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1. Protein expression and purification of myosins: Single-headed chicken myosin Va containing one IQ motif with bound LC-1sa light chain and single-headed porcine myosin VI (T406A) mutant with bound calmodulin light chain were purified from Sf9 cells by FLAG affinity chromatography, as described previously[1,2]. Muscle myosin II and actin were purified from rabbit skeletal muscle using established protocols[3,4]. Phalloidin (1:1 with monomeric actin) was used to stabilize actin filaments.

2. ATPase assays: Steady-state ATPase assays were performed using an NADH enzyme-linked assay[5]. Briefly, a mixture of actin-myosin was incubated with compounds for 30 minutes at 0° C in KMg50 Buffer (10 mM imidazole pH 7.0, 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA and 1 mM DTT). Equal volumes of mixture of MgATP and assay components were mixed and quickly centrifuged prior to measurement. Time course (5 minute intervals) of fluorescence decrease, due to NADH oxidation, was measured using a Fusion multi-well plate reader (Falcon 384-well plate, λ_{ex}=340 nM, 440 nm emission filter). 40 U/mL lactate dehydrogenase, 200 u/mL pyruvate kinase, 1 mM phosphor(enol)pyruvate, 0.5 mM NADH and 1 mM MgATP was used in the assay. The final reaction volume was 20 µL with 5% (v/v) DMSO for compound testing. 30 nM of myosin V was used with 2 µM of actin, 50 nM of myosin II and myosin VI were used with 10 µM of actin for all the experiments described in the main text.

3. Measurement of apparent K_I: The Michaelis constant (K_m) and maximum catalytic rate (k_{cat}) in the absence of compounds were determined by fitting the [ATP]- or [actin]-dependence of myosin V ATPase activity to a rectangular hyperbola (Equation 1).

$$\frac{v}{[M]_{tot}} = \frac{k_{cat}[S]}{K_m + [S]} \qquad \text{Equation 1,}$$

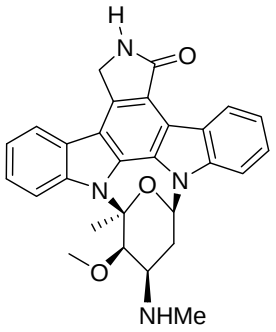
where $[M]_{tot}$ is the total myosin concentration and [S] is the substrate (ATP or Actin).

Compounds were treated as reversible inhibitors as is the case for known myosin inhibitors blebbistatin[6] and BDM[7]. The [compound]-dependence of myosin V ATPase inhibition was fit to the equation 2 according to previously described methods[8] and yielded apparent inhibitor binding constants (K_I). Values of K_I were very similar to IC_{50} values (within ~10-20%) obtained by fitting the data to a Hill slope equation (fits not shown).

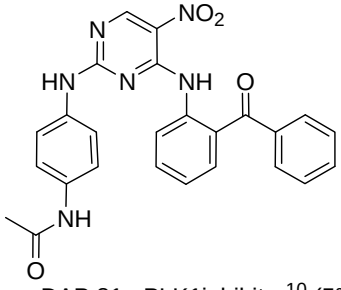
$$\frac{v}{[\text{myosin}]_{total}} = \frac{k_{cat}[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_I}\right)} \qquad \text{Equation 2,}$$

where [I] is the inhibitor concentration and K_I is the apparent inhibitor binding constant.

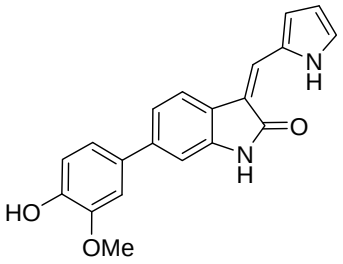
4. Collection of kinase inhibitors: The compounds listed below were tested against myosin V. Values in parenthesis indicate the average percent inhibition at 100 μM inhibitor concentration (n=3 independent experiments); myosin V= 30 nM and actin= 2 μM.



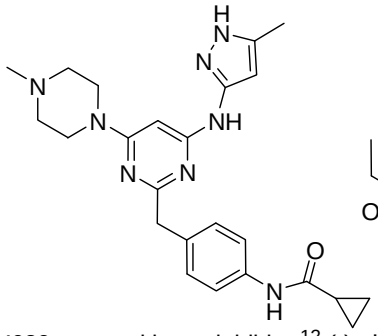
Staurosporine, multi kinase inhibitor[9] (37%)



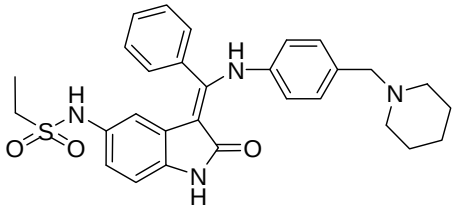
DAP 81, PLK1inhibitor[10] (5%)



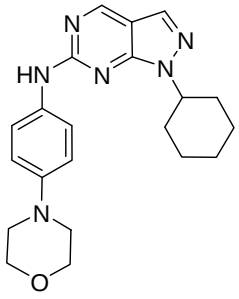
CHK1 inihibitor[11] (40%)



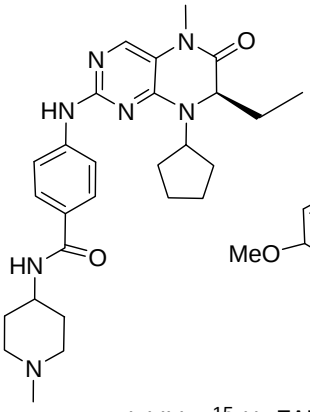
VX680, aurora kinase inhibitor[12] (-)



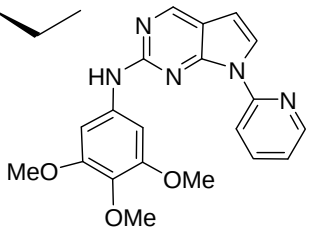
Hesperadin, aurora kinase inhibitor[13] (20%)



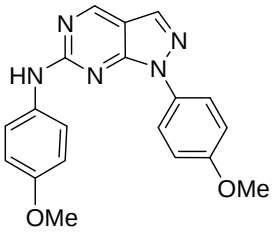
Cell cycle kinase inhibitor[14] (8%)



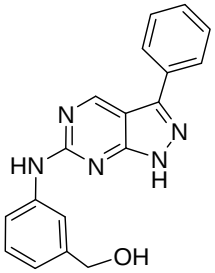
BI-2356, PLK1inhibitor[15] (-)



FAK inhibitor[16] (10%)



Mitotic kinase modulator[17] (7%)



Cell cycle kinase inhibitor[18](34%)

5. General Information for inhibitor synthesis:

A. General Procedures. Reactions were generally run in capped 1 dram vials (4 mL) stirred with Teflon®-coated magnetic stir bars. Moisture- and air-sensitive reactions were performed in flame-dried round bottom flasks, fitted with rubber septa or glass gas adapters, under a positive pressure of nitrogen. Moisture- and air-sensitive liquids or solutions were transferred via nitrogen-flushed syringe or stainless steel cannula. Where necessary, solutions were deoxygenated by bubbling with

nitrogen using a gas dispersion tube. Concentration of solvents was accomplished by rotary evaporation using a Büchi rotary evaporator, equipped with a dry ice-acetone condenser, at 5-75 mm Hg at temperatures between 35 and 50 °C. Experiments were monitored by thin layer chromatography (TLC). The maintenance of 30 to 150 °C reaction temperatures was accomplished by the use of an oil bath, or a 12-well (16 mm) aluminum heating block which could achieve temperatures up to 200 °C.

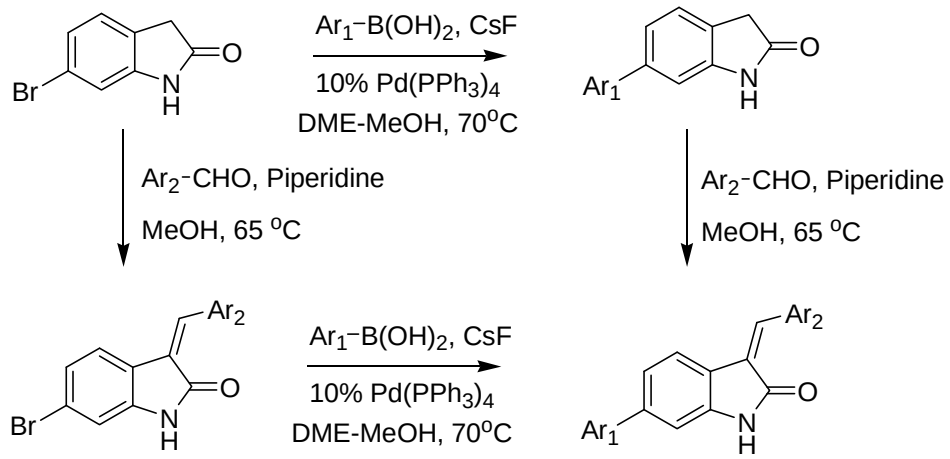
B. Materials. Solvents were purchased as Sure/Seal Aldrich bottles and reagents were used without further purification.

C. Chromatography. Analytical thin layer chromatography (TLC) was performed using Whatman 250 micron aluminum backed UV F_{254} precoated silica gel flexible plates. Subsequent to elution, ultraviolet illumination at 254 nm allowed for visualization of UV active materials. Staining with *p*-anisaldehyde, basic potassium permanganate solution, or Verghn's reagents allowed for further visualization. Flash column chromatography was performed using a Teledyne ISCO CombiFlash® Companion® chromatography instrument and RediSep® Columns (machine-packed with 35 to 60 micron silica gel).

D. Physical Data. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on Bruker DPX 400 MHz nuclear magnetic resonance spectrometers. Chemical shifts for ^{1}H NMR spectra are reported as in units of parts per million (ppm) relative to tetramethylsilane (δ 0.0) using the residual solvent signal as an internal standard or tetramethylsilane itself: chloroform-*d* (δ 7.26, singlet), dimethylsulfoxide-d_6 (δ 2.50, quintet), methanol-d_4 (δ 3.30, quintet), and deuterium oxide-d_2 (δ 4.80, singlet). Liquid chromatography mass spectral analyses were obtained using a Waters MicroMassZQ mass spectrometer, with an electron spray ionization (ESI) probe, connected to a Waters 2795 HT Separation Module Alliance HT HPLC system running MassLynx (V4.0). The system used a Waters 996 Photodiode Array Detector set to 254 nm for peak detection, and a Symmetry® C18 (3.5 micron) 2.1 x 50 mm column for separation (mobile phase for positive mode: solvent A: water with 0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid). Values are reported in units of mass to charge (*m/z*).

6. Synthesis of oxindoles:

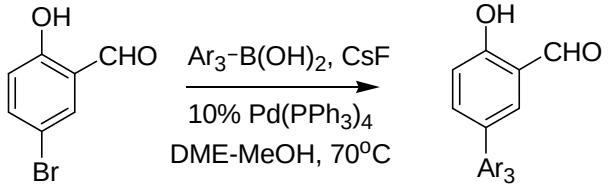
A. General Approach: Oxindoles are synthesized based on reported protocol.[11] Briefly, an exchangeable sequence of palladium catalyzed Suzuki coupling and Knoevenagel condensation was employed on 6-Bromooxindole to create diversity on oxindole skeleton. Suzuki coupling was performed using commercially available boronic acids and for Knoevenagel condensation, various aromatic aldehydes were used. A general scheme is shown below.



General synthetic route to various oxindoles

Procedure for Suzuki coupling: A solid mixture of 6-Bromooxindole (0.25 mmol), boronic acid (0.3 mmol), $Pd(PPh_3)_4$ (0.025 mmol) and CsF (0.3 mmol) was dried under vacuum for half an hour followed by the addition of 3 mL degassed solvent (DME:MeOH=2:1). Reaction mixture was heated at 70 ^oC for 8 hrs. Completion of the reaction was determined by TLC. Upon completion, reaction was diluted with ethyl acetate (40 mL) and filtered to remove the inorganic residues. Organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Crude material, after solvent evaporation, was purified by silica gel chromatography (elution with petroleum ether-ethyl acetate solvent system) to obtain pure compound (yield varied from 50% to 70%).
Procedure for Knoevenagel condensation: 0.25 mmol of 6-Bromooxindole (or Suzuki coupling product of 6-Bromooxindole) and 0.3 mmol of aromatic aldehyde were dissolved in 3 mL dry methanol. 0.3 mmol of piperidine was added dropwise and reaction mixture was heated at 65 ^oC for 2 hrs. Upon cooling, product precipitated out from the solvent. It was filtered and washed with ice-cold ethyl acetate-petroleum ether (1:1) and dried under vacuum. Yield of this reaction varied from 65% to 80%.

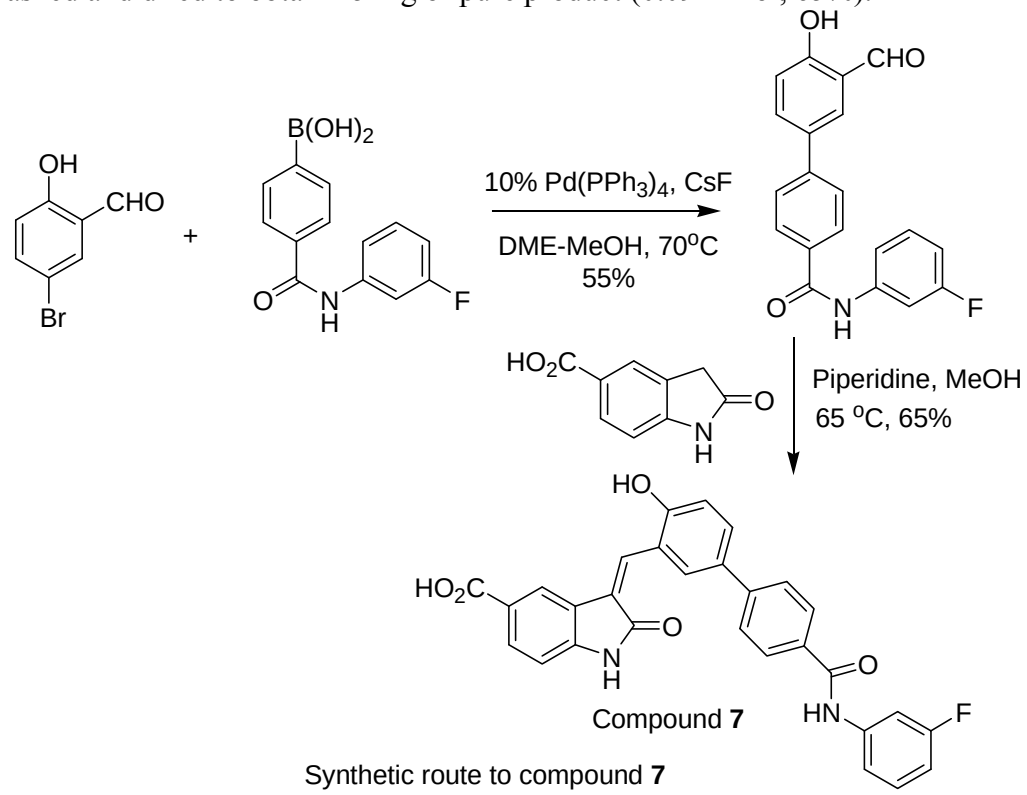
B. Synthesis of non-commercial aromatic aldehydes: Many of the aldehydes used are not readily available from commercial sources. All such aldehydes were synthesized from 5-Bromosalicylaldehyde using various aromatic boronic acids employing Suzuki coupling. Reaction procedure is exactly same as described above. A general scheme is shown below.



Synthetic route to non-commercial aromatic aldehydes

C. Synthesis of compound 7: As a representative example, we describe the synthesis of compound **7**. A mixture of 50 mg of 5-Bromosalicylaldehyde (0.25 mmol), 78 mg of N-3-Fluorophenyl 4-boronobenzamide (0.3 mmol), 29 mg of $Pd(PPh_3)_4$ (0.025 mmol and 45 mg CsF (0.3 mmol) in 3 mL

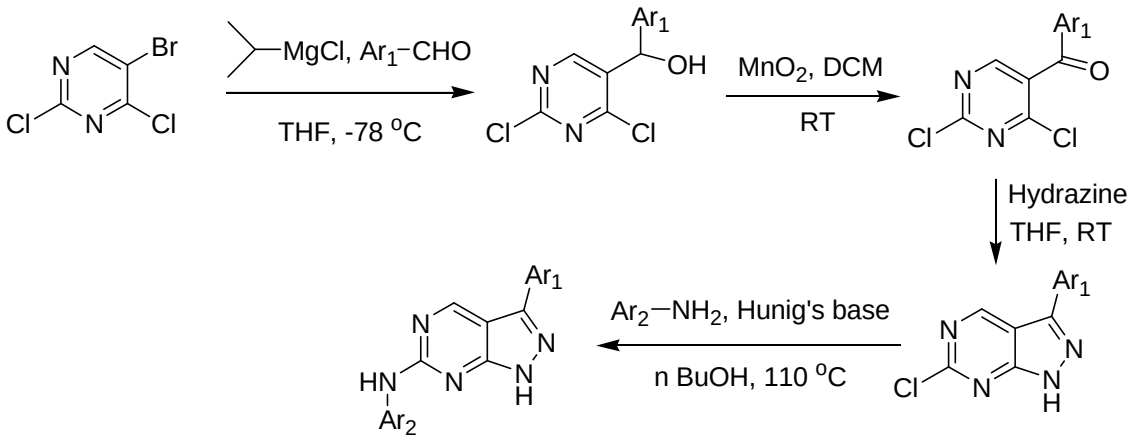
degassed solvent (DME:MeOH=2:1) was heated at 70 ^{o}C for 10 hrs. The crude material was purified by silica gel column chromatography using petroleum ether-ethyl acetate solvent system (2:3) to obtain 46 mg of pure aldehyde (0.14 mmol, 55%). The aldehyde was heated at 65 ^{o}C with 21 mg of 5-carboxyoxindole (0.12 mmol) and 14 µL of piperidine (0.14 mmol) in 1.5 mL of dry methanol for 2 hrs. Upon cooling, an orange colored compound **7** was precipitated out which was filtered, washed and dried to obtain 45 mg of pure product (0.09 mmol, 65%).



Synthetic route to compound **7**

7. Synthesis of Pyrazolopyrimidines:

A. General Approach: Pyrazolopyrimidines are synthesized based on reported protocol.[18] Briefly, a metal-halogen exchange reaction between 5-Bromo-2,4-dichloropyrimidine and isopropylmagnesium chloride followed by nucleophilic addition to an aromatic aldehyde provided the benzyl alcohol which was subsequently oxidized to ketone by MnO_2. Hydrazine mediated pyrazole ring formation followed by nucleophilic substitution by primary aromatic amine furnished the desired final product. A general scheme is shown below.



General synthetic route to various pyrazolopyrimidines

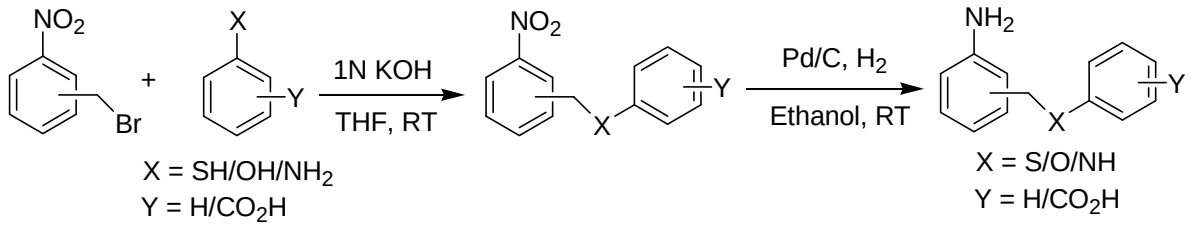
Procedure for metal-halogen exchange reaction: To an anhydrous THF (5 mL) solution of 5-Bromo-2,4-dichloropyrimidine (1 mmol) at -78 ^{o}C, isopropylmagnesium chloride in THF (1.25 mmol) was added dropwise and reaction was continued for 45 minutes while temperature was increased to -40 ^{o}C under nitrogen atmosphere. A THF solution of aromatic aldehyde (1 mmol) was added dropwise and temperature was increased to 0 ^{o}C and stirred for another 2 hrs. Reaction was quenched with saturated NH_4Cl solution and extracted with ethyl acetate. Organic phase was washed with water and brine and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography (yield varied from 60% to 80%).

Procedure for oxidation: Benzyl alcohol (0.5 mmol) was dissolved in dry DCM (6 mL) and activated MnO_2 (5 mmol) added and reaction was stirred for 20 hrs at room temperature. Upon completion, MnO2 was filtered off; filtrate was evaporated to dryness and the crude product was purified by silica gel column chromatography (yield varied from 60% to 75%).

Procedure for pyrazole ring formation: Into a THF (3 mL) solution of ketone (0.25 mmol), anhydrous hydrazine (0.25 mmol) was added dropwise at room temperature. Reaction was continued for 1 hr and filtered off any solid residue that was formed. Filtrate was evaporated to dryness and the crude product was purified by silica gel column chromatography (yield varied from 50% to 65%).

Nucleophilic addition of primary aromatic amines: Into a mixture of substituted chloropyrazolopyrimidine (0.1 mmol) and primary aromatic amine (0.1 mmol) in n-BuOH (1.5 mL) was added Hunig's base (0.12 mmol). The vial was closed tightly and stirred at 110 ^{o}C for 26 hrs. Upon completion, solvent was removed under vacuum and the crude product was purified by silica gel column chromatography (yield varied from 45% to 60%). In many cases, crude reaction mixture was diluted with 1 mL ethyl acetate-petroleum ether (1:1) to precipitate the required product followed by washing with cold ethyl acetate.

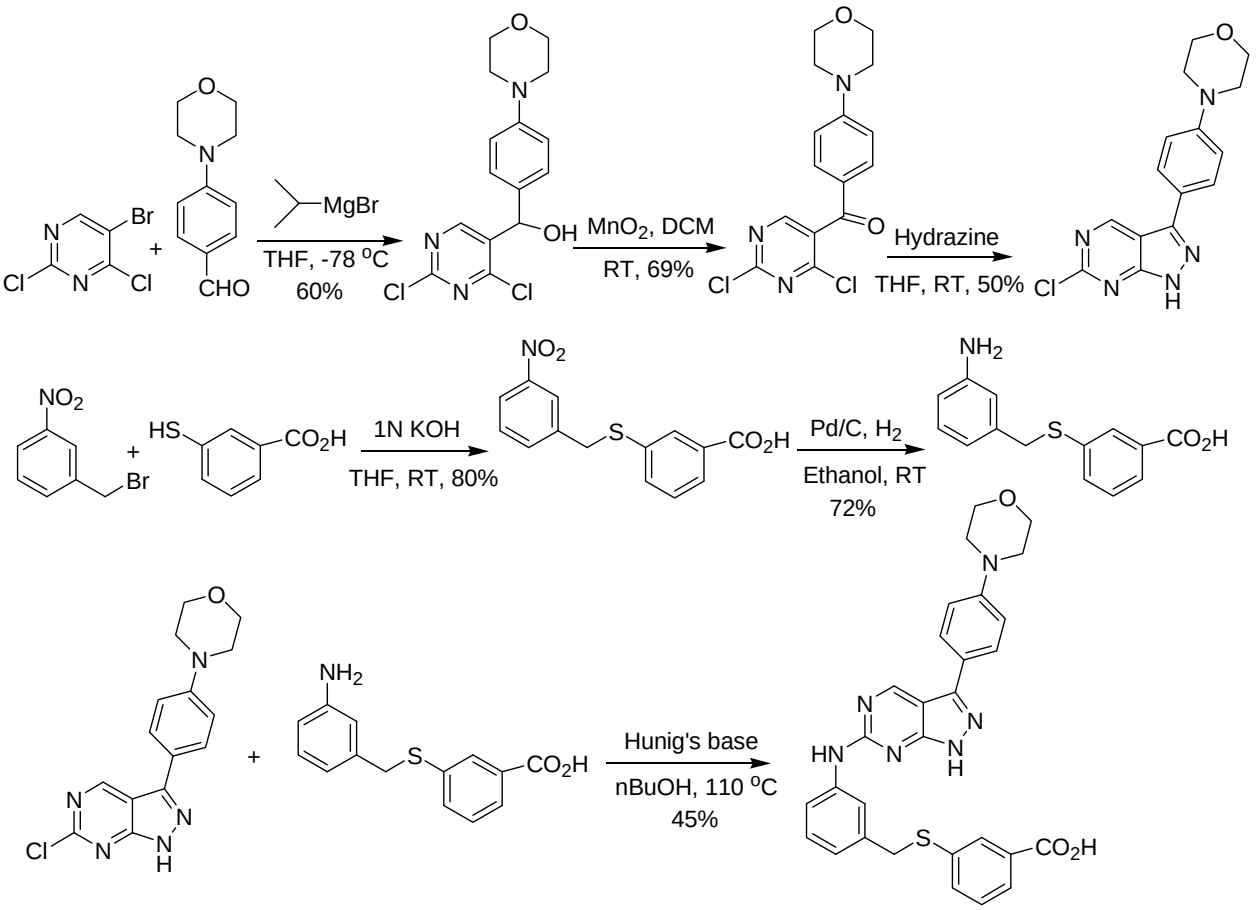
B. Synthesis of non-commercial primary aromatic amines: Many of the primary aromatic amines used in the present work are not commercially available. All such amines were synthesized from nitrobenzylbromide and substituted phenol/thiophenol/aniline using base-mediated nucleophilic substitution reaction. Finally, nitro group was reduced to primary amine. A general scheme is shown below.



Synthetic route to non-commercial primary aromatic amines

Procedure for nucleophilic aromatic substitution: Into a THF (3 mL) solution of Nitrobenzyl bromide (0.5 mmol) and substituted phenol/thiol/aniline (0.5 mmol) was added 1N KOH (1.5 mmol) and stirred at room temperature for 16-32 hrs. Upon completion, excess base was neutralized with 1N HCl and extracted with ethyl acetate. Organic layer was washed with water and brine and dried over Na_2SO_4. Crude material was purified by silica gel column chromatography using ethyl acetate-dichloromethane (yield varied from 75% to 85%).
Procedure for nitro reduction: To an ethanolic solution of nitro compound (0.3 mmol), Pd/C (10% w/w, 0.03 mmol) was added and connected with Hydrogen gas. Reaction was stirred for 4 hrs and catalyst was filtered off. The filtrate was removed under vacuum and crude product was purified by silica gel column chromatography using methanol-dichloromethane (yield varied from 65% to 80%).

C. Synthesis of MyoVin-1 (8): As a representative example, we describe the synthesis of MyoVin-1 (**8**). Synthesis of pyrazolopyrimidine was done essentially the same way as described above. 228 mg of Bromo-2,4-dichloropyrimidine (1 mmol) was subjected to metal-halogen exchange reaction with isopropylmagnesium chloride in THF (1.25 mmol) followed by addition of 168 mg of 4-morpholinobenzaldehyde (1 mmol). The crude product was purified by silica gel chromatography (ethyl acetate-petroleum ether (1:1) to obtain 204 mg of alcohol (0.6 mmol, 60%). The alcohol (170 mg, 0.5 mmol) was oxidized with MnO_2 (435 mg, 5 mmol) in DCM (6 mL) for 26 hrs. The crude product was purified by silica gel chromatography (ethyl acetate-petroleum ether (1:2) to obtain 116 mg of ketone (0.35 mmol, 69%). Into a THF (3 mL) solution of ketone (85 mg, 0.25 mmol), hydrazine (8 µL, 0.25 mmol) was added and stirred for 1 hr. The crude product was purified by silica gel chromatography (ethyl acetate-petroleum ether (3:2) to obtain 39 mg of ketone (0.125 mmol, 50%).
Synthesis of primary aromatic amine was accomplished as described earlier. 3-Nitrobenzyl alcohol (108 mg. 0.5 mmol) and 3-mercaptobenzoic acid (77 mg, 0.5 mmol) was stirred in presence of 1N KOH (1.5 mmol) in THF for 24 hrs. Crude material was purified by silica gel column chromatography using 1:2 ethyl acetate-dichloromethane to obtain 115 mg of product (0.5 mmol, 80%). 87 mg of the product (0.3 mmol) was dissolved in 3 mL ethanol and added Pd/C (10 mg, 0.03 mmol). After hydrogenation, crude product was purified by silica gel column chromatography (methanol-dichloromethane, 1:9) to obtain 56 mg of amine (0.22 mmol, 72%).
Finally, 31 mg of pyrazolopyrimidine (0.1 mmol) and 26 mg of amine (0.1 mmol) were heated in nBuOH for 26 hrs. Reaction was cooled and added 1 mL of ethyl acetate-petroleum ether (1:1) to precipitate the required product and washed with cold ethyl acetate. It was dried to obtain 24 mg of the MyoVin-1 (**8**) (0.045 mmol, 45%).



MyoVin-1 (**8**)

Synthetic route to MyoVin-1(compound **8**)

8. SAR data for oxindoles: Inhibition of actin-stimulated ATPases of myosin V at 50 μM compound's concentration. Values in parenthesis indicate the average percent inhibition (n=3 independent experiments); myosin V= 30 nM and actin= 2 μM.

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 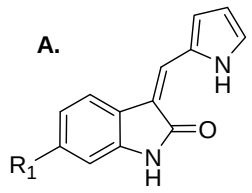 </td>
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 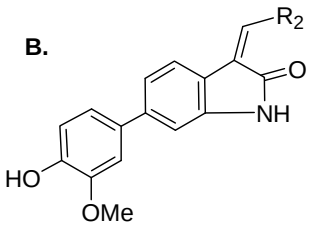 </td>
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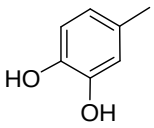
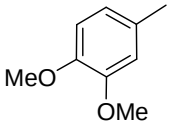
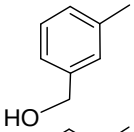
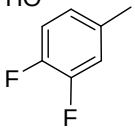
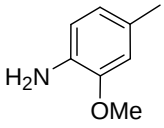
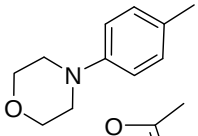
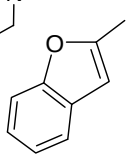
  (20%)
  (15%)
  (17%)
  (9%)
  (21%)
  (13%)
  (6%)</td>
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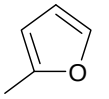
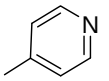
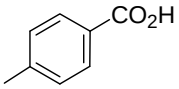
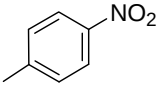
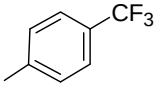
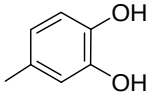
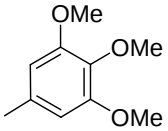
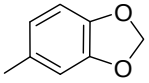
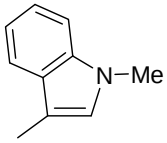
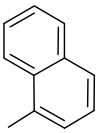
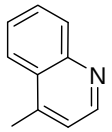
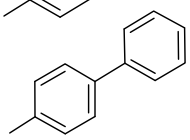
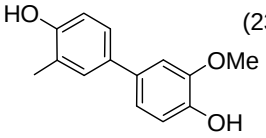
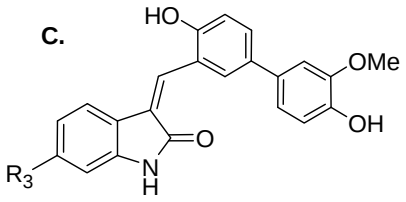
  (15%)
  (11%)
  (19%)
  (10%)
  (17%)
  (8%)
  (6%)
  (7%)
  (10%)
  (6%)
  (14%)
  (16%)
  (19%)
  (23%)</td>
</tr>
</table>

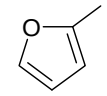
8. SAR data for oxindoles (continued):

C.

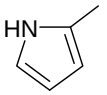


R_3=

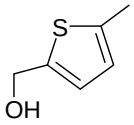
Br— (35%)



(21%)

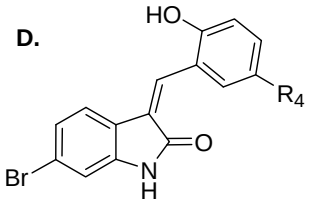


(23%)

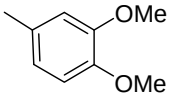


(21%)

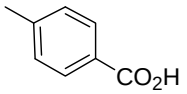
D.



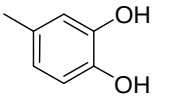
R_4=



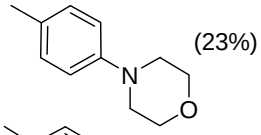
(20%)



(33%)



(34%)



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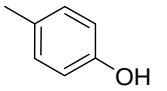

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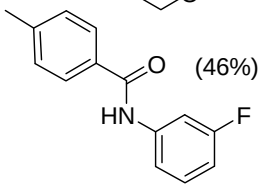
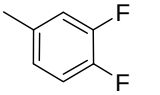
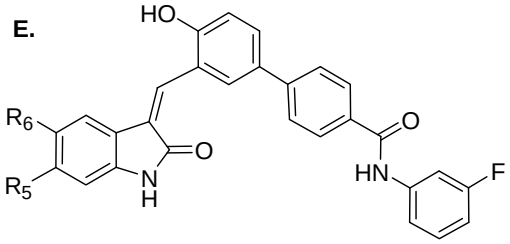


(46%)



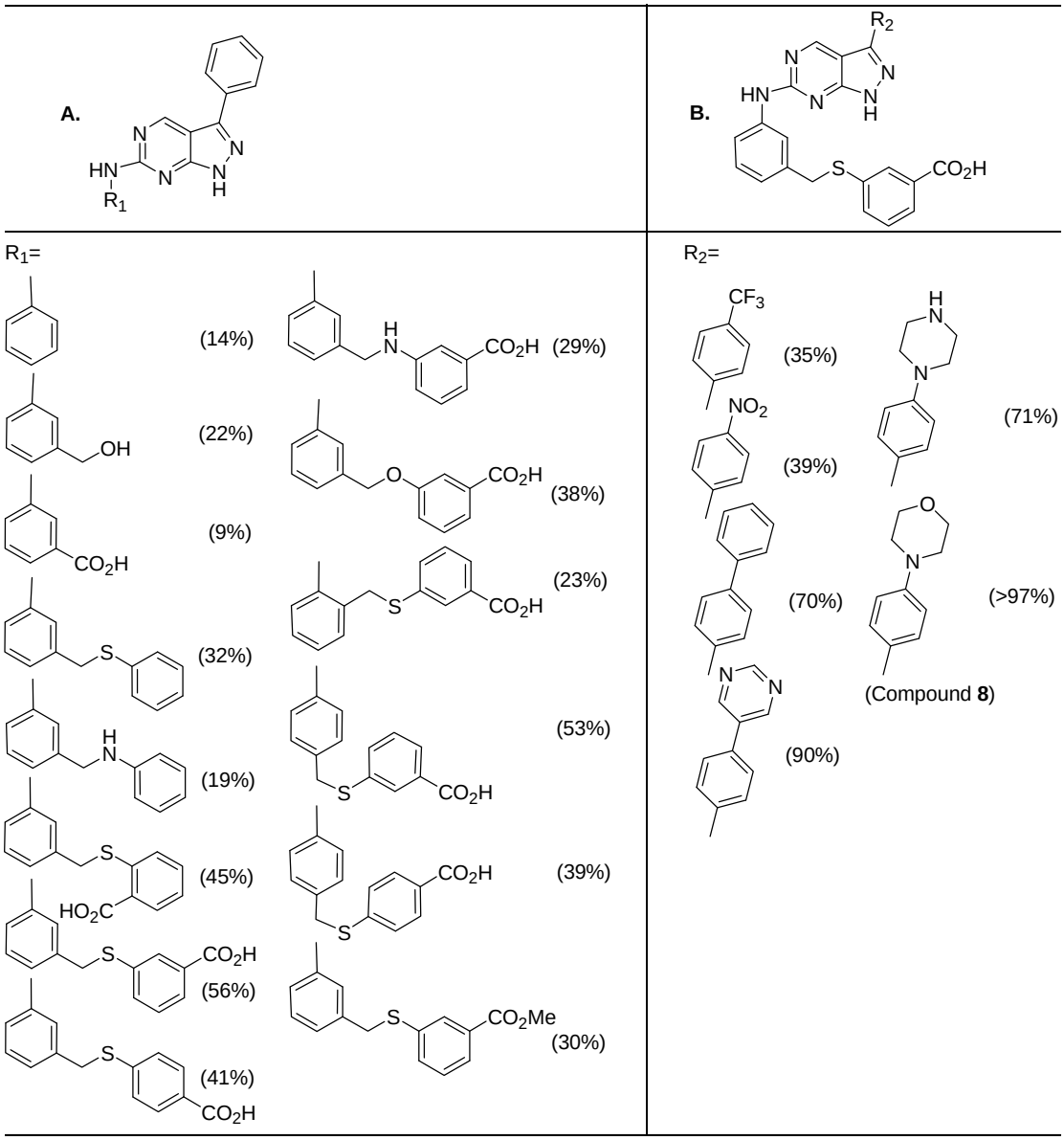
(26%)

E.

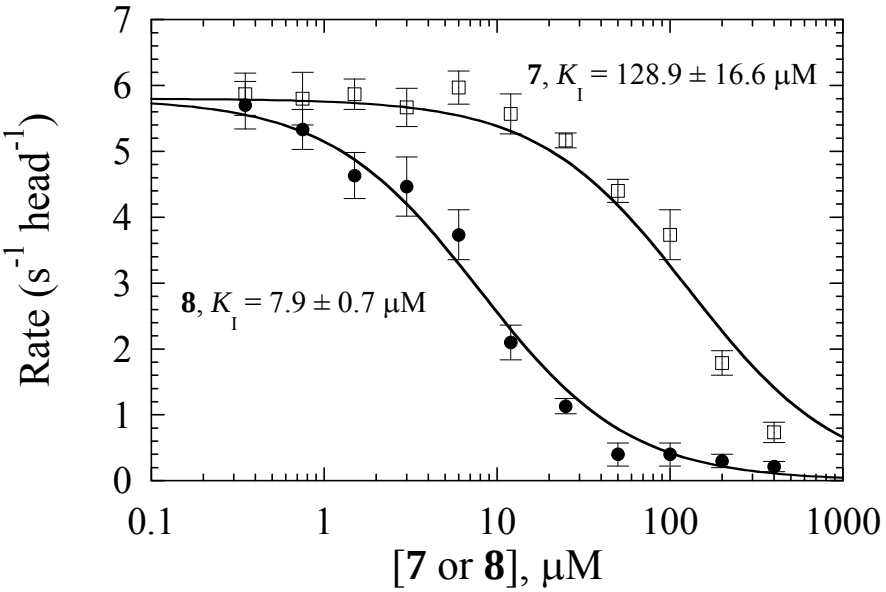


R_5=F, R_6=H	(32%)
R_5=H, R_6=F	(37%)
R_5=H, R_6=NH_2	(30%)
R_5=H, R_6=CN	(55%)
R_5=H, R_6=$CONH_2$	(65%)
R_5=H, R_6=CO_2H (compound **7**)	(75%)

9. SAR data for pyrazolopyrimidines: Average percent inhibition of actin-stimulated ATPase activity of myosin V at 50 μM compound's concentration (n=3 independent experiments); myosin V= 30 nM and actin= 2 μM.

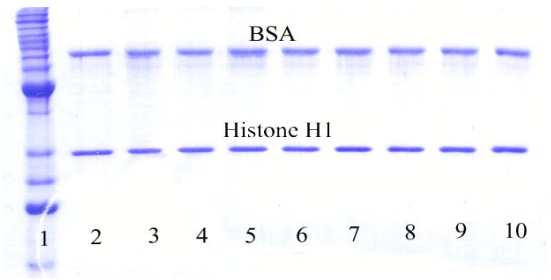


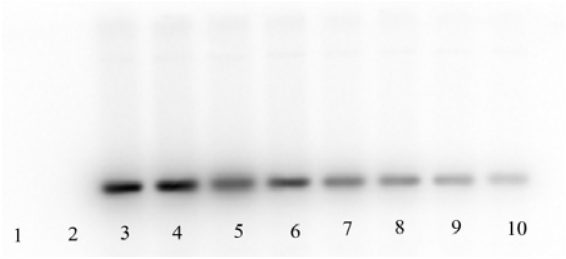
10. Analysis of detergent effects on inhibition: The presence of 0.1% Triton-X-100 increases the K_I of **7** by ~10 fold whereas the effect on compound **8** was ~1.3 fold.

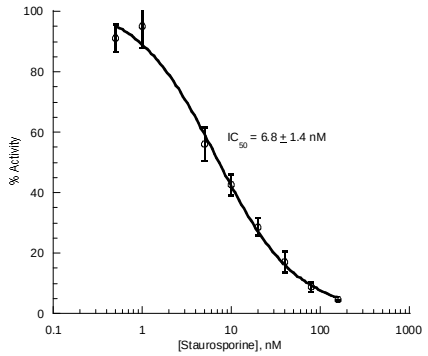


11. CDK1 assay: CDK1 activity was assayed in kinase buffer (20 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 1 mM DTT, 5 mM EDTA, 1 mM Na_3VO_4, 5 mM β-glycerophosphate, 0.05mg/mL BSA). Human recombinant Histone H1 (1 μg, *NEW ENGLAND BioLabs Inc.),* 20 ng recombinant CDK1 (Upstate Biotechnoly) and the appropriate amount of compound in DMSO (5% (v/v) final) were added to half the reaction volume (10 μl) and allowed to incubate for 30 min on ice. Then 10 μl of kinase buffer containing unlabeled ATP (10 μM final) and [γ-^{32}P]ATP (2.5 μCi final) was added. Reactions were mixed, incubated for 45 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were scanned using Typhoon 9400 Variable Mode Imeger (Amersham Bioscience) and quantified with ImageQuant software (Molecular Dynamics).

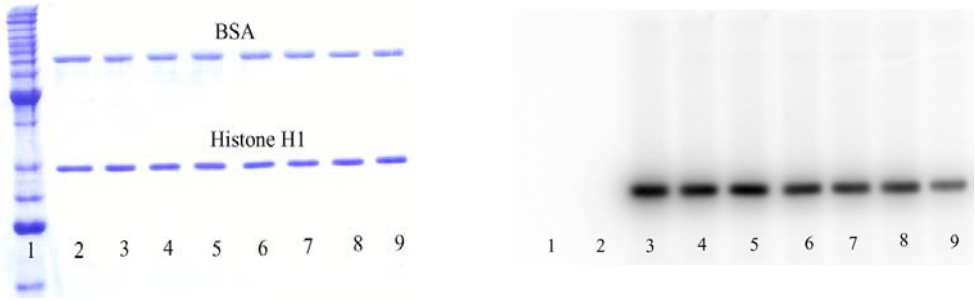
a) Staurosporine dose-response data: A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. The IC_{50} of staurosporine was measured as 6.8 nM. Detail description of the lanes: **1**. protein ladder; **2**. no kinase; **3**. DMSO; **4**. Staurosporine (1 nM); **5**. Staurosporine (5 nM); **6**. Staurosporine (10 nM); **7**. Staurosporine (20 nM); **8**. Staurosporine (40 nM); **9**. Staurosporine (80 nM); **10**. Staurosporine (160 nM).





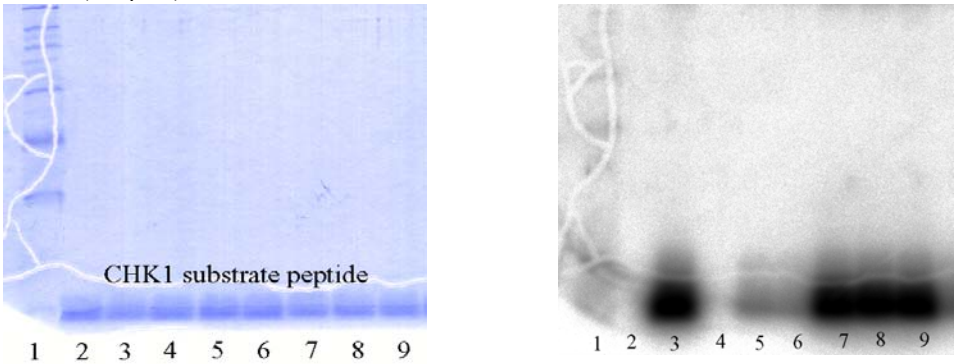


b) MyoVin-1 (8) dose-response data: A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. Detail description of the lanes: **1**. protein ladder; **2**. no kinase; **3**. DMSO; **4**. MyoVin-1 (12.5 μM); **5**. MyoVin-1 (25 μM); **6**. MyoVin-1 (50 μM); **7**. MyoVin-1 (100 μM); **8**. MyoVin-1 (200 μM); **9**. MyoVin-1 (400 μM).

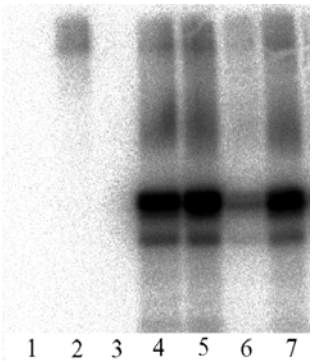


12. CHK1 assay: CHK1 activity was assayed in kinase buffer (20 mM MOPS (pH 7.2), 10 mM $MgCl_2$, 1 mM DTT, 5 mM EGTA, 1 mM Na_3VO_4, 25 mM β-glycerophosphate). CHKtide (4 μg, CHK1 substrate, Upstate Biotechnology), 31 ng recombinant CHK1 (Upstate Biotechnoly) and the appropriate amount of compound in DMSO (5% (v/v) final) were added to half the reaction volume (10 μl) and allowed to incubate for 30 min on ice. Then 10 μl of kinase buffer containing unlabeled ATP (10 μM final) and [γ-^{32}P]ATP (2.5 μCi final) was added. Reactions were mixed, incubated for 30 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (18.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were quantified with ImageGauge software (Fujifilm).

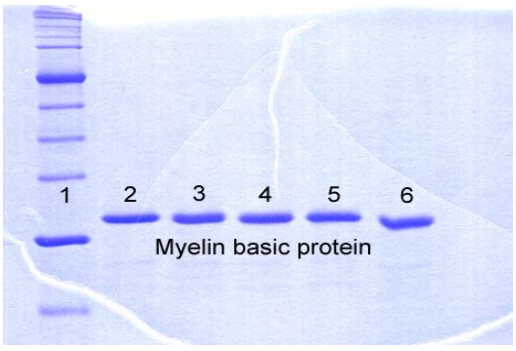
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. Detail description of the lanes: **1**. protein ladder; **2**. no kinase; **3**. DMSO; **4**. Comp. ***4*** (40 nM); **5**. Comp. ***4*** (20 nM); **6**. Comp. ***4*** (10 nM);); **7**. MyoVin-1 (100 μM); **8**. MyoVin-1 (50 μM); **9**. MyoVin-1 (25 μM).

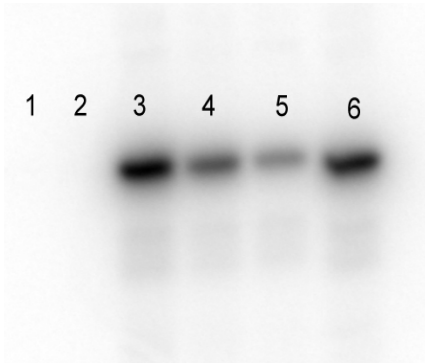


13. PLK1 assay: Plk1 activity was assayed in kinase buffer (50 mM Tris (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT, 2 mM EGTA, 0.5 mM Na_3VO_4, 20 mM NaF, 20 mM β-glycerophosphate). Casein (3 µg), 100 ng recombinant *Xenopus laevis* Plk1 (expressed and purified similarly to the method described[19]) and the appropriate amount of compound in DMSO (5% (v/v) final) were added to half the reaction volume (10 µl) and allowed to incubate for 30 min on ice. Then 10 µl of kinase buffer containing unlabeled ATP (10 µM final) and [γ-^{32}P]ATP (2.5 µCi final) was added. Reactions were mixed, incubated for 40 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were quantified with ImageGauge software (Fujifilm).
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. Detail description of the lanes: **1**. protein ladder; **2**. no casein; **3**. no kinase; **4**. DMSO; **5**. DMSO; **6**. DAP-81 (5 µM);); **7**. MyoVin-1 (100 µM).

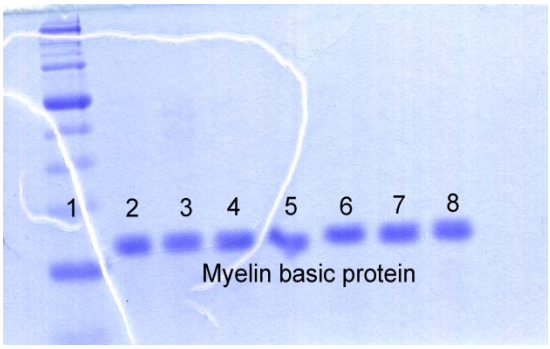


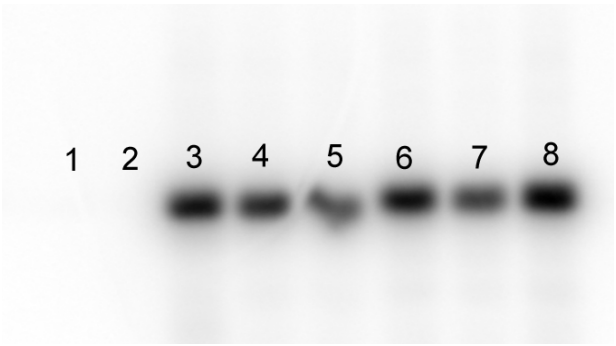
14. Abl kinase assay: Abl kinase activity was assayed in kinase buffer (50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 1 mM EGTA, 1 mM Na_3VO_4, 5 mM β-glycerophosphate, 0.01% Brij 35). Myelin basic protein$_f$ (1 µg), 2 ng recombinant Abl Protein Tyrosine Kinase (Abl) and the appropriate amount of compound in DMSO (5% v/v) were added to half the reaction volume (10 µl) and allowed to incubate for 30 min on ice. Then 10 µl of kinase buffer containing unlabeled ATP (10 µM final) and [γ-^{32}P]ATP (2.5 µCi final) was added. Reactions were mixed, incubated for 40 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were scanned using Typhoon 9400 Variable Mode Imeger (Amersham Bioscience) and quantified with ImageQuant software (Molecular Dynamics).
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below.
Detail description of the lanes: **1**: Protein Ladder. **2**: No Kinase. **3**: DMSO. **4**: Staurosporine (0.25 µM). **5**. Staurosporine (0.5 µM). **6**: MyoVin-1 (100 µM)





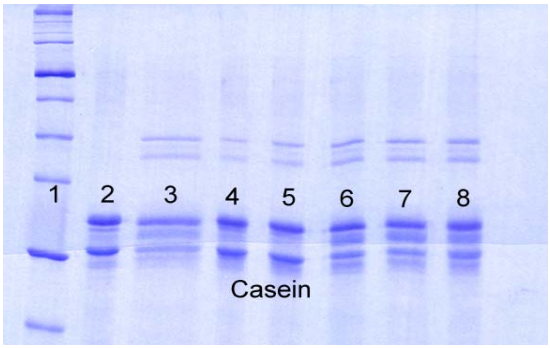
15. p42 MAP kinase (Erk2) assay: p42 MAP kinase activity was assayed in kinase buffer (50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 1 mM EGTA, 1 mM Na_3VO_4, 5 mM β-glycerophosphate, 0.01% Brij 35). Myelin basic protein$_f$ (1 µg), 3 ng recombinant p42 MAP Kinase (Erk2) and the appropriate amount of compound in DMSO (5% v/v) were added to half the reaction volume (10 µl) and allowed to incubate for 30 min on ice. Then 10 µl of kinase buffer containing unlabeled ATP (10 µM final) and [γ-^{32}P]ATP (2.5 µCi final) was added. Reactions were mixed, incubated for 40 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gel bands were scanned using Typhoon 9400 Variable Mode Imeger (Amersham Bioscience) and quantified with ImageQuant software (Molecular Dynamics).
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. Detail description of the lanes: **1**: Protein Ladder. **2**: No Kinase. **3**: DMSO. **4**: Staurosporine (2.5 µM). **5**. Staurosporine (5 µM). **6**: MyoVin-1 (100 µM). **7**: MyoVin-1 (150 µM). **8**: DMSO.

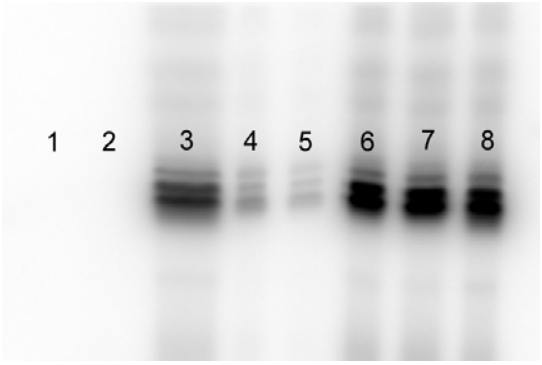




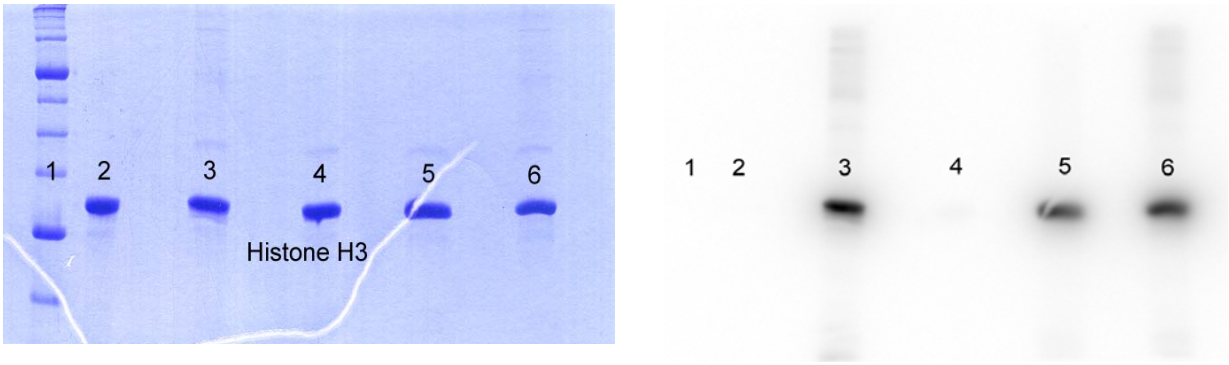
16.

Casein kinase II assay: Casein kinase II activity was assayed in kinase buffer (50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 1 mM EGTA, 1 mM Na_3VO_4, 5 mM β-glycerophosphate, 0.01% Brij 35). Casein (4 µg), 100 ng recombinant casein kinase II and the appropriate amount of compound in DMSO (5% v/v) were added to half the reaction volume (10 µl) and allowed to incubate for 30 min on ice. Then 10 µl of kinase buffer containing unlabeled ATP (10 µM final) and [γ-^{32}P]ATP (2.5 µCi final) was added. Reactions were mixed, incubated for 40 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were scanned using Typhoon 9400 Variable Mode Imeger (Amersham Bioscience) and quantified with ImageQuant software (Molecular Dynamics).
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below. Detail description of the lanes: **1**: Protein Ladder. **2**: No Kinase. **3**: DMSO. **4**: Casein kinase II inhibitor (2.5 µM). **5**. Casein kinase II inhibitor (5 µM). **6**: MyoVin-1 (100 µM). **7**: MyoVin-1 (150 µM). **8**: DMSO.

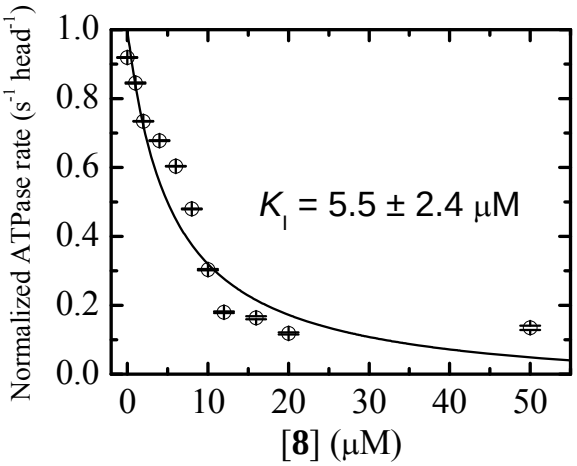




17. Aurora B kinase assay: Aurora B kinase activity was assayed in kinase buffer (50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 1 mM EGTA, 1 mM Na_3VO_4, 5 mM β-glycerophosphate, 0.01% Brij 35). Histone H3 (1 µg), 30 ng recombinant aurora B kinase and the appropriate amount of compound in DMSO (5% v/v) were added to half the reaction volume (10 µl) and allowed to incubate for 30 min on ice. Then 10 µl of kinase buffer containing unlabeled ATP (10 µM final) and [γ-^{32}P]ATP (2.5 µCi final) was added. Reactions were mixed, incubated for 40 min at 32 °C and stopped by the addition of Laemmli sample buffer. Proteins were resolved by SDS-PAGE (12.5%). Gels were dried and exposed to a phosphorimager screen. Gel bands were scanned using Typhoon 9400 Variable Mode Imeger (Amersham Bioscience) and quantified with ImageQuant software (Molecular Dynamics).
A representative Coomassie blue stained gel and corresponding autoradiogram are shown below.
Detail description of the lanes: **1**: Protein Ladder. **2**: No Kinase. **3**: DMSO. **4**: Hesperadin (0.5 µM). **5**: MyoVin-1 (150 µM). **6**: MyoVin-1 (100 µM).



18. Inhibition of Two-headed myosin V: Steady-state ATPase of double-headed myosin V, an active construct that contains the two catalytic domains and twelve associated light chains of native myosin V,[20] was measured at 25° C in KMg50 Buffer using an NADH enzyme-linked assay[5] as described above under section **2**. The actin concentration was 2.5 µM with a final myosin concentration of 80 nM heads (i.e. 40 nM double-headed myosin). Uncertainty bars represent standard errors in the ATPase timecourse fits. A K_I of 5.5 ± 2.4 µM was obtained from the best fit of the data to a hyperbola.



19. References:

Full reference for 23 in the manuscript:

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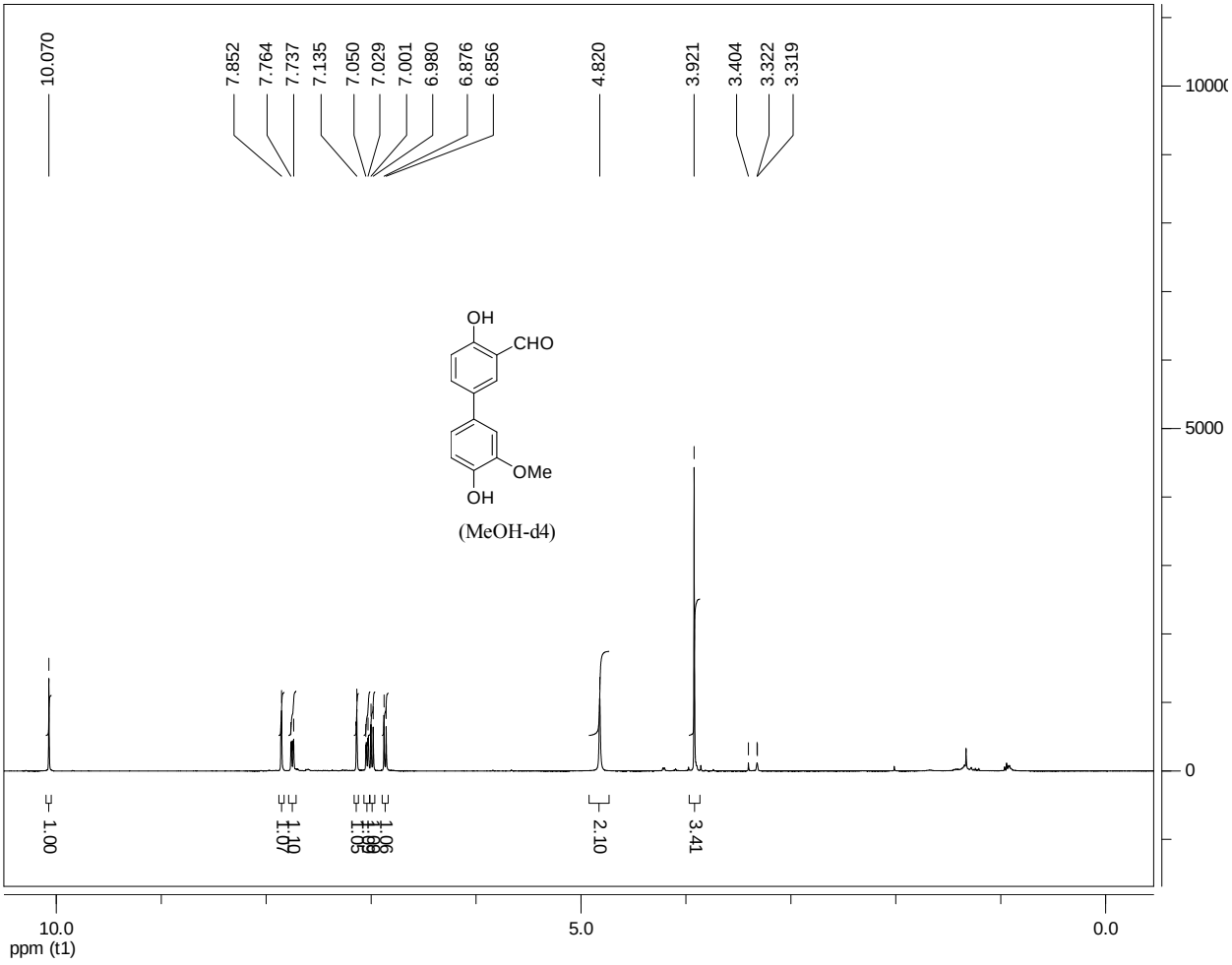
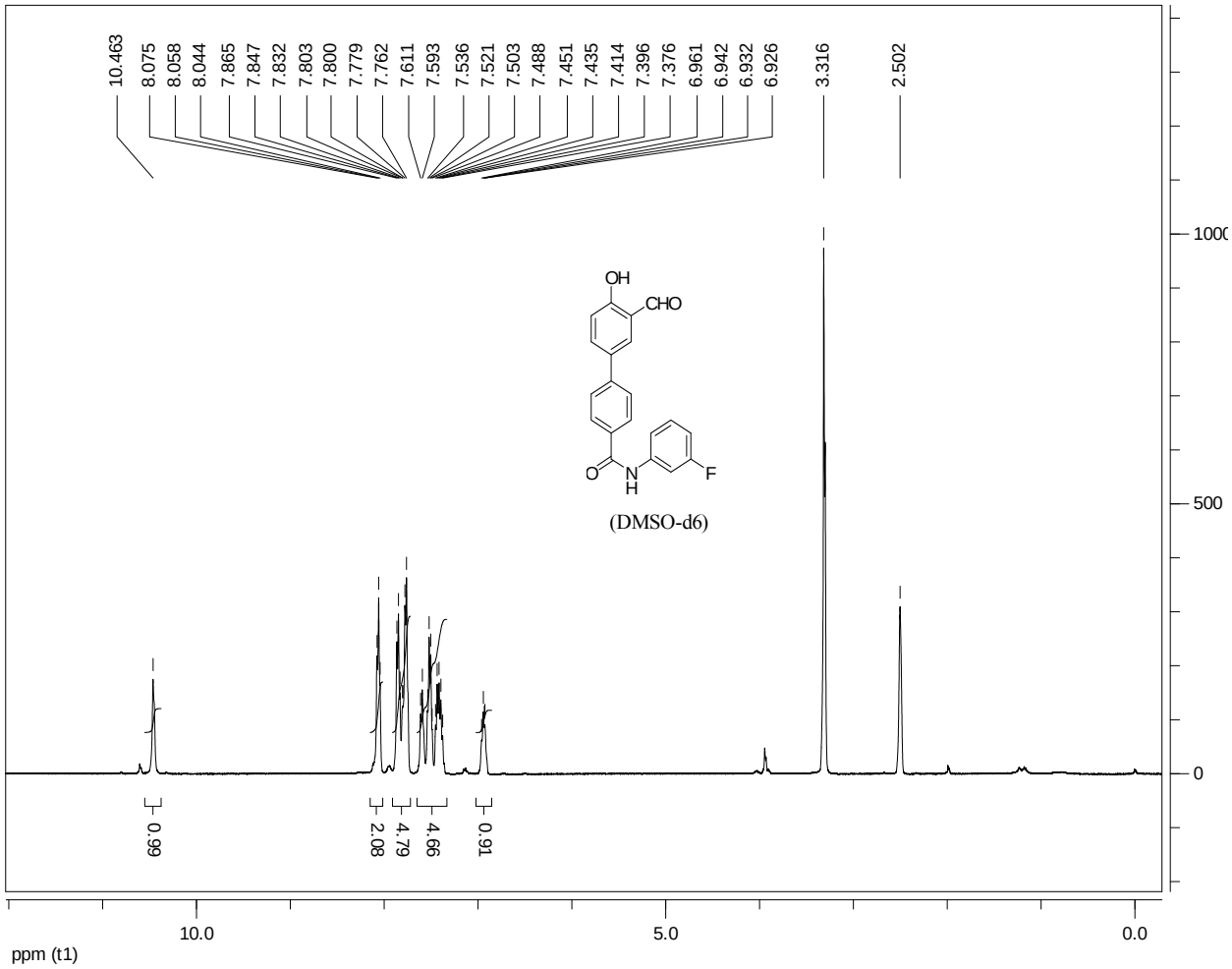
21. Characterization data and NMR spectra for selected compounds



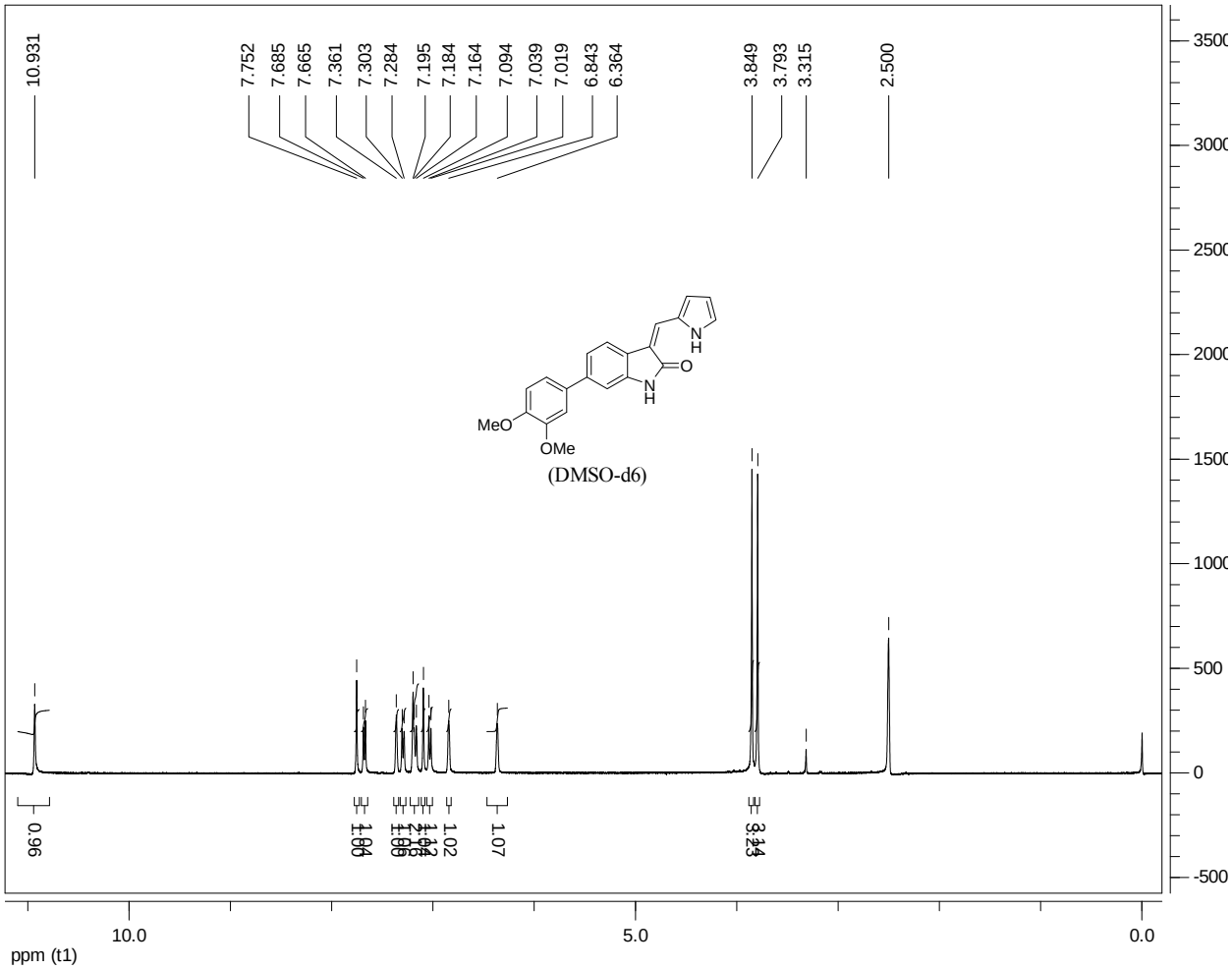
^{1}H NMR (400 MHz, MeOH-d_4) δ 10.07 (1H, s), 7.85 (1H, s), 7.75 (1H, d, *J*=10.8 Hz), 7.14 (1H, s), 7.04 (1H, d, *J* =10 Hz), 7.0 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 6.4 Hz), 4.82 (2H, s), 3.92 (3H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 245.48.



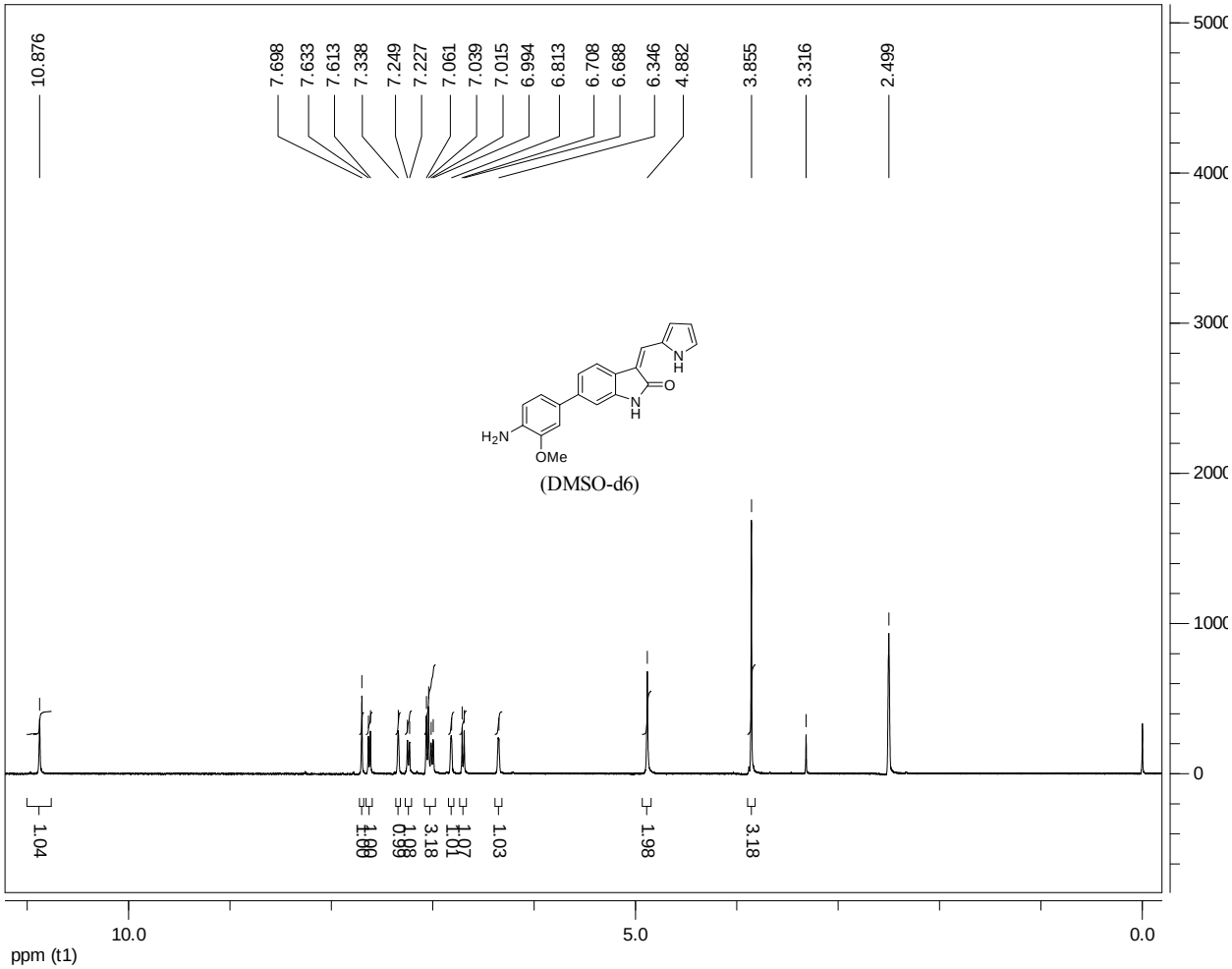
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (1H, s), 8.07 (1H, d, *J*=6.8 Hz), 8.04 (1H, d, *J* =5.6 Hz), 7.86-7.83 (2H, m), 7.8-7.76 (2H, m), 7.6 (1H, d, *J* = 7.2 Hz), 7.54-7.37 (3H, m), 6.94 (1H, t, *J* = 7.6 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 336.52.



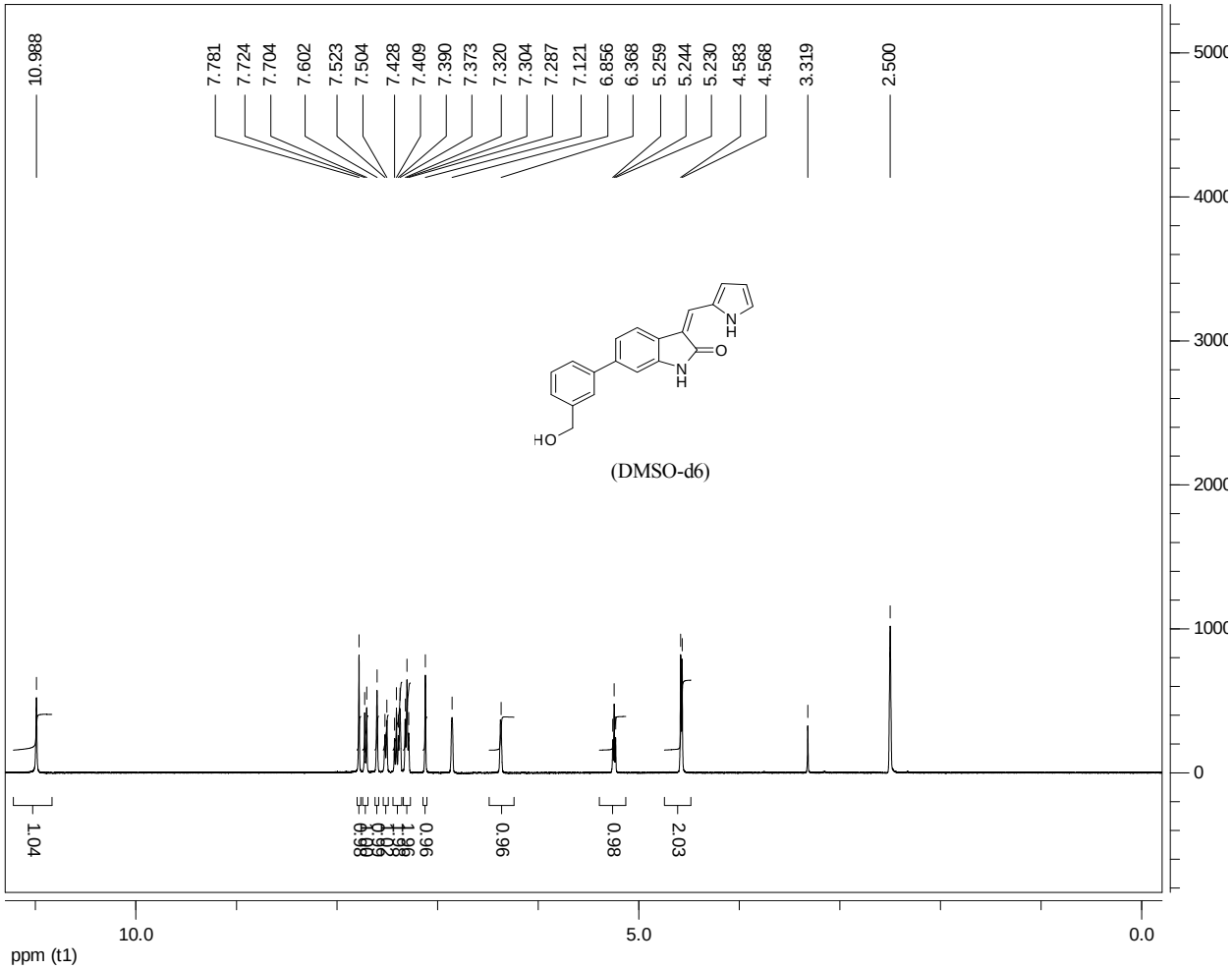
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (1H, s), 7.75 (1H, s), 7.68 (1H, d, *J* = 8 Hz), 7.36 (1H, s), 7.29 (1H, d, *J* = 7.6 Hz), 7.2 (1H, s), 7.17 (1H, d, *J* = 8 Hz), 7.09 (1H, s), 7.03 (1H, d, *J* = 8 Hz), 6.84 (1H, s), 6.36 (1H, s), 3.85 (3H, s), 3.79 (3H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 347.41.



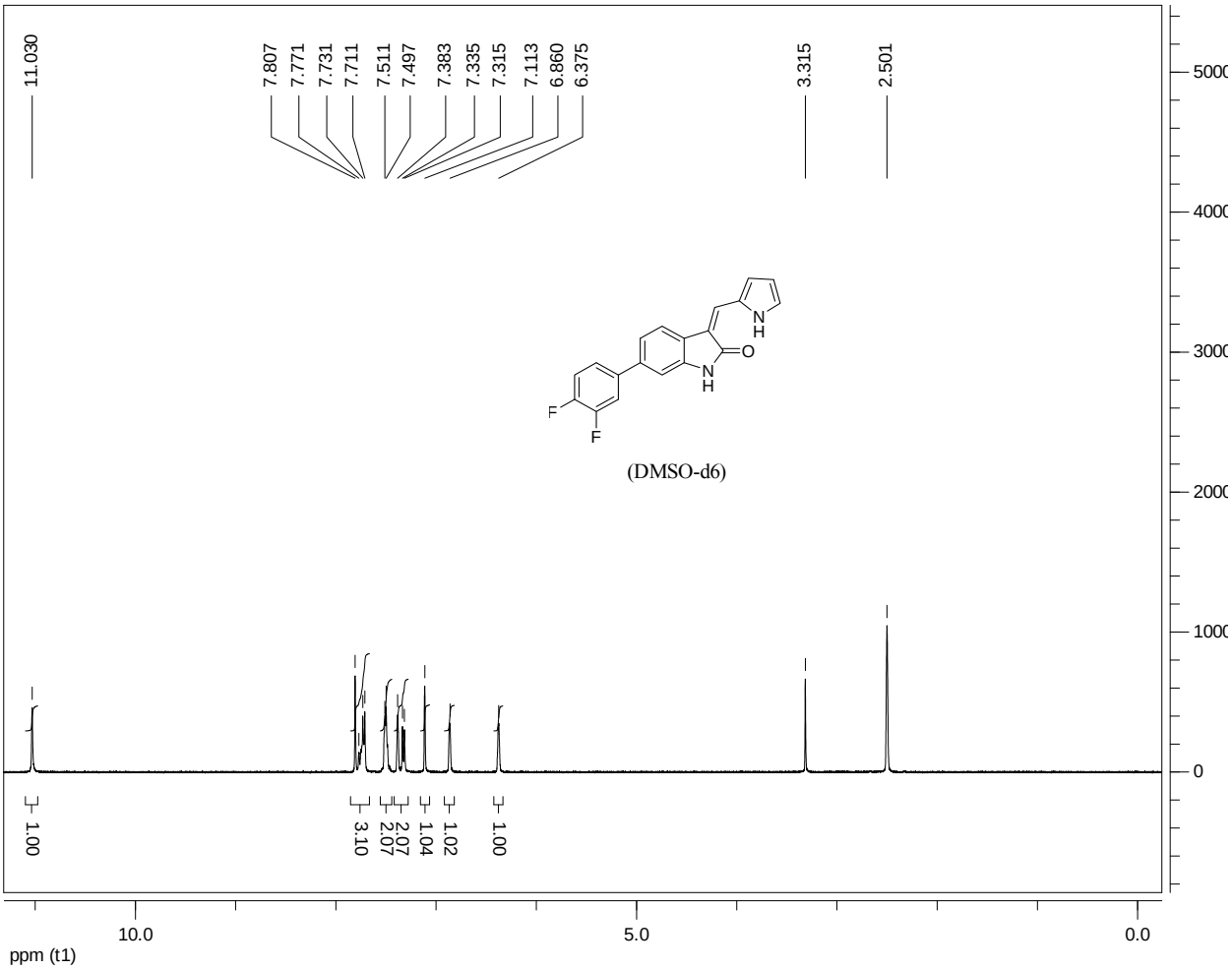
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (1H, s), 7.7 (1H, s), 7.62 (1H, d, *J* = 8 Hz), 7.34 (1H, s), 7.24 (1H, d, *J* = 8.8 Hz), 7.05 (1H, d, *J* = 8.8 Hz), 7.01 (1H, d, *J* = 8.4 Hz), 6.81 (1H, s), 6.7 (1H, d, *J* = 8 Hz), 6.35 (1H, s), 4.88 (2H, s), 3.86 (3H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 332.33.



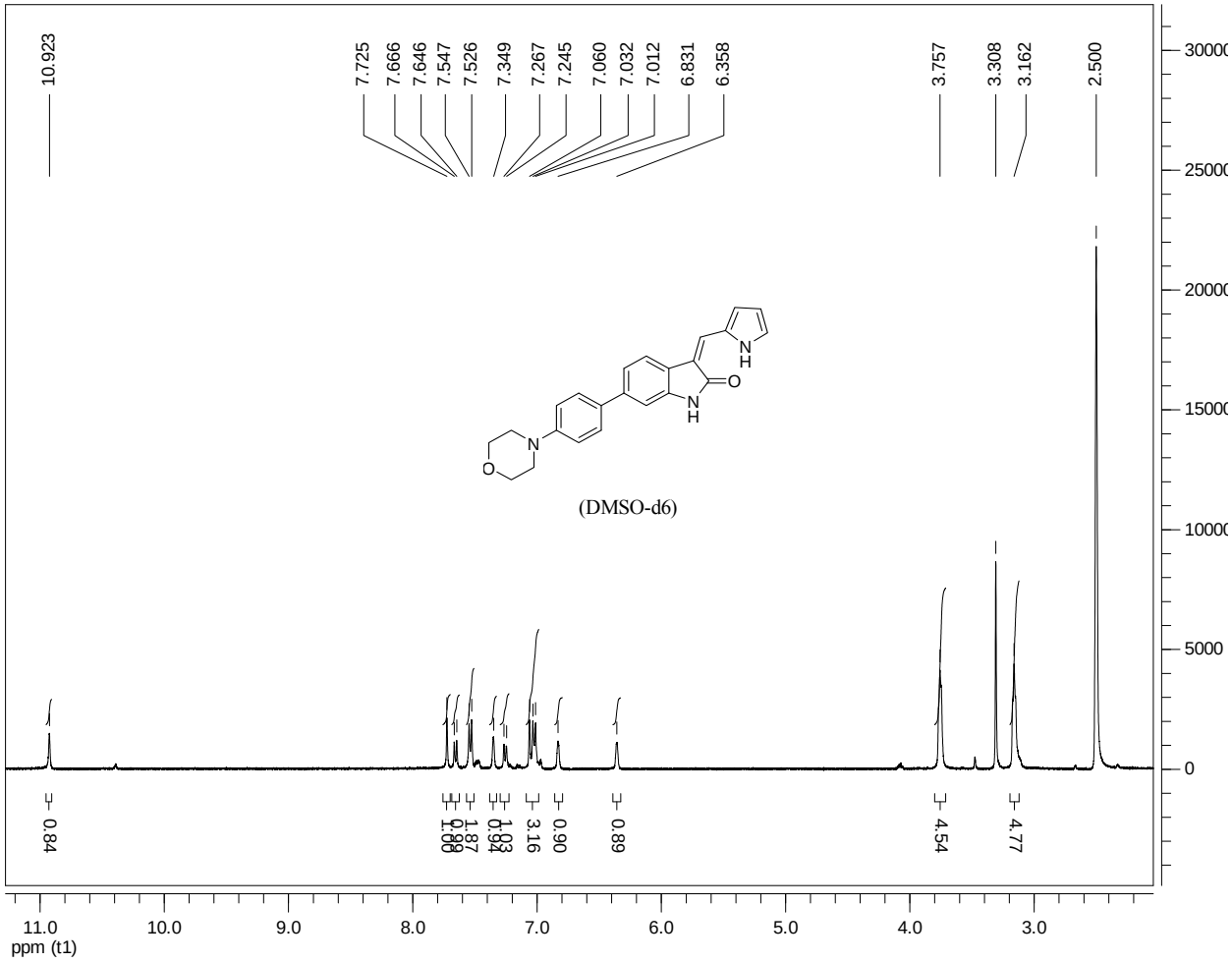
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (1H, s), 7.78 (1H, s), 7.71 (1H, d, *J* = 8 Hz), 7.6 (1H, s), 7.51 (1H, d, *J* = 7.6 Hz), 7.41 (1H, t, *J* = 7.6 Hz), 7.37 (1H, s), 7.3 (2H, t, *J* = 6.4 Hz), 7.12 (1H, s), 6.86 (1H, s), 6.37 (1H, s), 5.24 (1H, t, *J* = 6 Hz), 4.58 (2H, d, *J* = 6 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 317.24.



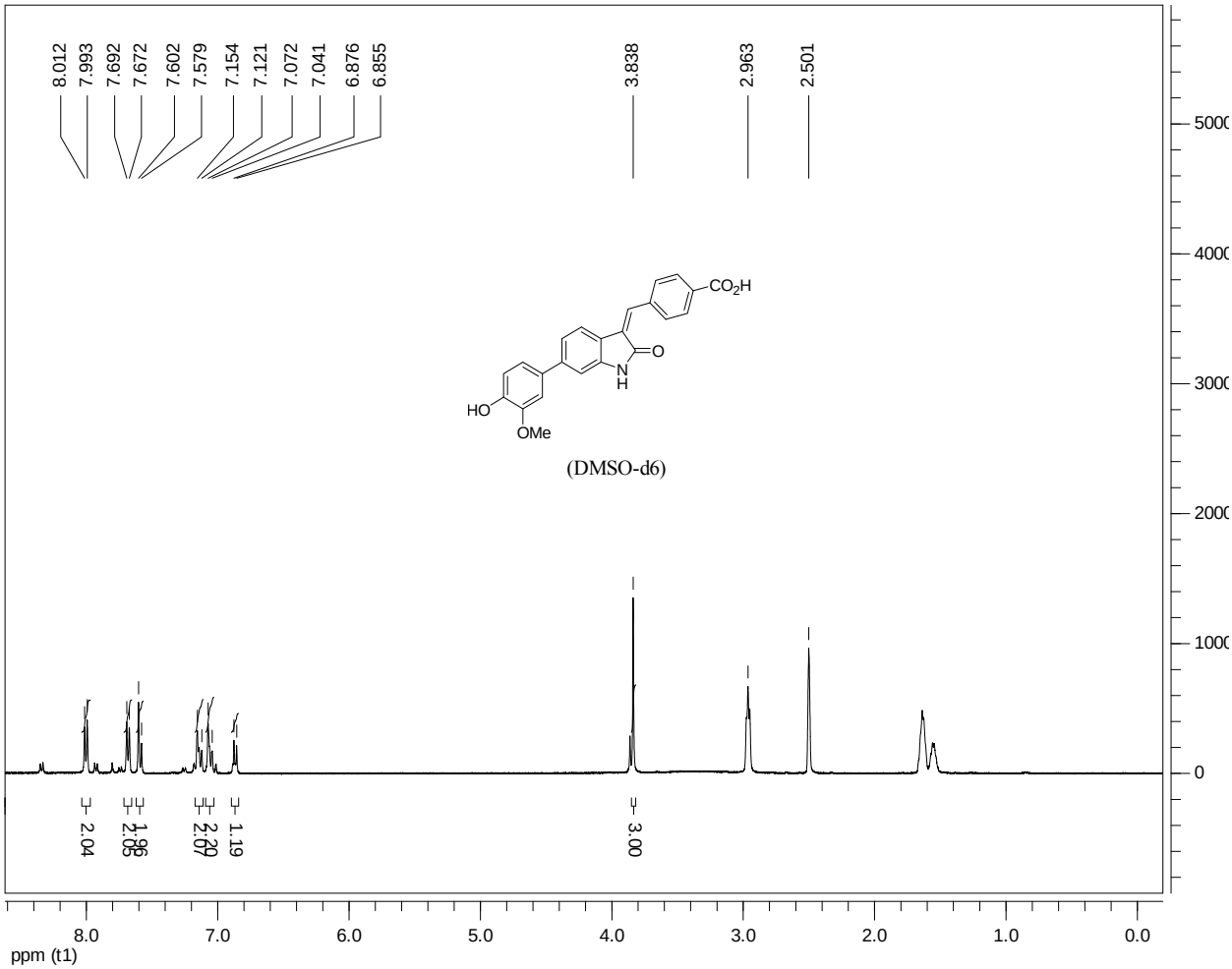
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (1H, s), 7.81 (1H, s), 7.77-7.71 (2H, m), 7.51-7.45 (2H, m), 7.38 (1H, s), 7.33 (1H, d, *J* = 8 Hz), 7.11 (1H, s), 6.86 (1H, s), 6.38 (1H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 323.19.



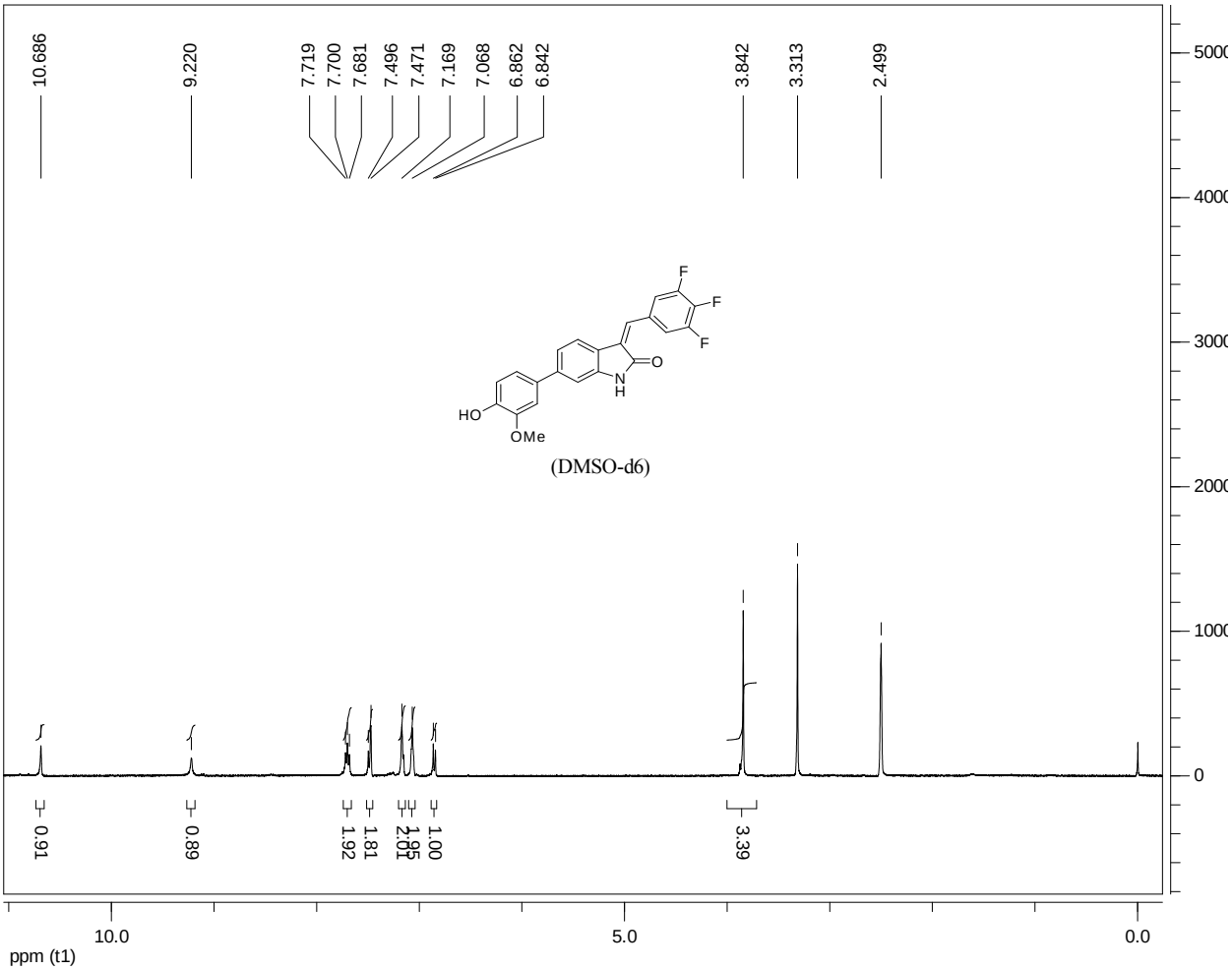
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.9 (1H, s), 7.73 (1H, s), 7.66 (1H, d, *J* = 8 Hz), 7.54 (2H, d, *J* = 8.4 Hz), 7.35 (1H, s), 7.26 (1H, d, *J* = 8.8 Hz), 7.06 (1H,s), 7.02 (2H, d, *J* = 8 Hz), 6.83 (1H, s), 6.36 (1H, s), 3.76 (4 H, s), 3.16 (4H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 372.57.

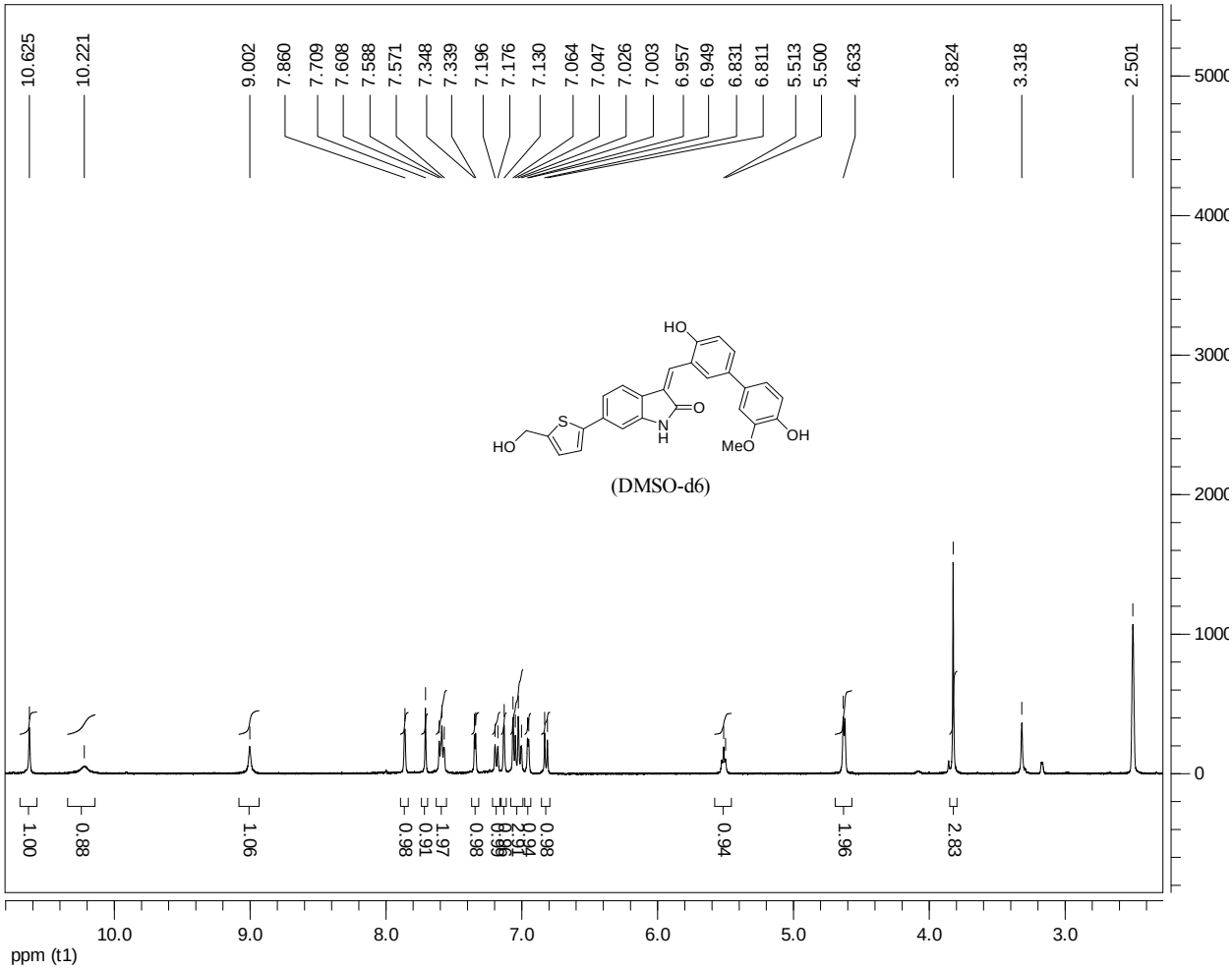


^{1}H NMR (400 MHz, DMSO-d_6) δ 8.0 (2H, d, *J* = 7.6 Hz), 7.68 (2H, d, *J* = 8 Hz), 7.6 (1H, s), 7.59 (1H, d, *J* = 8.4 Hz), 7.15-7.12 (2H, m), 7.07 (1H, s), 7.06 (1H, d, *J* = 8.8 Hz), 6.87 (1H, d, *J* = 8.4 Hz), 3.84 (3H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 388.5.

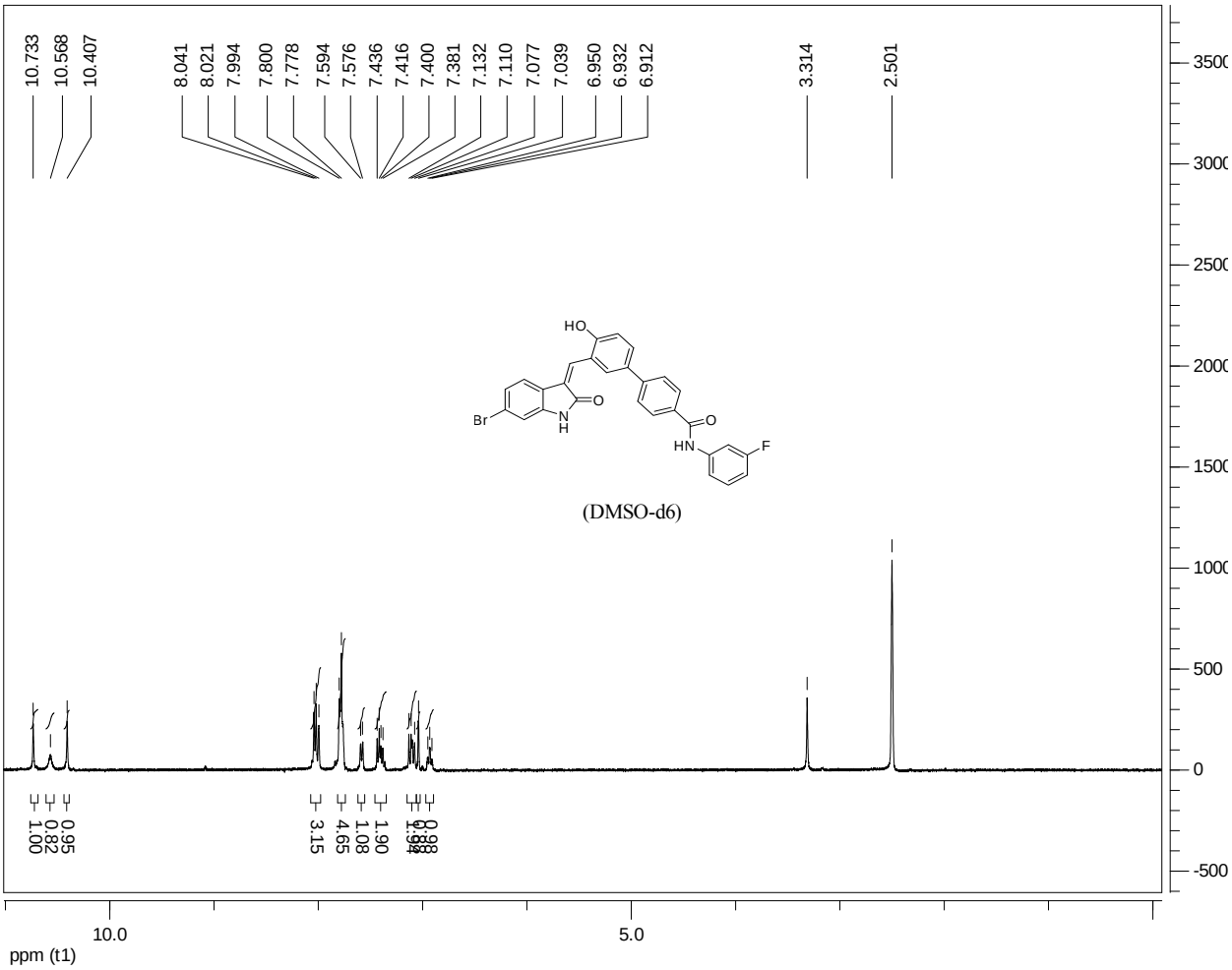


^{1}H NMR (400 MHz, DMSO-d_6) δ 10.7 (1H, s), 9.22 (1H, s), 7.7 (2H, t, *J* = 7.6 Hz), 7.49 (1H, d, *J* = 8.4 Hz), 7.47 (1H, s), 7.17 (2H, m), 7.07 (2H, m), 6.85 (1H, d, *J* = 8 Hz), 3.84 (3H, s) ppm.
LRMS (ESI) *m/z* $[M+H]^+$ = 398.61.



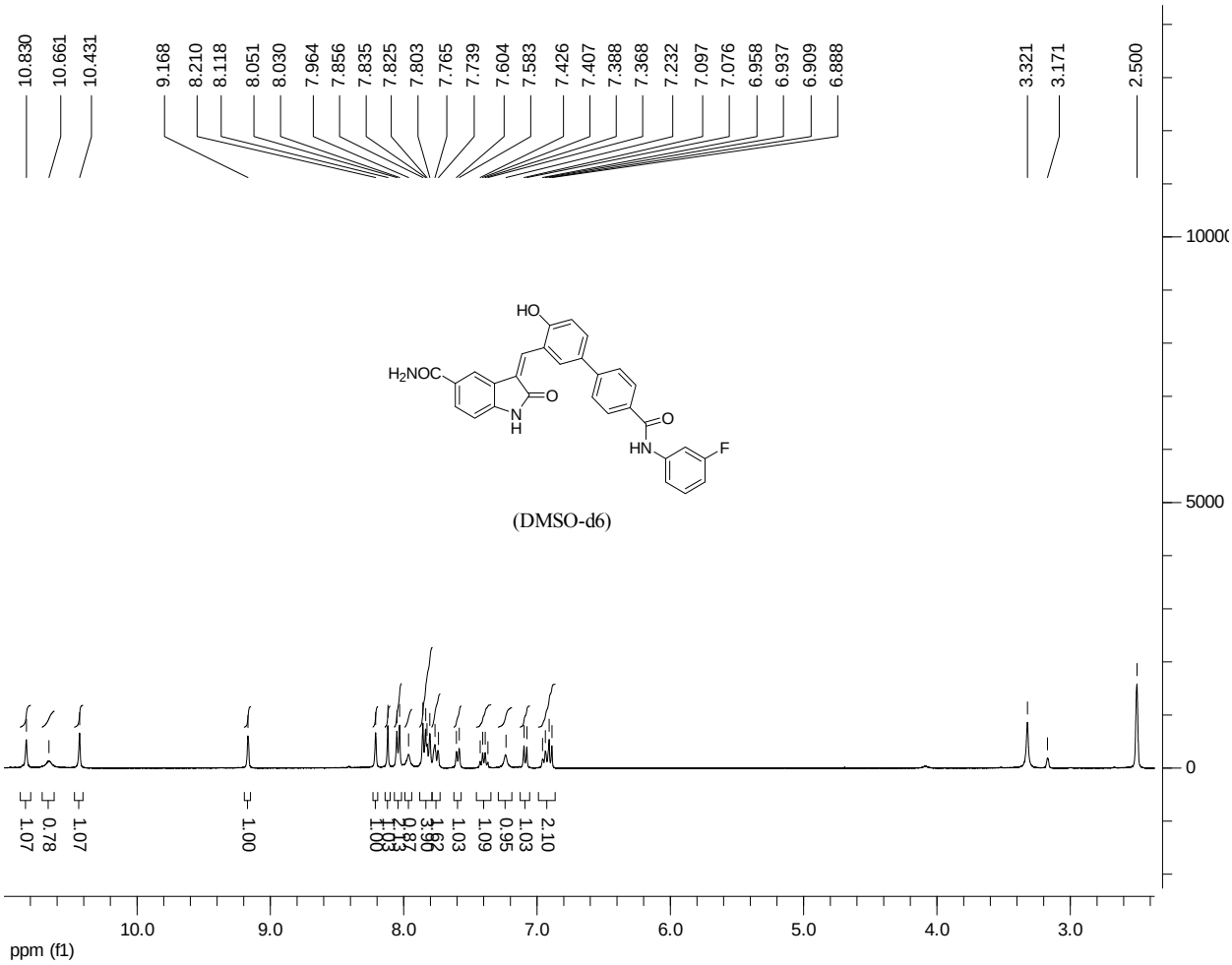
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.6 (1H, s), 10.2 (1H, br s), 9.0 (1H, s), 7.86 91H, s), 7.7 (1H, s), 7.6 (1H, d, *J* = 8 Hz), 7.58 (1H, d, *J* = 6.8 Hz), 7.35 (1H, d, *J* = 3.6 Hz), 7.19 (1H, d, *J* = 8 Hz), 7.13 (1H, s), 7.06 (1H, d, *J* = 6.8 Hz), 7.04 (1H, d, *J* = 8.4 Hz), 7.01 (1H, d, *J* = 8.4 Hz), 6.96 (1H, d, *J* = 3.2 Hz), 6.82 (1H, d, *J* = 8 Hz), 5.5 (1H, t, *J* = 5.2 Hz), 4.63 (2H, d, *J* = 5.2 Hz), 3.82 (3H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 472.23.

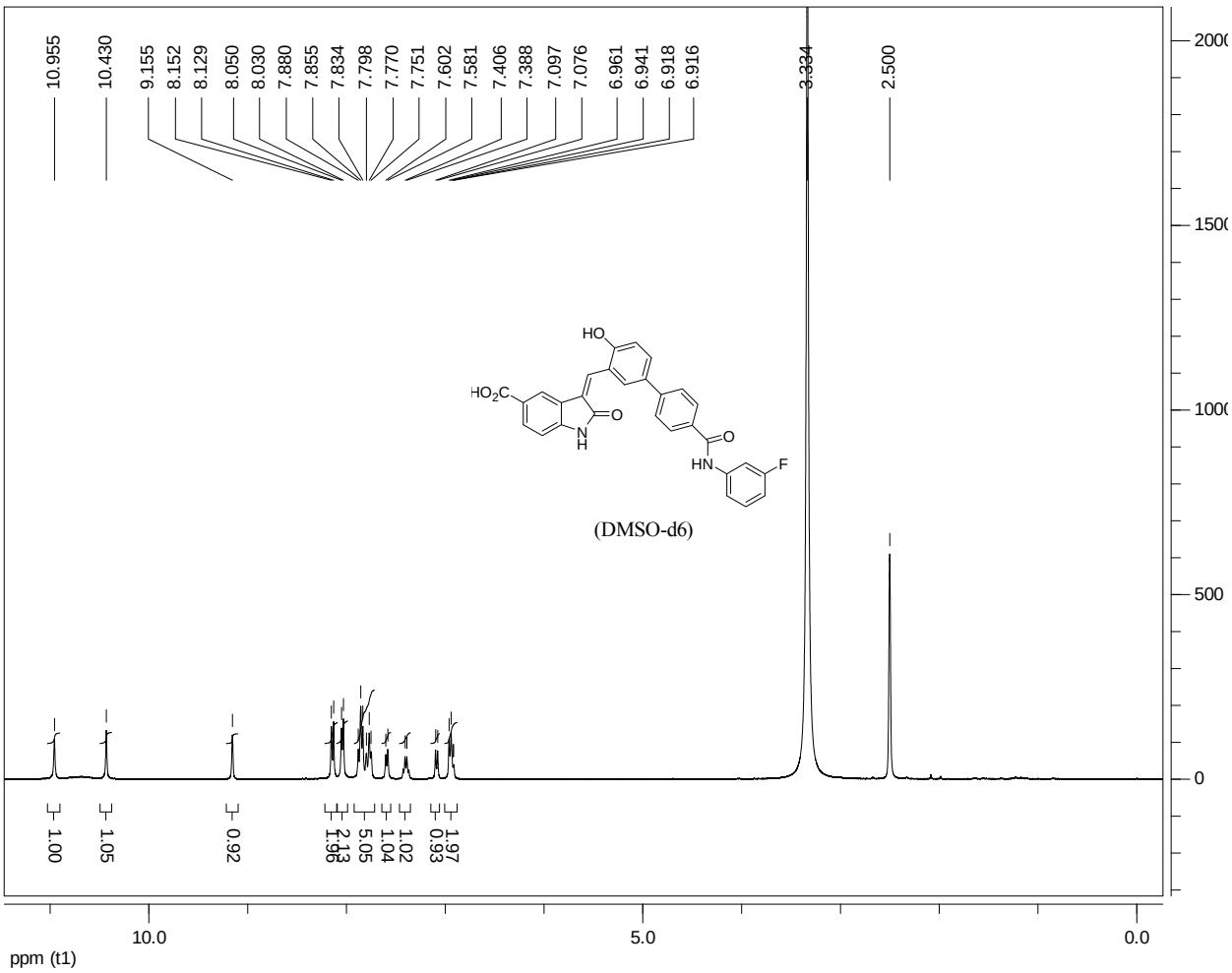


^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (1H, s), 10.57 (1H, br s), 10.4 (1H, s), 8.04-7.99 (3H, m), 7.8-7.77 (4H, m), 7. 58 (1H, d, *J* = 7.2 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.39 (1H, d, *J* = 7.6 Hz), 7.12 (1H, d, *J* = 8.8 Hz), 7.09 (1H, d, *J* = 8 Hz), 7.04 (1H, s), 6.93 (1H, t, *J* = 8.8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 528.01/530.05 (bromine pattern).

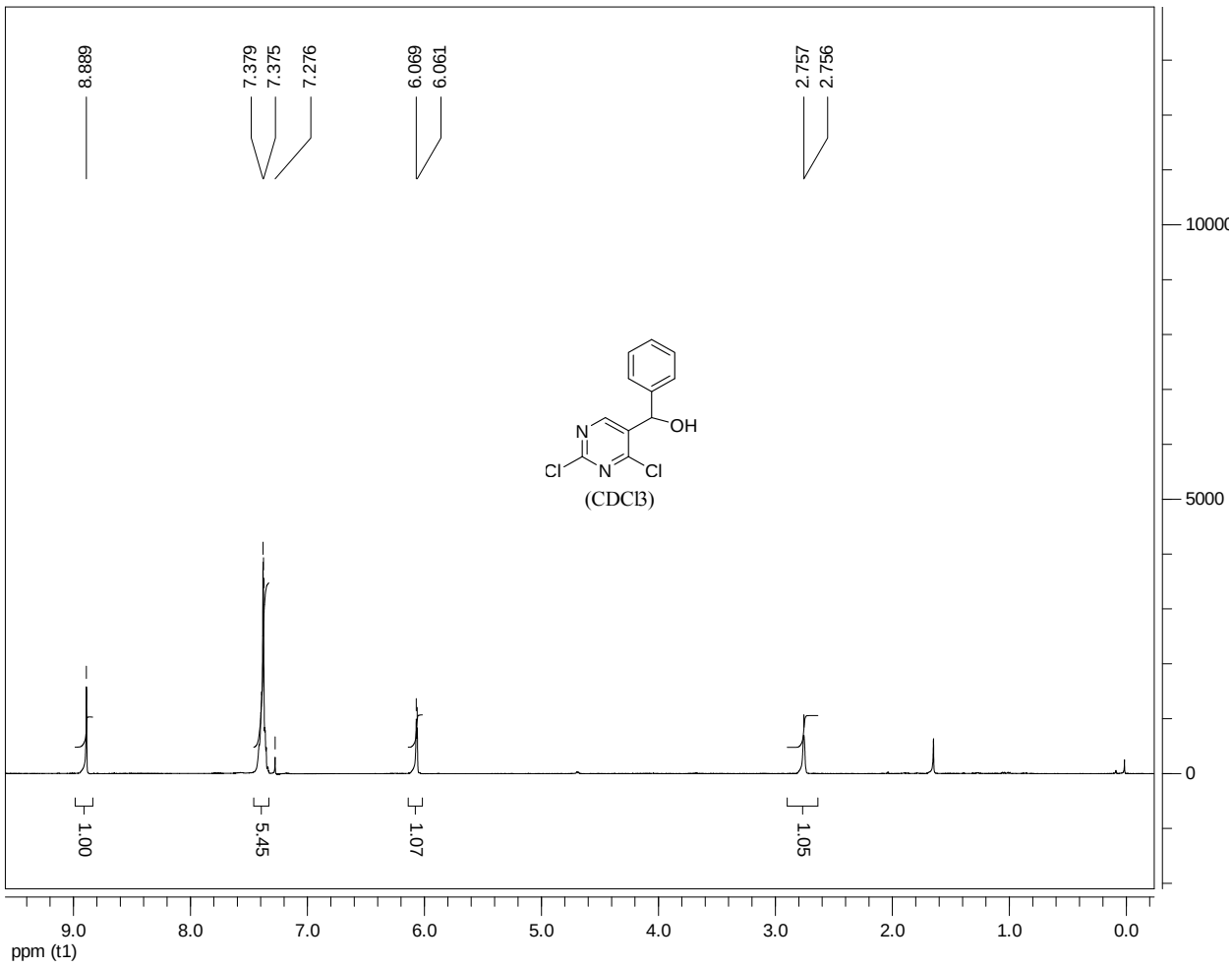


^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (1H, s), 10.66 (1H, br s), 10.43 (1H, s), 9.17 (1H, s), 8.21 (1H, s), 8.12 (1H, s), 8.04 (2H, d, *J* = 8.4 Hz), 7.96 (1H, br s), 7.85 (2H, d, *J* = 8.4 Hz), 7.81 (1H, d, *J* = 8.4 Hz), 7.8 (1H, s), 7.75 (1H, d, *J* = 8.4 Hz), 7.59 (1H, d, *J* = 8.4 Hz), 7.4 (1H, q, *J* = 8 Hz), 7.23 (1H, br s), 7.09 (1H, d, *J* = 8.4 Hz), 6.95 (1H, d, *J* = 8.4 Hz), 6.9 (1H, d, *J* = 8.4 Hz) ppm.
LRMS (ESI) *m/z* $[M+H]^+$ = 494. 14



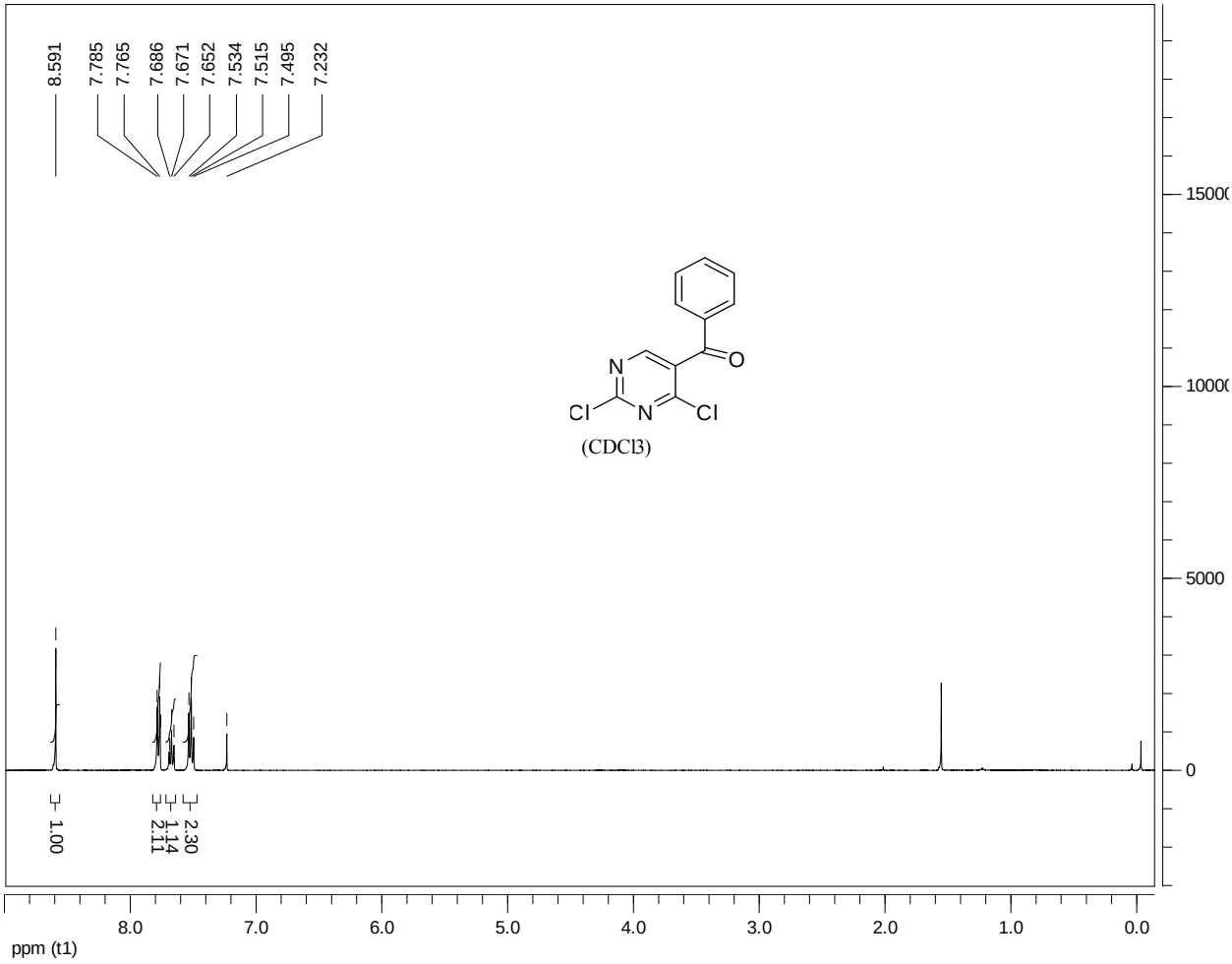
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (1H, s), 10.43 (1H, s), 9.16 (1H, s), 8.14 (2H, d, *J* = 9.2 Hz), 8.04 (2H, d, *J* = 8 Hz), 7.87 (1H, d, *J* = 10 Hz), 7.86 (1H, s), 7.84 (1H, d, *J* = 8.4 Hz), 7.8-7.75 (2H, m), 7.59 (1H, d, *J* = 8.4 Hz), 7.39 (1H, q, *J* = 7.2 Hz), 7.04 (1H, d, *J* = 8.4 Hz), 6.94-6.92 (2H, m) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 495.3



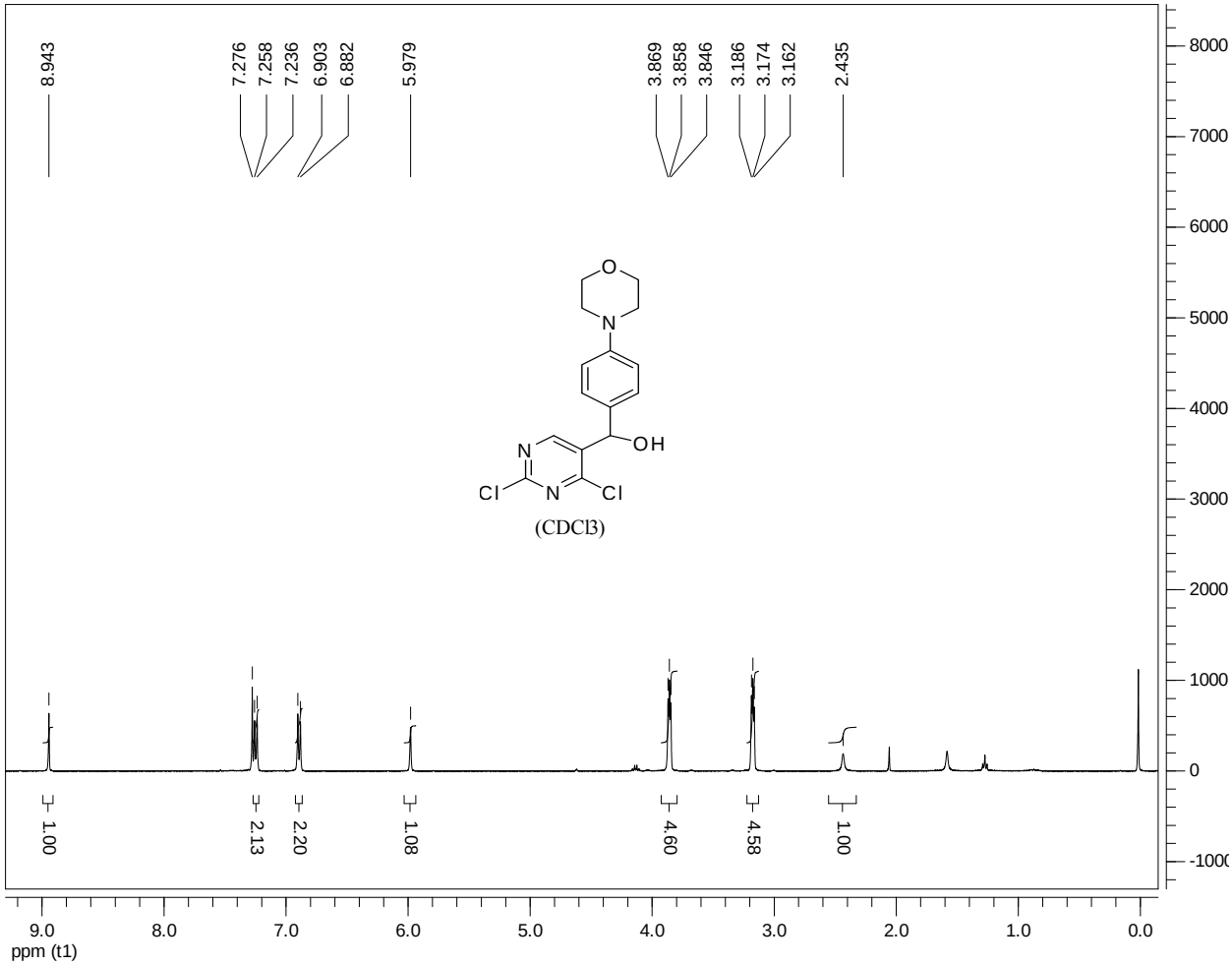
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (1H, s), 7.38 (5H, s), 6.07 (1H, s), 2.76 (1H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 256.27



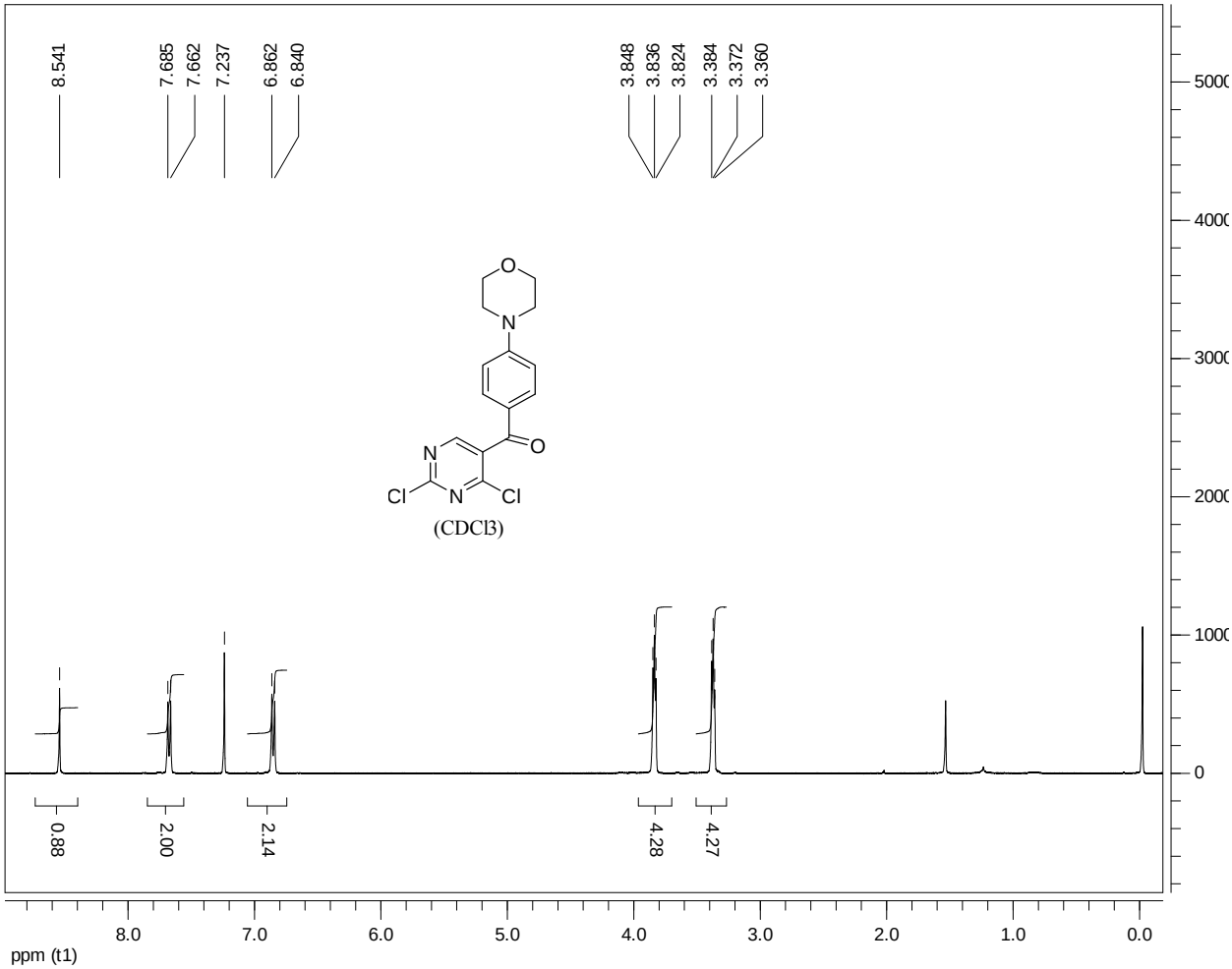
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (1H, s), 7.78 (2H, d, *J* = 8 Hz), 7.67 (1H, t, *J* = 7.6 Hz), 7.52 (2H, t, *J* = 8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 255.27



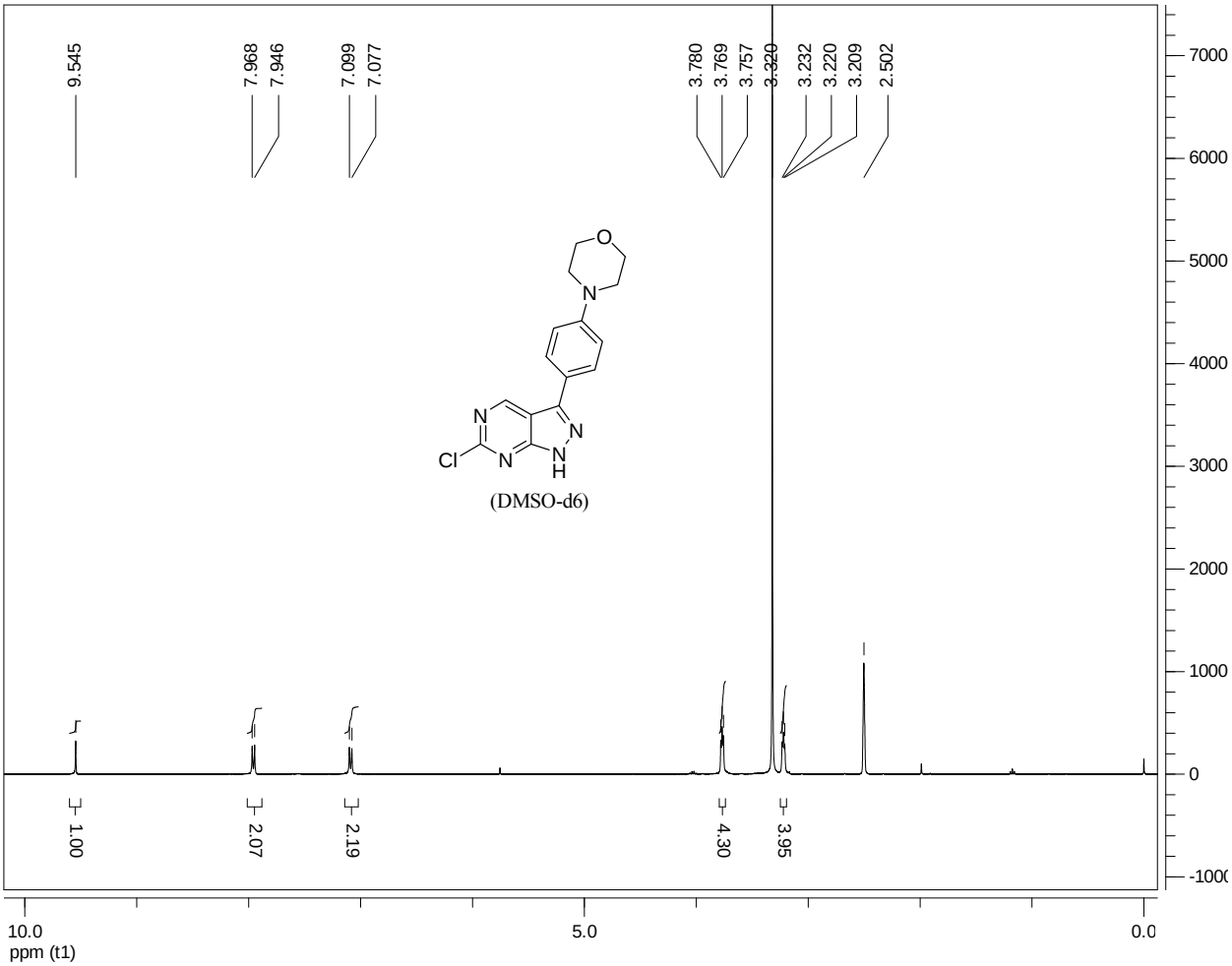
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (1H, s), 7.25 (2H, d, *J* = 8.8 Hz), 6.89 (2H, d, *J* = 8.4 Hz), 3.86 (4H, t, *J* = 4.8 Hz), 3.17 (4H, t, *J* = 4.8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 337.55



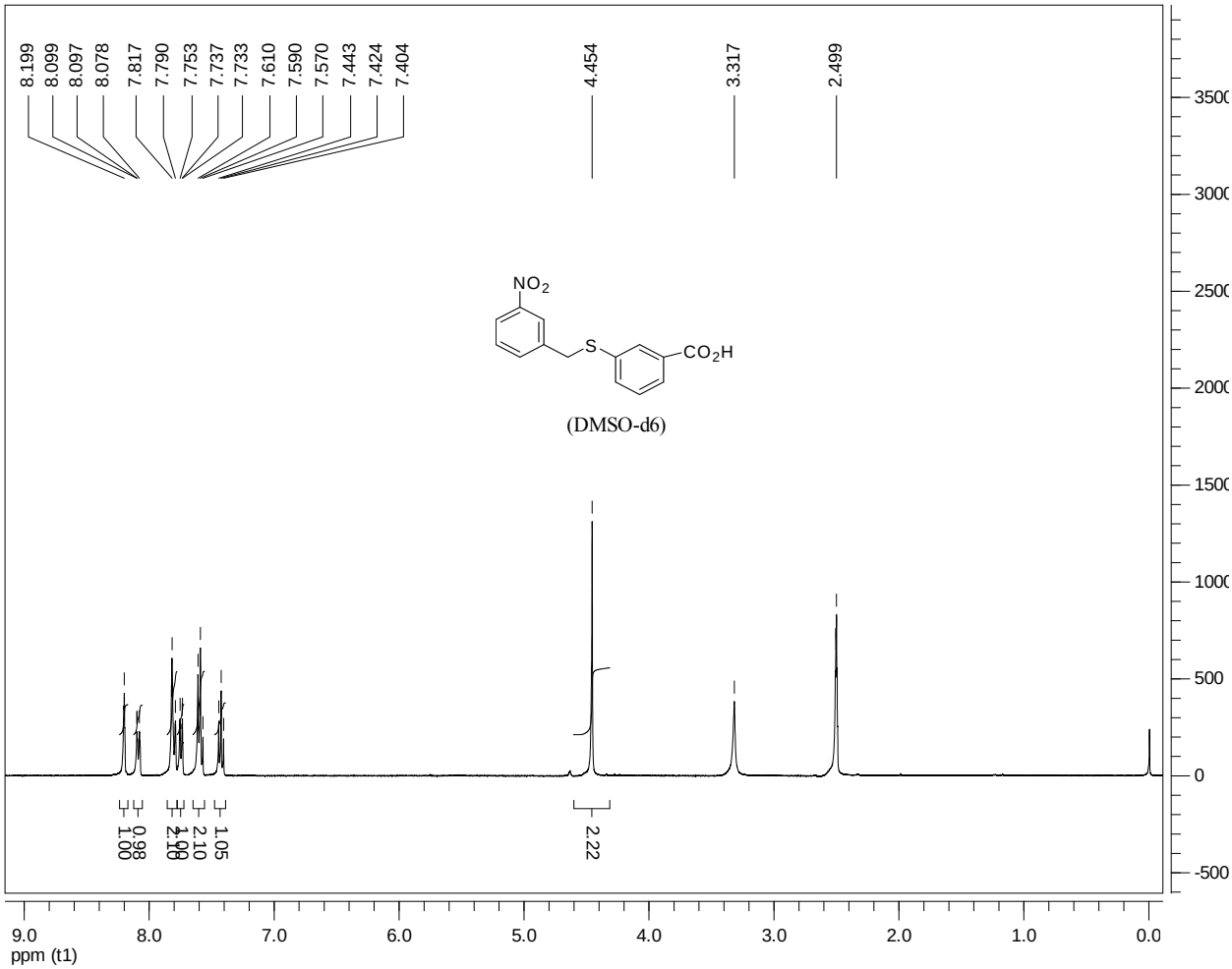
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (1H, s), 7.67 (2H, d, *J* = 9.2 Hz), 6.85 (2H, d, *J* = 8.8 Hz), 3.84 (4H, t, *J* = 4.8 Hz), 3.37 (4H, t, *J* = 4.8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 339.61



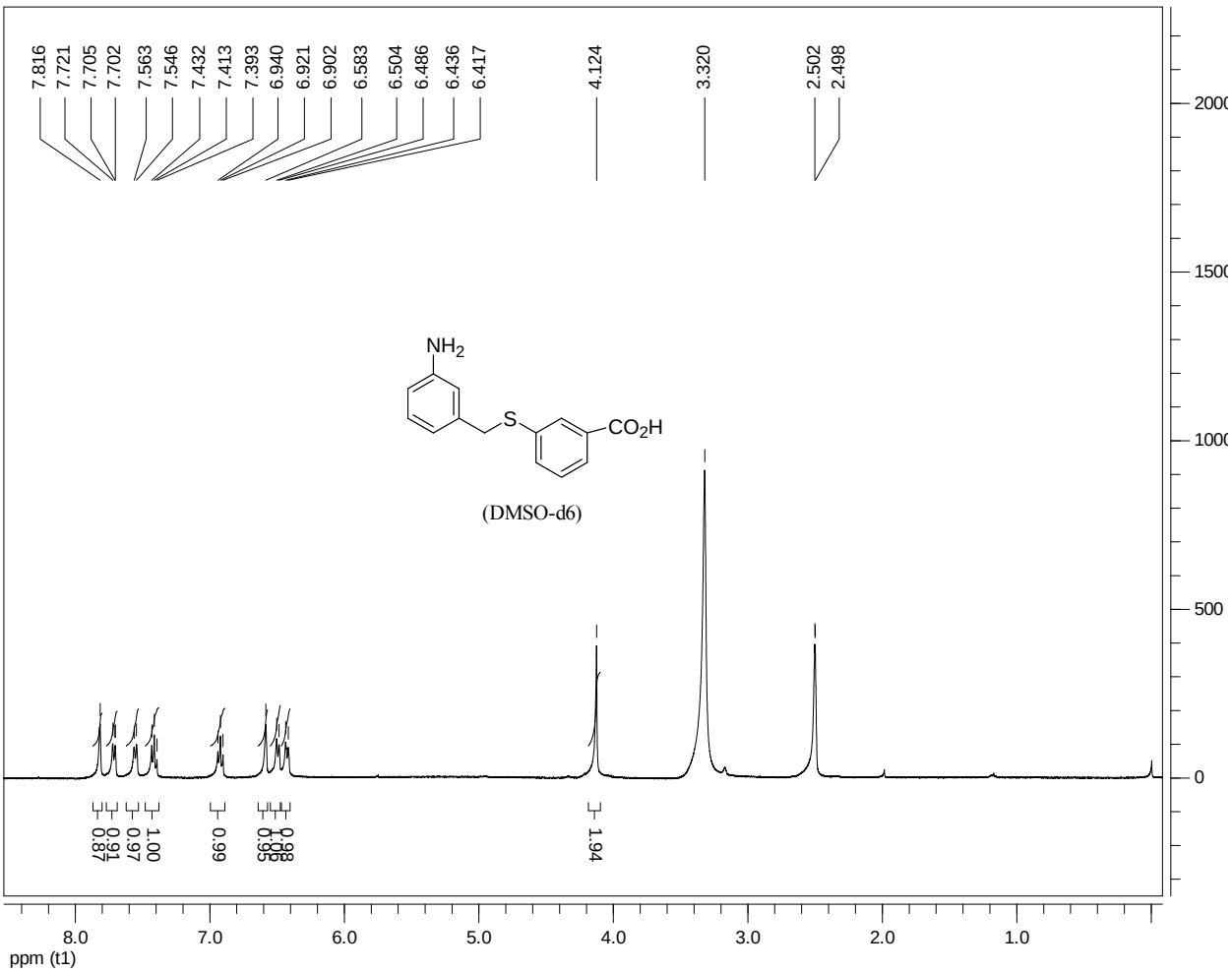
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.5 (1H, s), 7.96 (2H, d, *J* = 8.8 Hz), 7.09 (2H, d, *J* = 8.8 Hz), 3.77 (4H, t, *J* = 4.8 Hz), 3.22 (4H, t, *J* = 4.8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 316.81



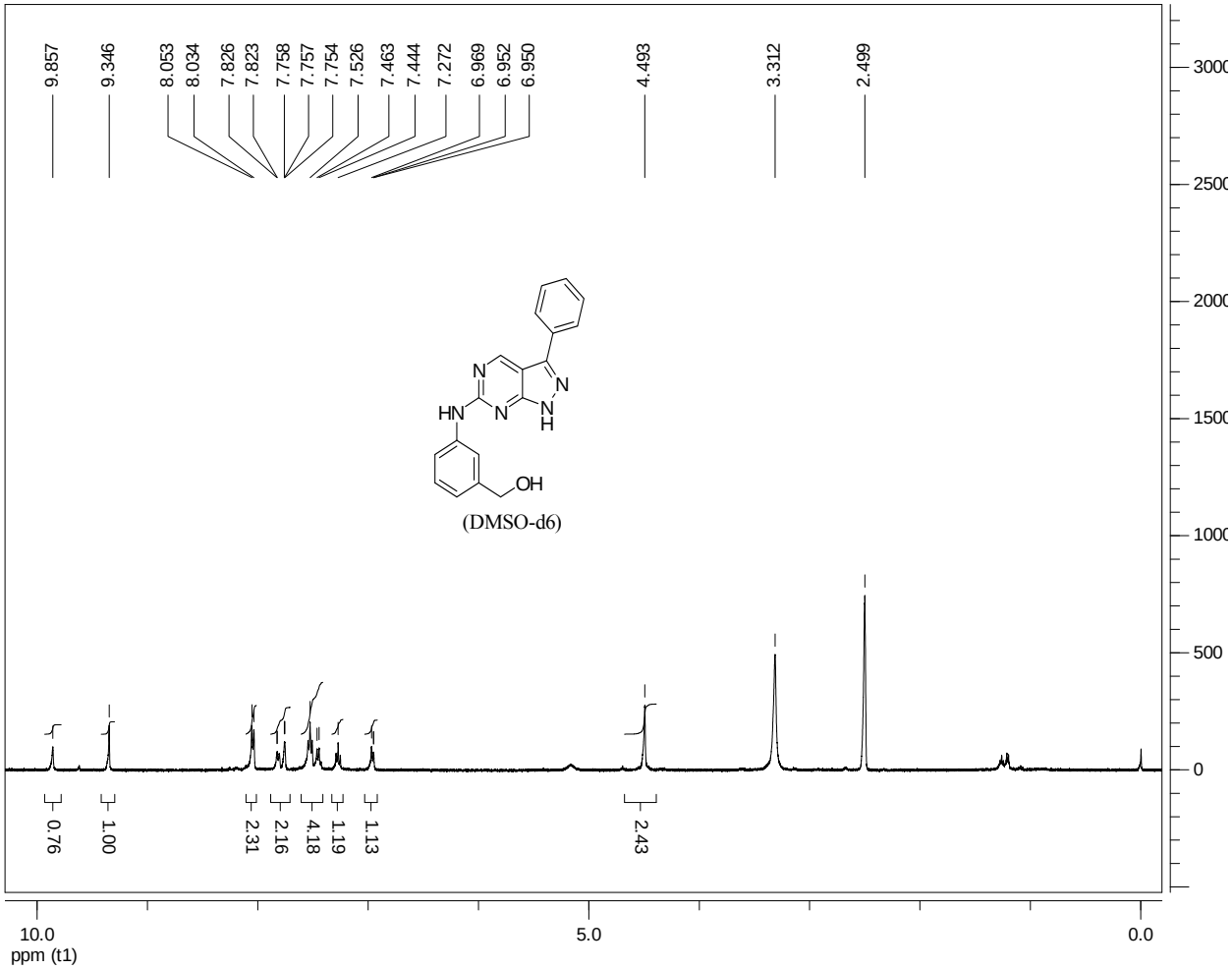
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.2 (1H, s), 8.09 (1H, d, *J* = 8.4 Hz), 7.82 (1H, s), 7.8 (1H, d, *J* = 8.4 Hz), 7.74 (1H, d, *J* = 8 Hz), 7.6 (1H, d, *J* = 8 Hz), 7.59 (1H, t, *J* = 8 Hz), 7.42 (1H, t, *J* = 8 Hz), 4.45 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 290.22



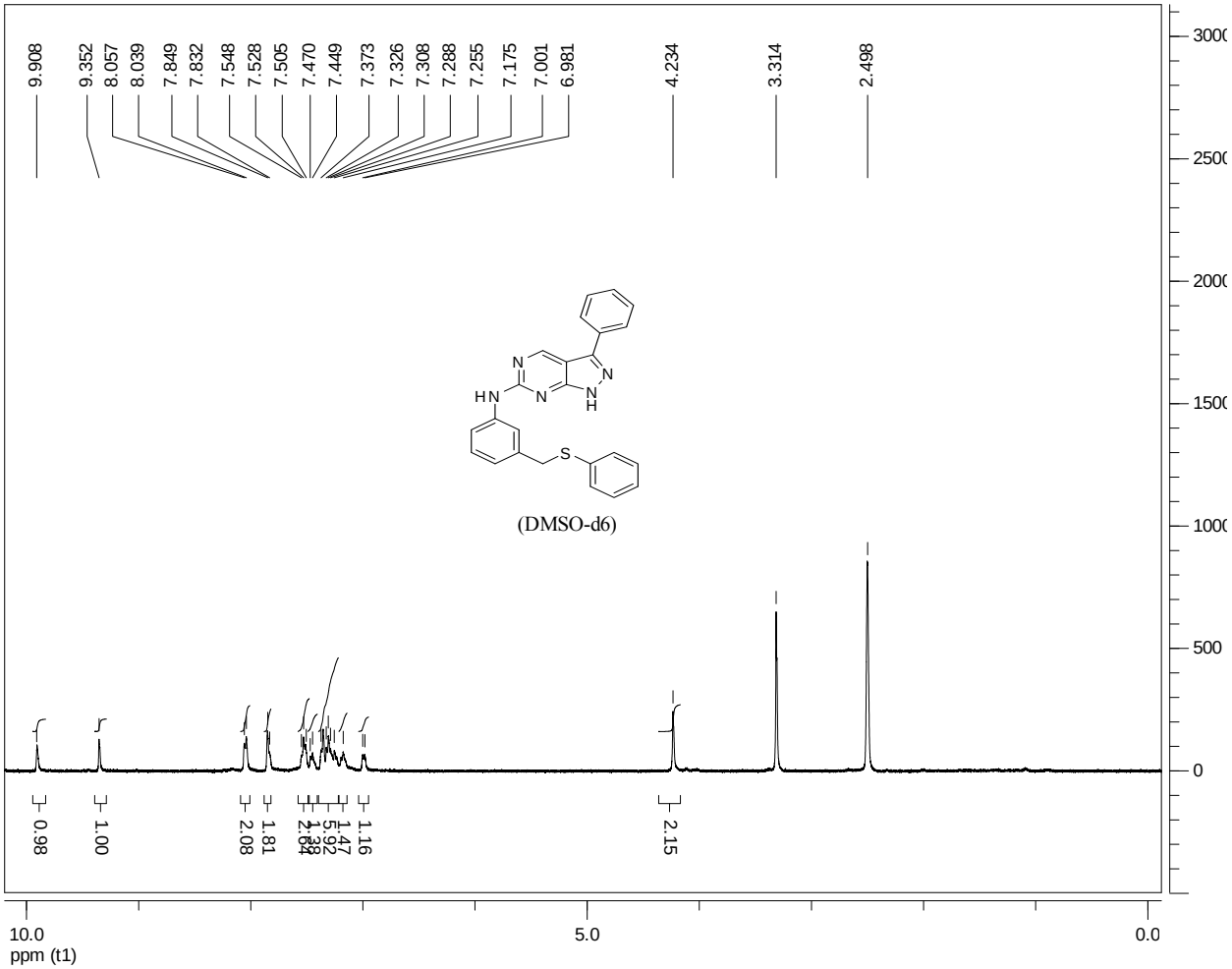
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (1H, s), 7.71 (1H, d, *J* = 7.6 Hz), 7.55 (1H, d, *J* = 6.8 Hz), 7.41 (1H, t, *J* = 8 Hz), 6.92 (1H, t, *J* = 7.6 Hz), 6.58 (1H, s), 6.5 (1H, d, *J* = 7.2 Hz), 6.43 (1H, d, *J* = 7.6 Hz), 4.12 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 260.05



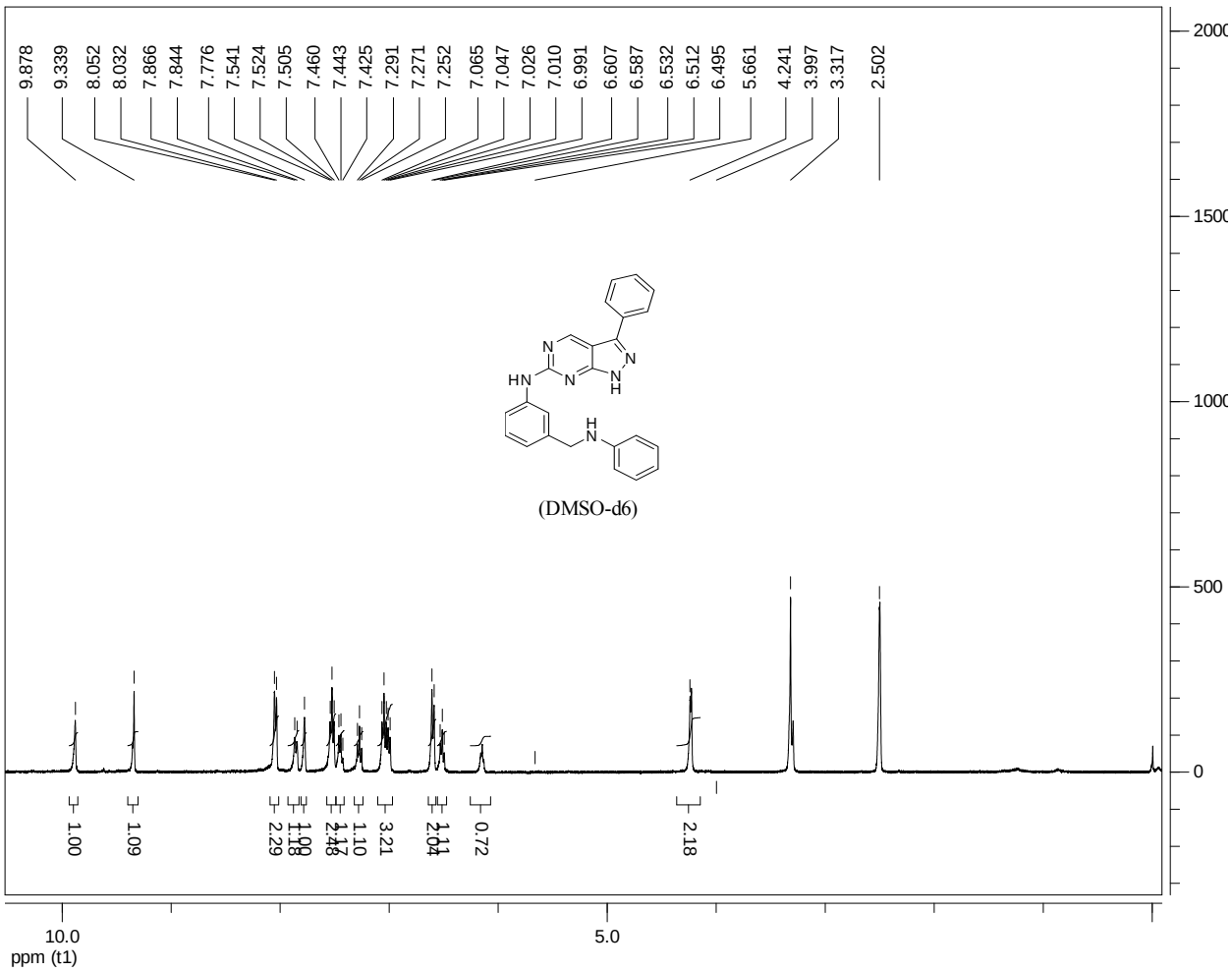
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (1H, s), 9.35 (1H, s), 8.04 (2H, d, *J* = 7.6 Hz), 7.82 (1H, d, *J* = 8 Hz), 7.76 (1H, s), 7.54-7.5 (3H, m), 7.44 (1H, t, *J* = 7.6 Hz), 7.27 (1H, t, *J* = 7.6 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 4.49 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 318.49



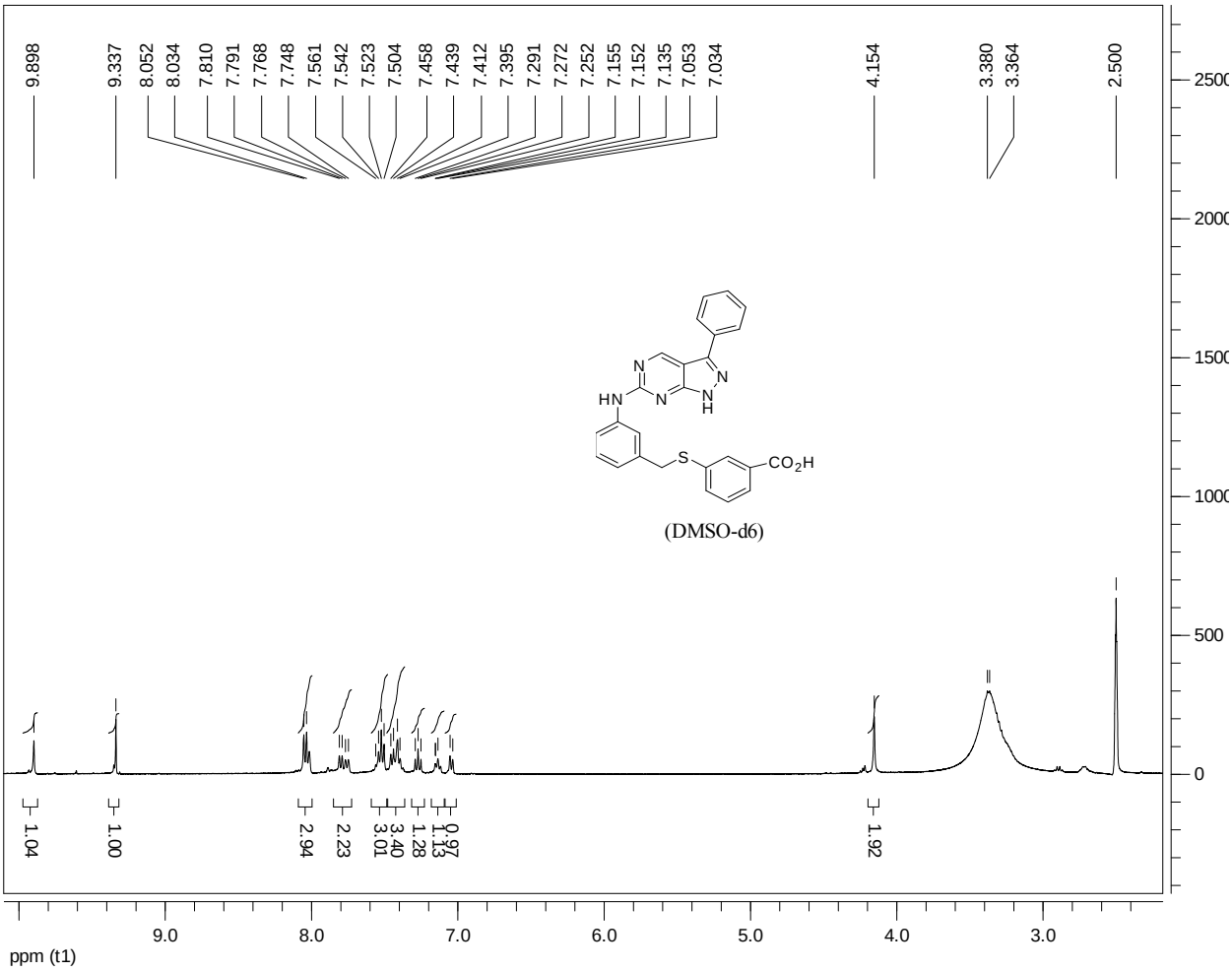
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.9 (1H, s), 9.35 (1H, s), 8.05 (2H, d, *J* = 7.2 Hz), 7.84 (2H, d, *J* = 6.8 Hz), 7.53 (2H, t, *J* = 8 Hz), 7.46 (1H, d, *J* = 8.4 Hz), 7.37-7.26 (6 H, m), 7.18 (1H, t, *J* = 7 Hz), 7.0 (1H, d, *J* = 8 Hz), 4.23 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 410.4



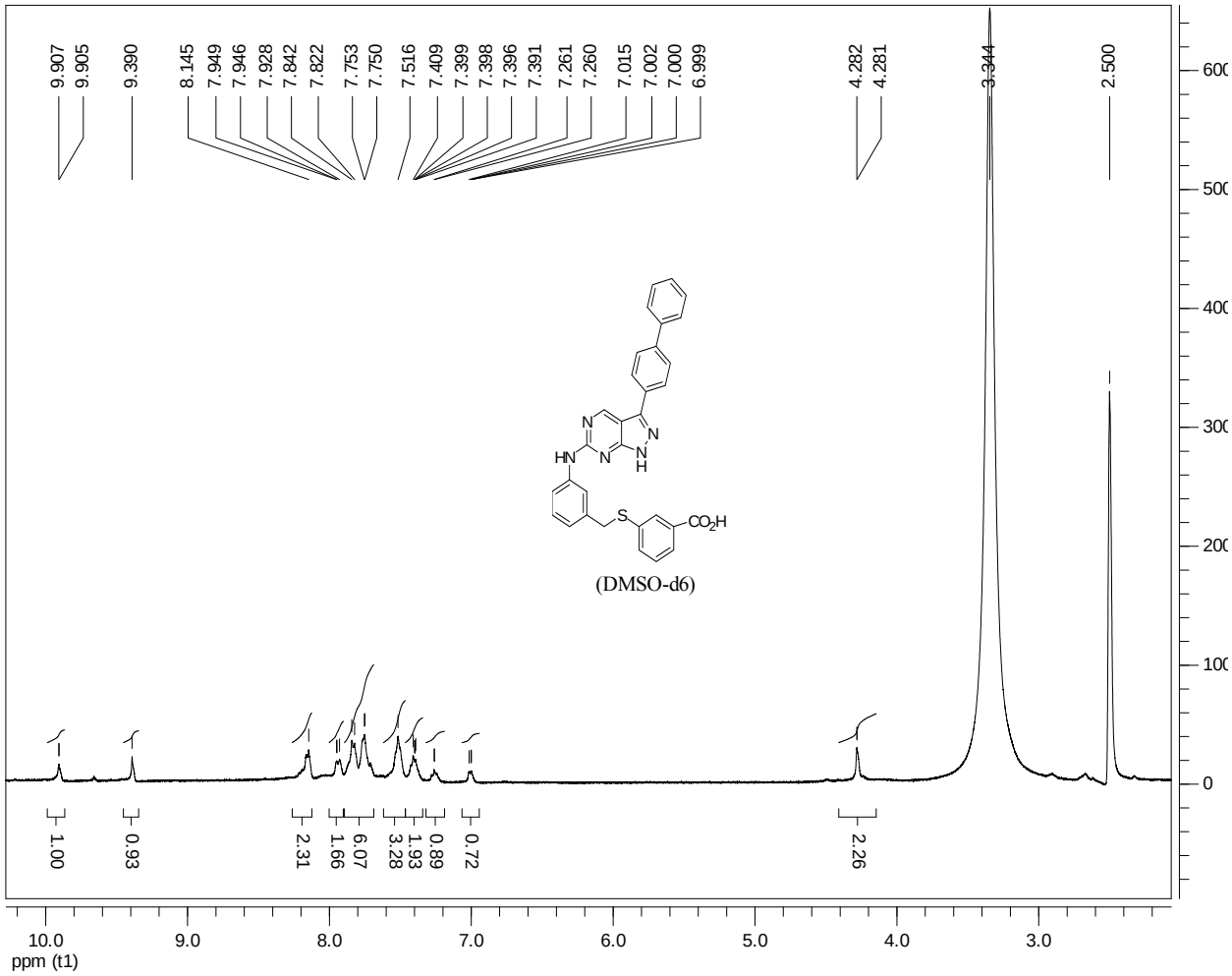
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (1H, s), 9.34 (1H, s), 8.04 (2H, d, *J* = 8 Hz), 7.86 (1H, d, *J* = 8.8 Hz), 7.78 ((1H, s), 7.52 (2H, t, *J* = 7.6 Hz), 7.44 (1H, t, *J* = 7.2 Hz), 7.27 (1H, t, *J* = 8 Hz), 7.06-6.99 (3H, m), 6.6 (2H, d, *J* = 8 Hz), 6.51 (1H, t, *J* = 8 Hz), 5.66 (1H, t, *J* = 7.6 Hz), 4,24 (2H, d, *J* = 5.6 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 393.71



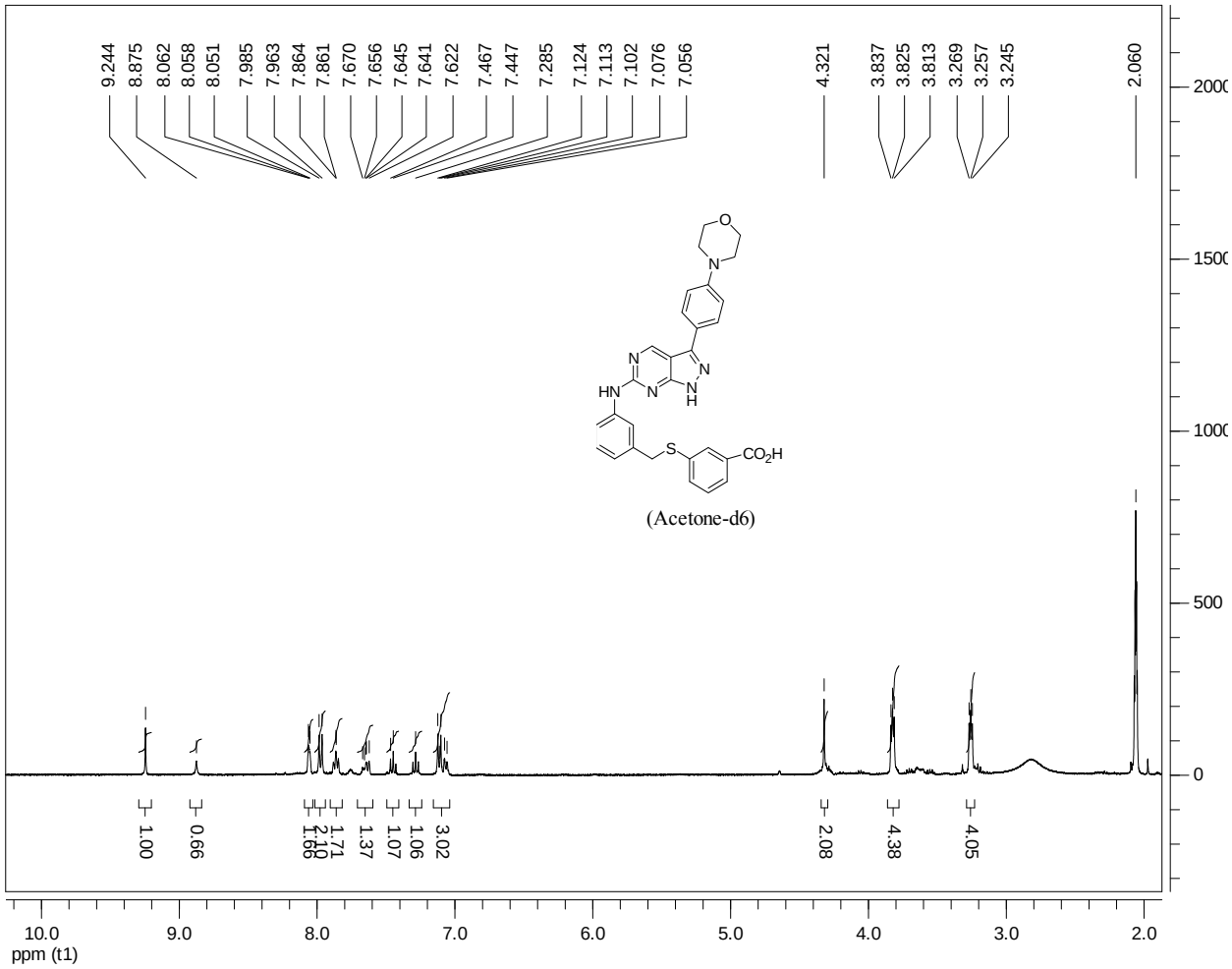
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.9 (1H, s), 9.34 (1H, s), 8.05-8.01 (2H, m), 7.8 (1H, d, *J* = 7.6 Hz), 7.76 (1H, d, *J* = 8 Hz), 7.56-7.5 (3H, m), 7.46-7.4 (3H, m), 7.27 (1H, t, *J* = 8 Hz), 7.13 (1H, t, *J* = 6.8 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 4.15 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 454.16



^{1}H NMR (400 MHz, DMSO-d_6) δ 9.9 (1H, s), 9.4 (1H, s), 8.28-8.15 (2H, m), 7.94 (2H, d, *J* = 8.4 Hz), 7.9-7.7 (6H, m), 7.6-7.48 (3H, m), 7.4-7.34 (2H, m), 7.26 (1H, t, *J* = 7.6 Hz), 7.0 (1H, d, *J* = 7.6 Hz), 4.28 (2H, s) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 530.23



^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (1H, s), 8.88 (1H, s), 8.05 (2H, s), 7.98 (2H, d, *J* = 8.8 Hz), 7.86 (2H, t, *J* = 8 Hz), 7.63 (1H, d, *J* = 7.6 Hz), 7.44 (1H, t, *J* = 8 Hz), 7.29 (1H, t, *J* = 8 Hz), 7.11 (2H, d, *J* = 8.8 Hz), 7.07 (1H, d, *J* = 8 Hz), 4.32 (2H, s), 3.83 (4H, t, *J* = 4.8 Hz), 3.26 (4H, t, *J* = 4.8 Hz) ppm.

LRMS (ESI) *m/z* $[M+H]^+$ = 539.7