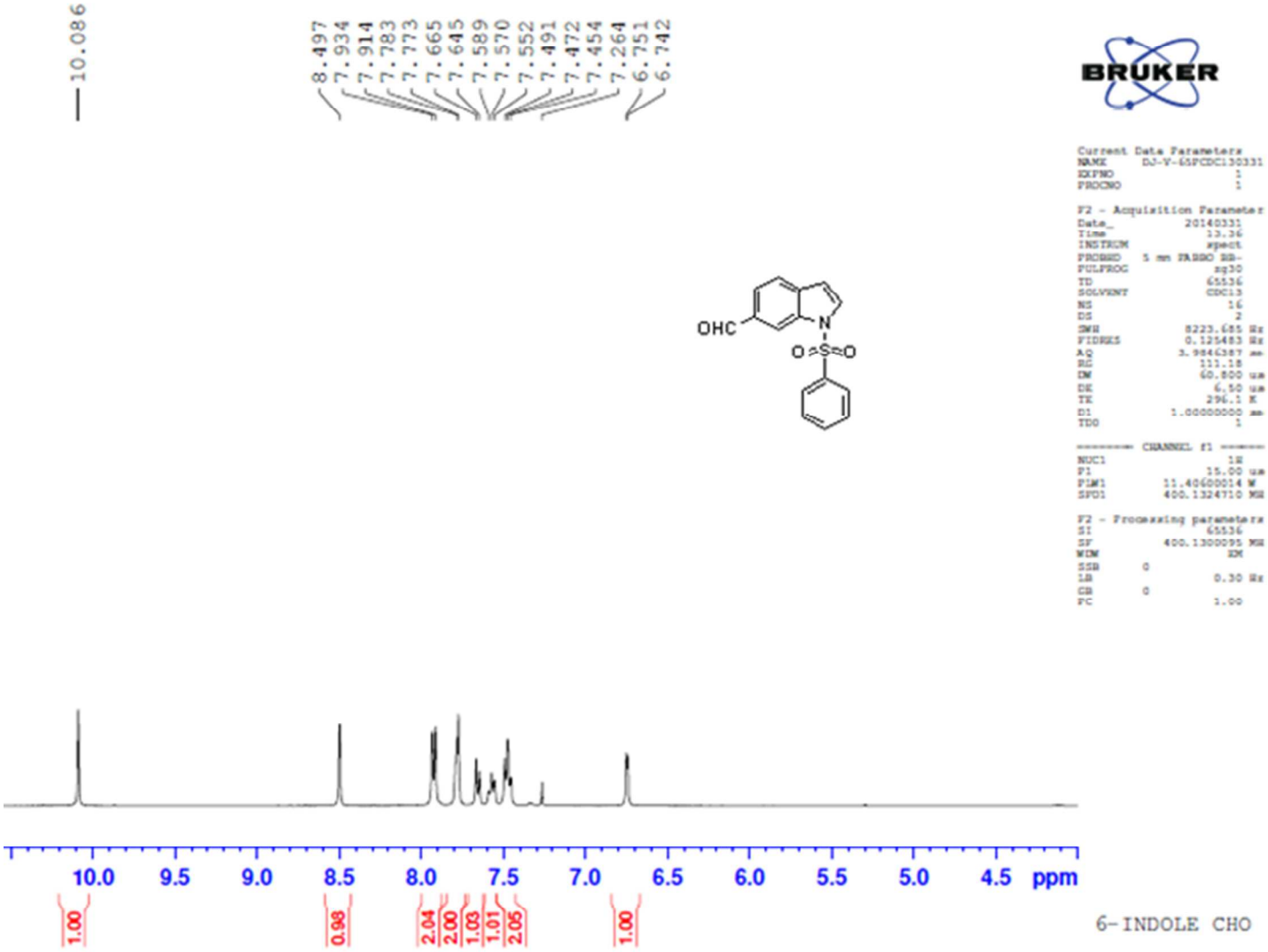
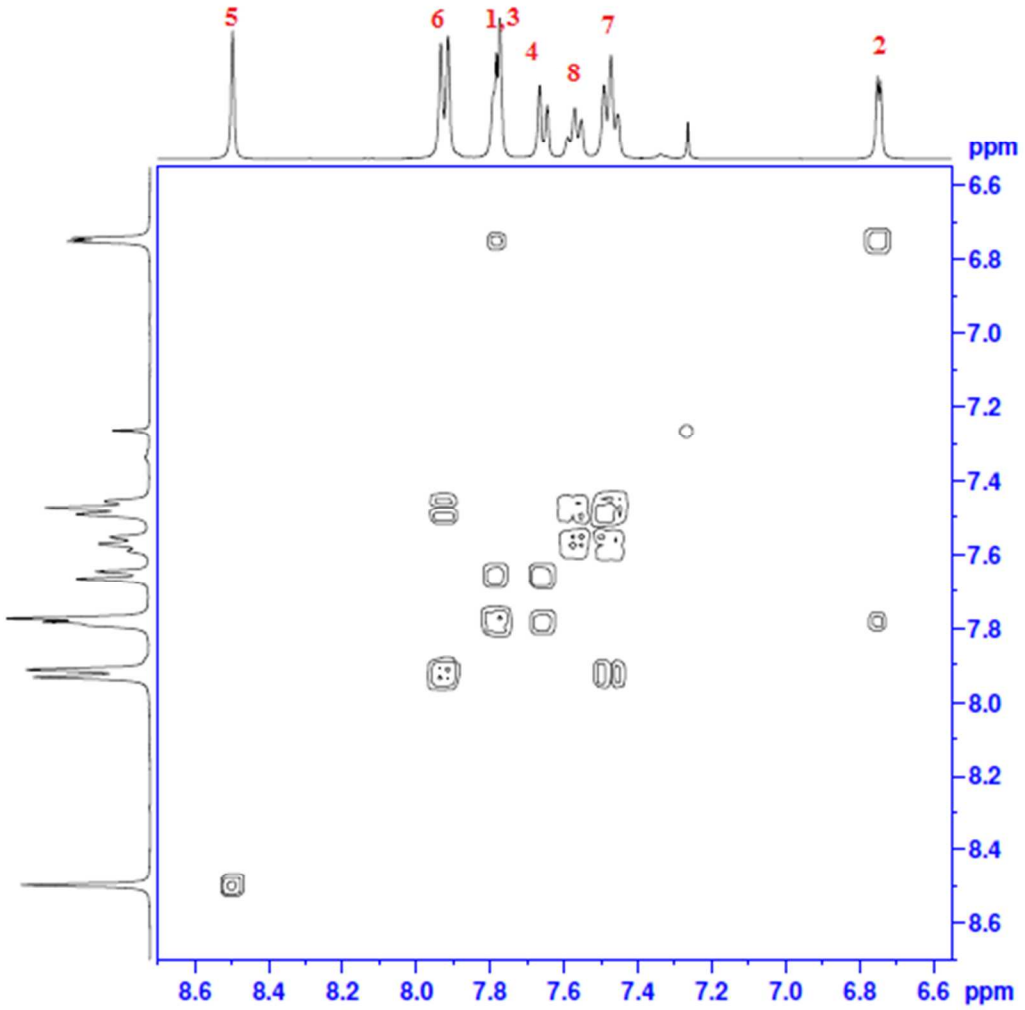


Compound **24**, 6-CHO: **^{1}H NMR** ($CDCl_3$, 400 MHz)



Compound **24**, 6-CHO: **^{1}H-^{1}H Cosy NMR** ($CDCl_3$, 400 MHz)

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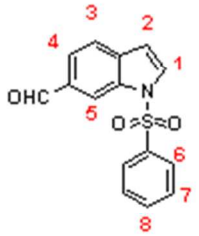
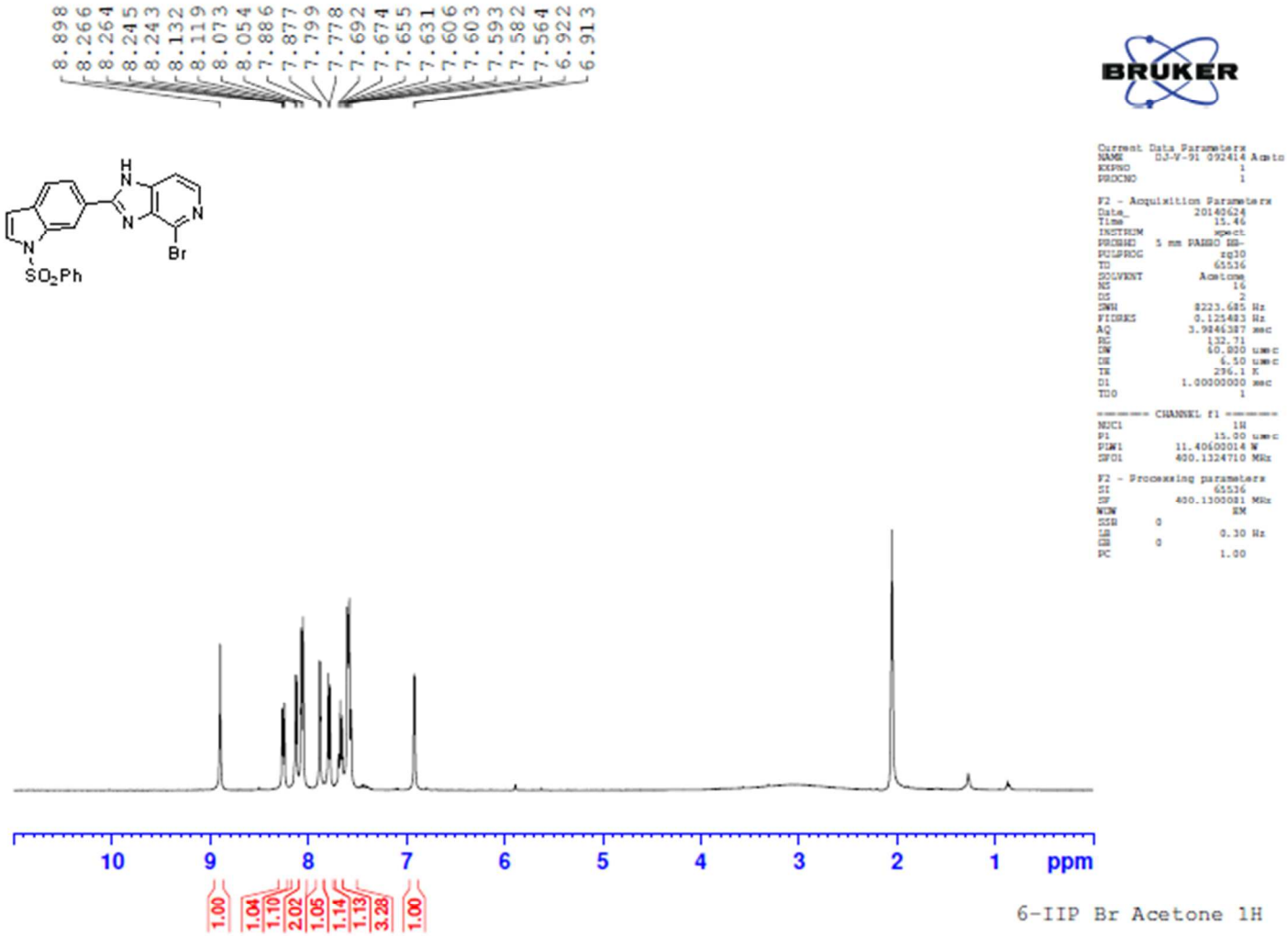
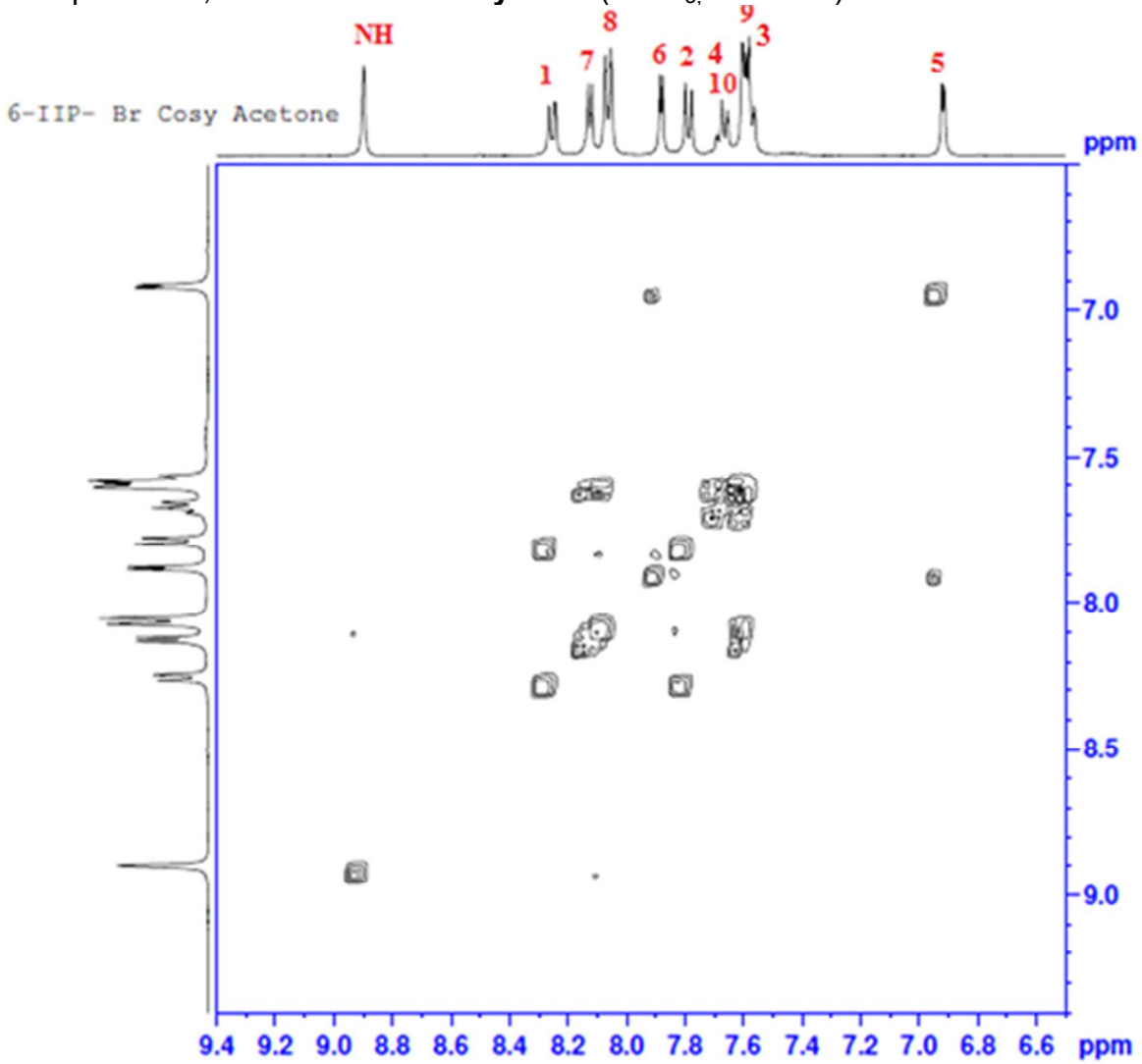
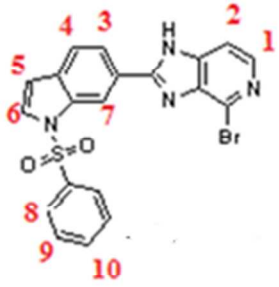


Compound **31**, 6-IIP-Br: **^{1}H NMR** (Acetone d_6, 400 MHz)

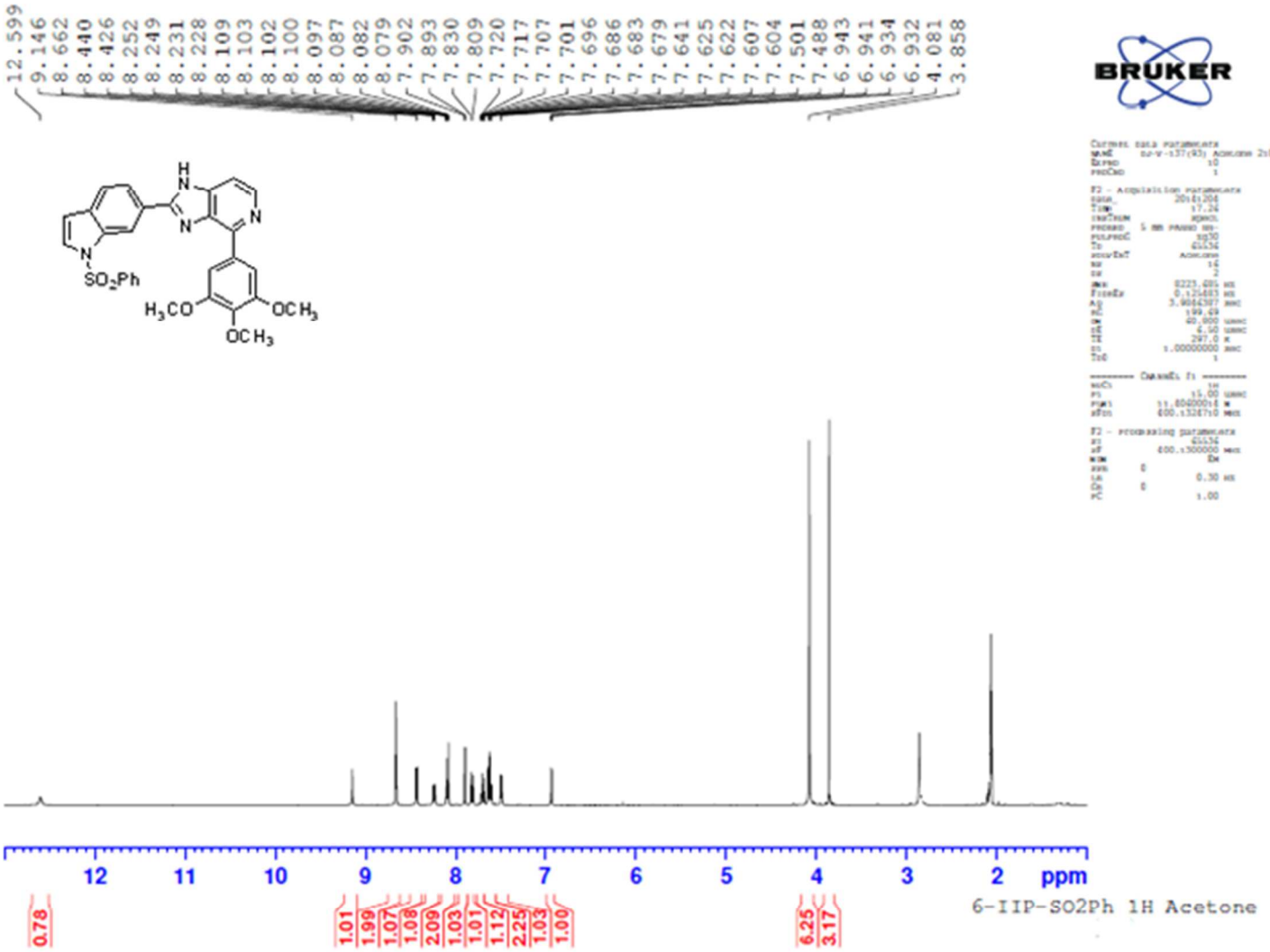


Compound **31**, 6-IIP-Br: **^{1}H-^{1}H Cosy NMR** ($CDCl_3$, 400 MHz)

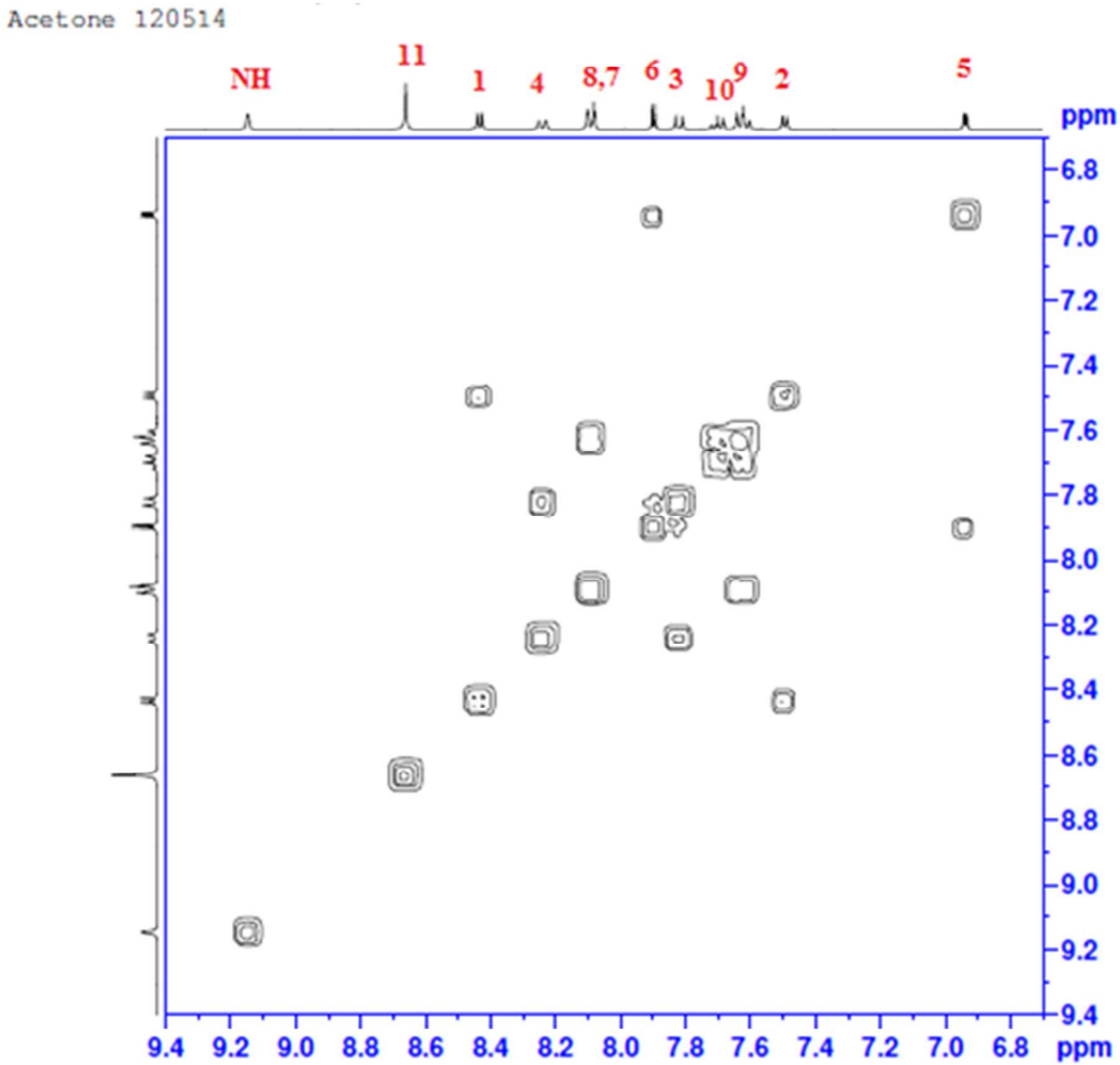


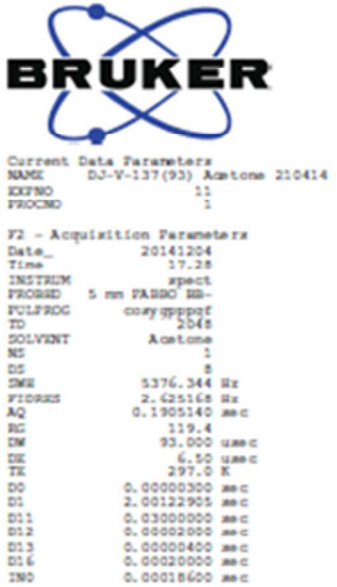


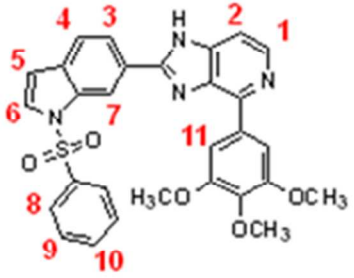
Compound **37**, 6-IIP-SO_2Ph: **^{1}H NMR** (Acetone d_6, 400 MHz)



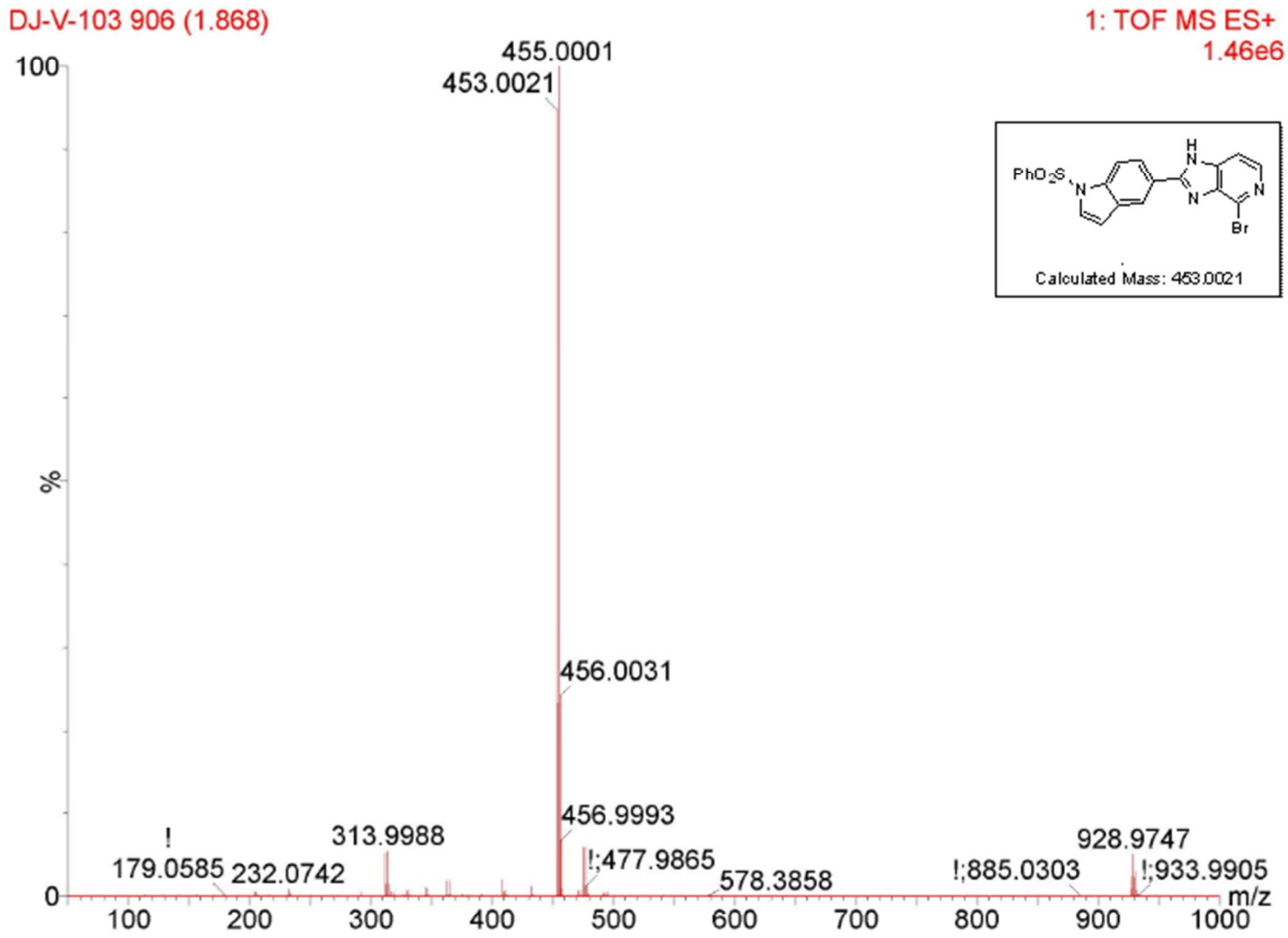
Compound **37**, 6-IIP-SO_2Ph: **^{1}H-^{1}H Cosy NMR** (Acetone d_6, 400 MHz)



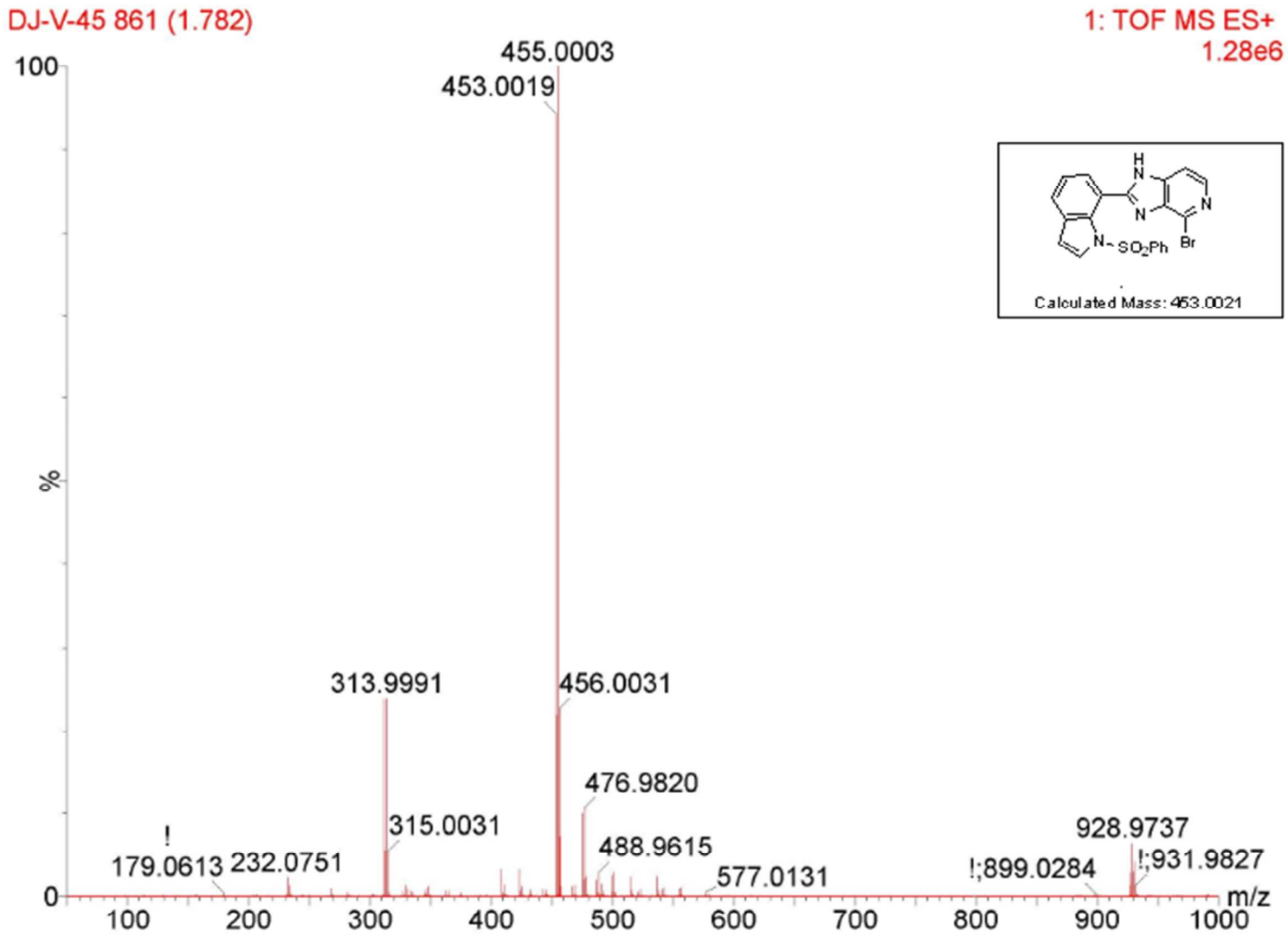




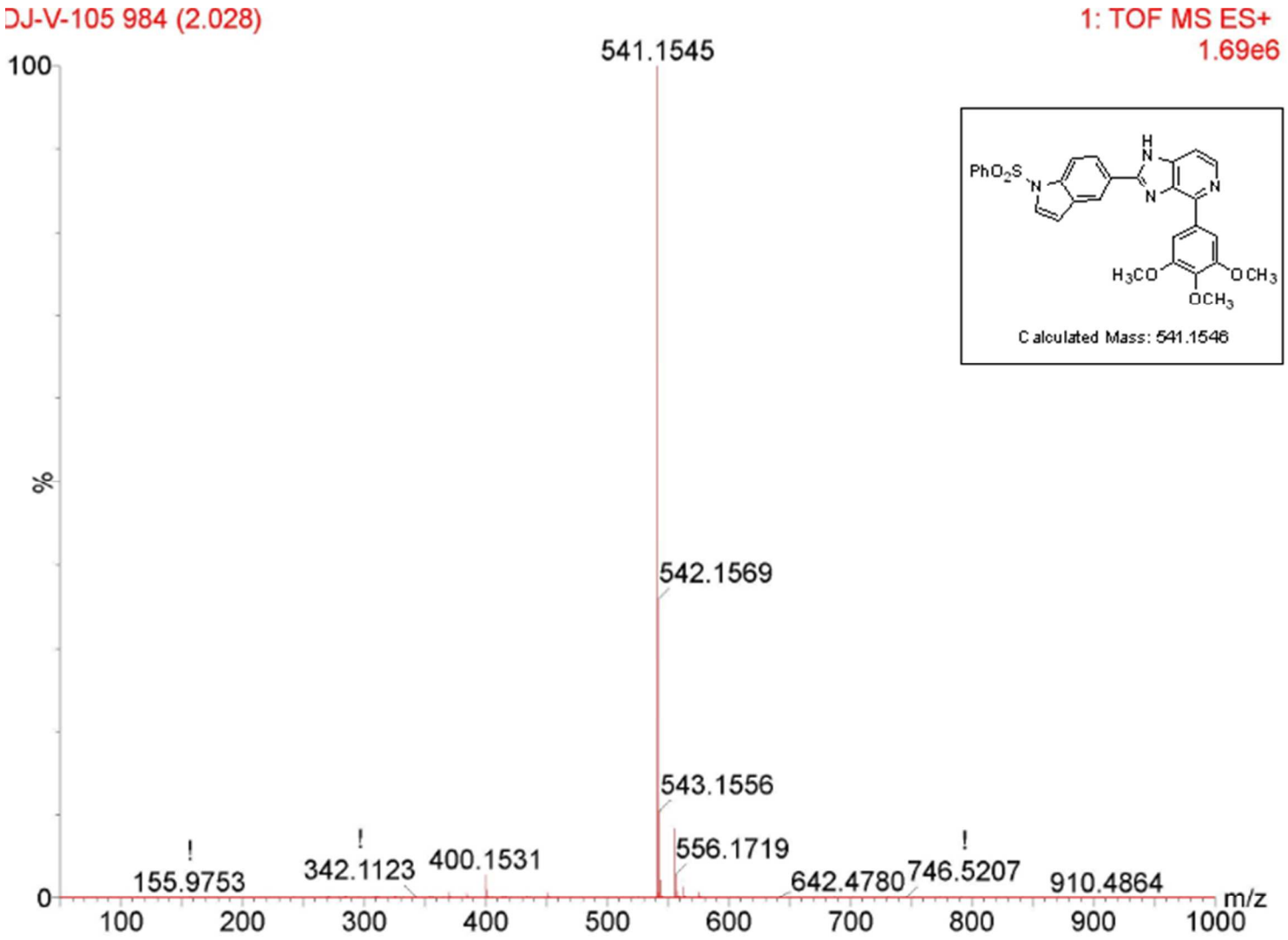
Compound **30**, 5-IIP-Br: **HRMS** Analysis



Compound **32,** 7-IIP-Br: **HRMS** Analysis



Compound **36,** 5-IIP-SO_2Ph: **HRMS** Analysis



Compound **38,** 7-IIP-SO_2Ph: **HRMS** Analysis