Supporting Information

New Quinazolinone Alkaloids within Rare Amino Acid Residue from Coral-Associated Fungus, *Aspergillus versicolor* LCJ-5-4

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List of Supporting Information

Experimental details ……..S3

The 18S rRNA sequences data of *Aspergillus versicolor* LCJ-5-4……....S9

Figure S1. The ^{1}H-NMR spectrum of cottoquinazoline B (**1**) in DMSO-d_6…….S10

Figure S2. The ^{13}C-NMR spectrum of cottoquinazoline B (**1**) in DMSO-d_6……..S11

Figure S3. The HMQC spectrum of cottoquinazoline B (**1**) in DMSO-d_6……S12

Figure S4. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline B (**1**) in DMSO-d_6……...S13

Figure S5. The HMBC spectrum of cottoquinazoline B (**1**) in DMSO-d_6……S14

Figure S6. The ^{1}H-NMR spectrum of cottoquinazoline C (**2**) in DMSO-d_6…….S15

Figure S7. The ^{13}C-NMR spectrum of cottoquinazoline C (**2**) in DMSO-d_6……S16

Figure S8. The HMQC spectrum of cottoquinazoline C (**2**) in DMSO-d_6……S17

Figure S9. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline C (**2**) in DMSO-d_6……...S18

Figure S10. The HMBC spectrum of cottoquinazoline C (**2**) in DMSO-d_6……S19

Figure S11. The ^{1}H-NMR spectrum of cottoquinazoline D (**3**) in DMSO-d_6…….S20

Figure S12. The ^{13}C-NMR spectrum of cottoquinazoline D (**3**) in DMSO-d_6……S21

Figure S13. The HMQC spectrum of cottoquinazoline D (**3**) in DMSO-d_6……S22

Figure S14. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline D (**3**) in DMSO-d_6……...S23

Figure S15. The HMBC spectrum of cottoquinazoline D (**3**) in DMSO-d_6……S24

Figure S16. HPLC profiles of acidic hydrolysates of **1** co-elution with D/L-Ala on chiral Crownpak CR (+) column……...……...……S25

Figure S17. HPLC profiles of acidic hydrolysates of **2** co-elution with D/L-Ile on chiral Crownpak CR (+) column……..……..……S26

FigureS18. HPLC profiles of acidic hydrolysates of **3** co-elution with 1-amino cyclopropane-1-carboxylic acid on chiral Crownpak CR (+) column…….……...S27

Experimental details

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. Melting points were obtained on a YANACO micro melting point apparatus. UV spectra were recorded on a Beckman DU® 640 spectrophotometer. IR spectra were recorded on a NICOLET NEXUS 470 spectrophotometer using KBr discs. ^{1}H, ^{13}C NMR, DEPT, and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ-values. ESIMS were measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. X-ray diffraction intensity data were collected with a MAC DIP-2030K diffractometer using graphite-monochromater Mo-Kα radiation (λ = 0.71073 Å) by the ω-scan technique [scan width 0–180°, $2\theta \leq 50°$]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10–40 μm) and over silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory, Qingdao, China). Semiprepartive HPLC was performed using an ODS column [Shin-pak ODS (H), 20×250 mm, 5 μm, 4 mL/min]. Amino acids were analyzed by a chiral HPLC column [Crownpak CR(+) (Daicel Chemical, Japan), 4×150 mm, 5 μm]. D/L-Ala, D/L-Ile and 1-aminocyclopropane-1- carboxylic acid were purchased from TCI Tokyo Chemical Industry Co., Ltd.

Fungal Material. *A. versicolor* LCJ-5-4 was isolated from the soft coral *Cladiella* sp. collected from Lingao in Hainan province, China. It was identified according to its morphological characteristics and analyses of 18S rRNA sequences (GenBank GU227343).

The voucher specimen is deposited in our laboratory at –80˚C. The producing strain was prepared on Potato Dextrose agar slants at 3.3% salt concentration and stored at 4˚C.

Fermentation and Extraction. *A. versicolor* LCJ-5-4 was grown under static conditions at 20 ^{o}C for 30 days in three hundred 1000 mL conical flasks containing liquid medium (300 mL/flask) composed of sorbitol (20 g/L), maltose (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), tryptophane (0.5g/L), yeast extract (3 g/L), and sea salt (33.3g/L), after adjusting its pH to 6.5. The fermented whole broth (about 90 L) was filtered through cheesecloth to separate into filtrate and mycelia. The filtrate was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with EtOAc to give an EtOAc solution, while the mycelia was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (140 g).

Purification. The extract (140 g), was separated into eight fractions on a silica gel vacuum liquid chromatograph (VLC) using step gradient elution with petroleum-$CHCl_3$ ether (0–100%) and then with MeOH-$CHCl_3$ (0–50%). Fraction 6 from the 50:1 of $CHCl_3$/MeOH eluent was further separated into 3 subfractions (Fr.6-1~6-3) on a silica gel VLC using step gradient elution with MeOH-$CHCl_3$ (0–50%). Fr.6-2 was subjected to silica gel VLC using step gradient elution with petroleum-acetone (0–100%) to afford ten subfractions (Fr.6-2-1~6-2-10). Fr.6-2-10 was further purified by semipreparative HPLC (40% acetonitrile/H_2O, 4.0 mL/min) to yield compounds **1** (5 mg) and **3** (10 mg). Fraction 7 from

the 30:1 of $CHCl_3$/MeOH was further separated into five subfractions (Fr.7-1~7-5) on a silica gel VLC using step gradient elution with MeOH-$CHCl_3$ (0–50%). Fr.7-5 was further purified by Sephadex LH-20, eluting with MeOH-$CHCl_3$ (1:1), and semipreparative HPLC (60% MeOH/H_2O, 4.0 mL/min) to yield **2** (5 mg).

Cottoquinazoline B (**1**): colorless prisms (MeOH), mp 273 °C (dec.); $[\alpha]_D^{25}$ +83 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.40), 223 (4.24), 265 (3.26), 303 (2.66), 313 (2.46) nm; IR (KBr) ν_{max} 3443, 3219, 2926, 2853, 1729, 1692, 1670, 1607, 1468, 1405, 1328, 1246, 1158, 1120, 1088, 773, 750 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 430.1526 $[M+H]^+$ (calcd. for $C_{23}H_{19}N_5O_4$: 430.1515).

Cottoquinazoline C (**2**): colorless prisms (MeOH), mp 263 °C (dec.); $[\alpha]_D^{25}$ +31 (*c* 1.0, MeOH), UV (MeOH) λ_{max} (log ϵ) 208 (4.52), 227 (4.42), 268 (3.52), 305(2.73), 317 (2.57) nm; IR (KBr) ν_{max} 3283, 2958, 2925, 2854, 1687, 1609, 1470, 1411, 1368, 1248, 1174, 1149, 1121, 1080, 1018, 776, 698 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 472.1984 $[M+H]^+$ (calcd. for $C_{26}H_{26}N_5O_4$: 472.1985).

Cottoquinazoline D (**3**): colorless prisms (MeOH), mp 270 °C (dec.); $[\alpha]_D^{25}$ +78 (*c* 0.2, MeOH), UV (MeOH) λ_{max} (log ϵ) 210 (4.91), 226 (4.72), 260 (4.17), 304 (2.97), 317 (2.75) nm; IR (KBr) ν_{max} 3443, 3007, 2957, 2926, 2853, 1690, 1609, 1478, 1433, 1411, 1366, 1312, 1248, 1181, 1097, 1060, 773, 755, 697 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 442.1501 $[M+H]^+$ (calcd. for $C_{24}H_{20}N_5O_4$: 442.1515).

Amino acids analyses of acidic hydrolysates of 1–3.[12,13] Each of compounds **1–3** (1 mg) was dissolved in 1 mL of 6N HCl and heated in a sealed tube at 110 °C for 12 h. The

hydrolysates were dried and reconstituted in 1 mL of H_2O. Each of the hydrolysates was then analyzed by chiral HPLC over Crownpak CR(+) column for alanine, isoleucine, 1-aminocyclopropane-1-carboxylic acid (flow rate 0.5 mL/min; solvent, aqueous $HClO_4$ pH = 1.5); detection, 201 nm; temperature 0 °C), respectively. The retention times of these authentic amino acids were as follows: D/L-Ala, t_R 3.5/4.4 min; D/L-Ile, t_R 11.2/13.4 min; 1-aminocyclopropane-1-carboxylic acid, t_R 3.5 min. The retention times of amino acids in hydrolysates of **1**–**3** were 3.5, 13.4, 3.5 min, respectively. Co-injection of the authentic samples with the hydrolysates confirmed that the amino acid residues in compounds **1**–**3** were D-Ala, L-Ile, and 1-aminocyclopropane-1-carboxylic acid, respectively (Fig. S16, S17, S18).

X-ray data of cottoquinazoline B (1). $C_{23}H_{19}N_5O_4$; Mr = 429.43; orthorhombic, space group $P2_12_12_1$, a = 7.5985 (8) Å, b = 14.3869 (16) Å, c = 17.7639 (18) Å, α = 90°, β = 90°, γ =90°, V = 1941.9(4) Å^3, Z = 4, D_{calc} = 1.469 g/cm^3, λ = 0.71073 Å, μ (Mo Kα) = 0.104 mm^{-1}, F (000) = 896 , T = 298(2) K. Of the 10208 reflections that were collected, 3405 were unique (Rint = 0.0967). The structure was solved by direct methods with SHELXL-97 and refined by full-matrix least-squares on F^2. Final refinement: data/restraints/parameters = 340/0/290; R_1 = 0.1208 (all data), wR_2 = 0.0962 (all data). Absolute structure parameter = 2.2 (18) and GOF = 1.049. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.220 and -0.193 e$^-$/Å^3, respectively. Crystallographic data (including structure factors) for compound **1** (CCDC805549) reported in this paper have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, CB2 1EZ, UK [fax: +44-0-1223-336033 or

e-mail: deposit@ccdc.cam.ac.uk].

X-ray data of cottoquinazoline D (3). $C_{24}H_{19}N_5O_4$; *Mr* = 441.44 ; orthorhombic, space group $P2_12_12_1$, *a* = 14.4532(14) Å, *b* = 18.7969 (18) Å, *c* = 7.2401(6) Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, V = 1967.0(3) $Å^3$, *Z* = 4, D_{calc} = 1.491 g/cm^3, λ = 0.71073 Å, μ (Mo Kα) = 0.105 mm^{-1}, *F* (000) = 920 , *T* = 298(2) K. Of the 10042 reflections that were collected, 2014 were unique (Rint = 0.0960). The structure was solved by direct methods with SHELXL-97 and refined by full-matrix least-squares on F^2. Final refinement: data/restraints/parameters = 2014/0/298; R_1 = 0.0835 (all data), wR_2 = 0.0814 (all data). Absolute structure parameter = 10 (10) and GOF = 1.028. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.168 and -0.184 $e^-/Å^3$, respectively. Crystallographic data (including structure factors) for compound **3** (CCDC805550) reported in this paper have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, CB2 1EZ, UK [fax: +44-0-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Cytotoxic Assays. Cytotoxicity was assayed by the MTT.[14] In the MTT assay, P388 and Hela cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cell suspension, 200 μL, at a density of 5×10^4 cell mL^{-1} was plated in 96-well microtiter plates and incubated for 24 h. Then, 2 μL of the test solutions (in MeOH) were added to each well and further incubated for 72 h. The MTT solution (20 μL, 5 mg/mL in IPMI-1640 medium) was then added to each well and incubated for 4 h. Old medium containing MTT (150 μL) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a

Spectra Max Plus plate reader at 540 nm. 5-FU (Fluorouracil) was used as the positive control.

Antimicrobial Assays. The antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans* were evaluated by an agar dilution method.[15] The tested strains were cultivated in LB agar plates for bacteria and in YPD agar plates for *Candida albicans* at 37 °C. Compounds **1**–**3** and positive controls were dissolved in 5% DMSO-H_2O at different concentrations from 100 to 0.05 μg/mL by the continuous 2-fold dilution methods. A 5 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no microbial growth could be observed. Ciprofloxacin lactate and ketoconazole was used as positive control for bacteria and *Candida albicans* with MIC values of 29.66, 3.71, 0.93, 7.42, 1.86 and 0.18 μM, respectively.

The 18S rRNA sequence data of *Aspergillus versicolor* LCJ-5-4

TGTCTAGTATAAGCAATCTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTA
TCGTTTATTTGATAGTACCTTACTACATGGATACCTGTGGTAATTCTAGAGCTAAT
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CTACTGCGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTA
GGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACT
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AGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTG
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GCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACAC
TGACAGGGCCAGCGAGTACATCACCTTGGCCGAGAGGCCCGGGTAATCTTGTTA
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CCTAGTAGGCACGAGTCATCAGCTCGTGCCGATTACGTCCCTGCCCTTTGTACAC
ACCGCCCGTCGCTACTACCGATTGAATGGCTCGGTGAGGCCTTCGGACTGGCGC
AGGAGGGTTGGCAACGACCCCCCCGCGCCGGAAAGTTGGTCAAACCCGGTCAT
TAGAGGAAGTAAAAA

Figure S1. The ^{1}H-NMR spectrum of cottoquinazoline B (**1**) in DMSO-d_6

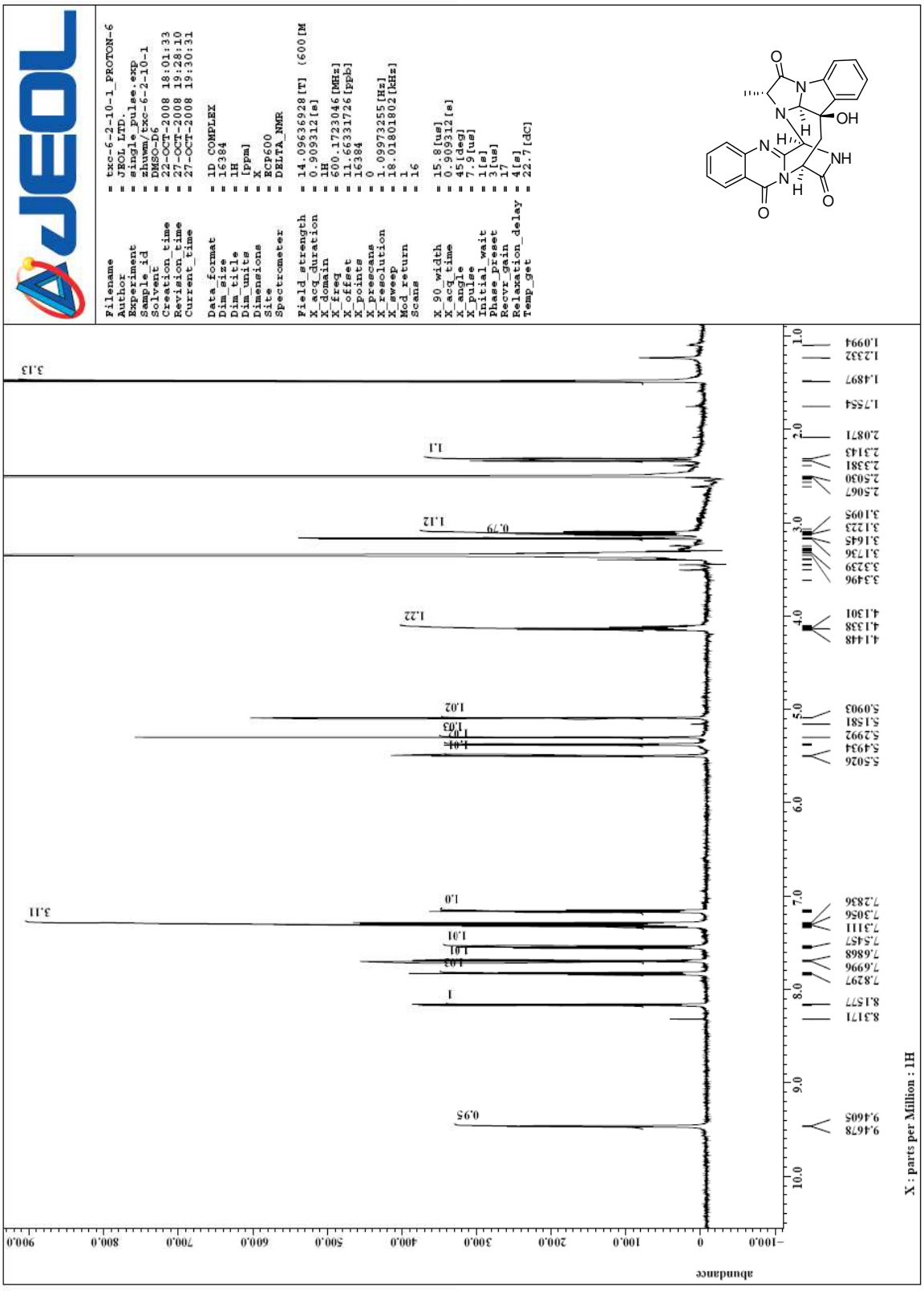

Figure S2. The ^{13}C-NMR spectrum of cottoquinazoline B (**1**) in DMSO-d_6

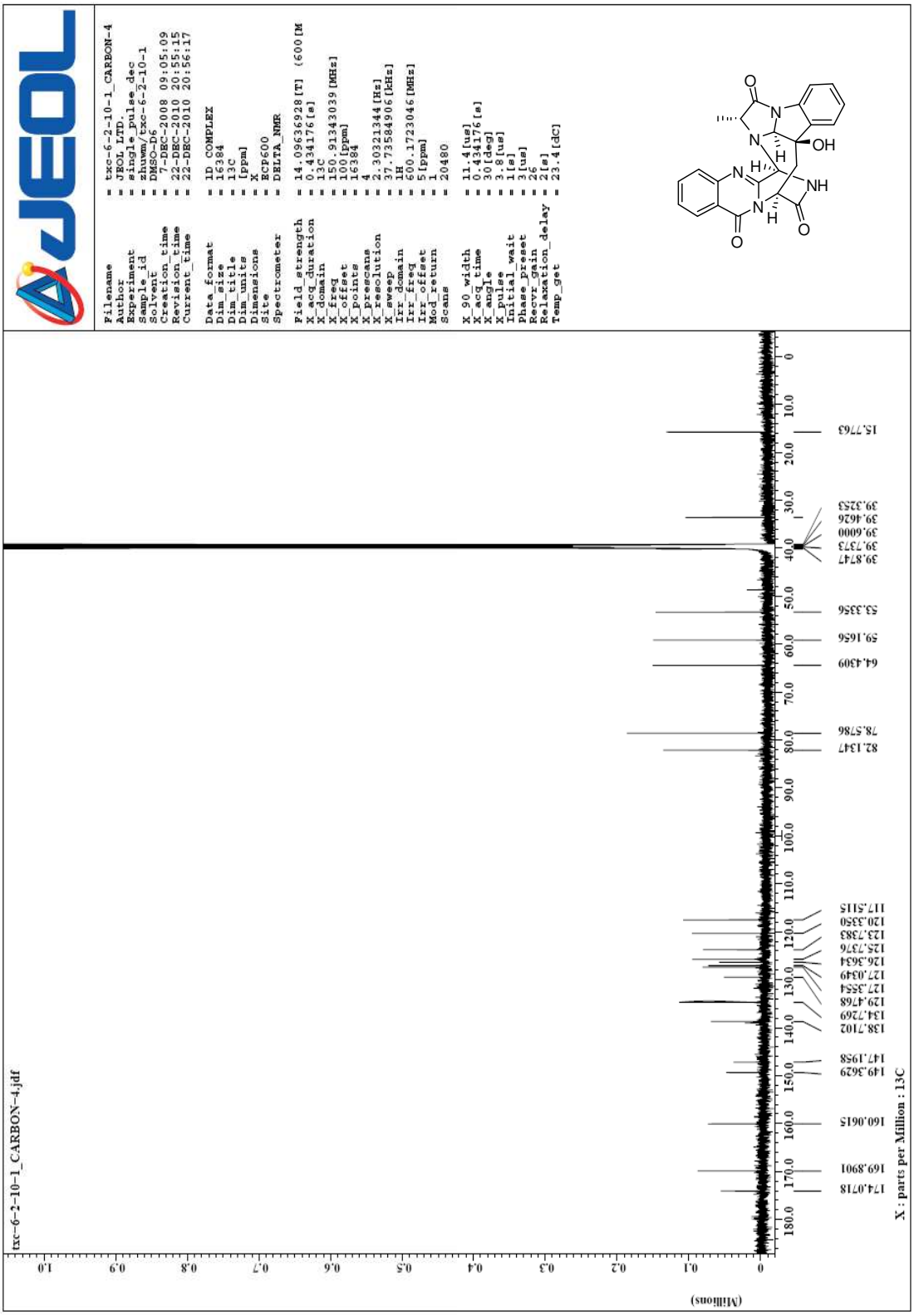

Figure S3. The HMQC spectrum of cottoquinazoline B (**1**) in DMSO-d_6

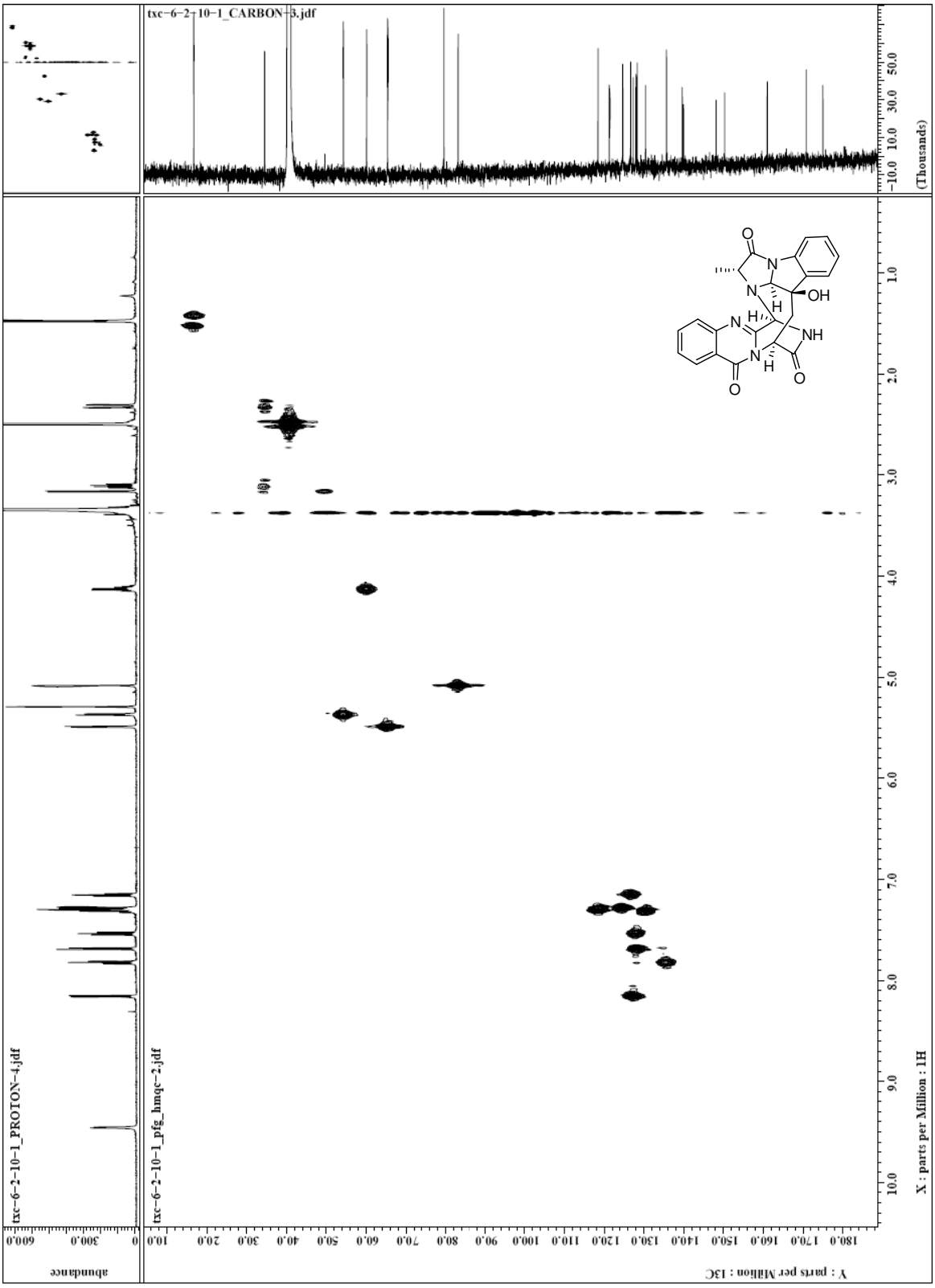

Figure S4. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline B (**1**) in DMSO-d_6

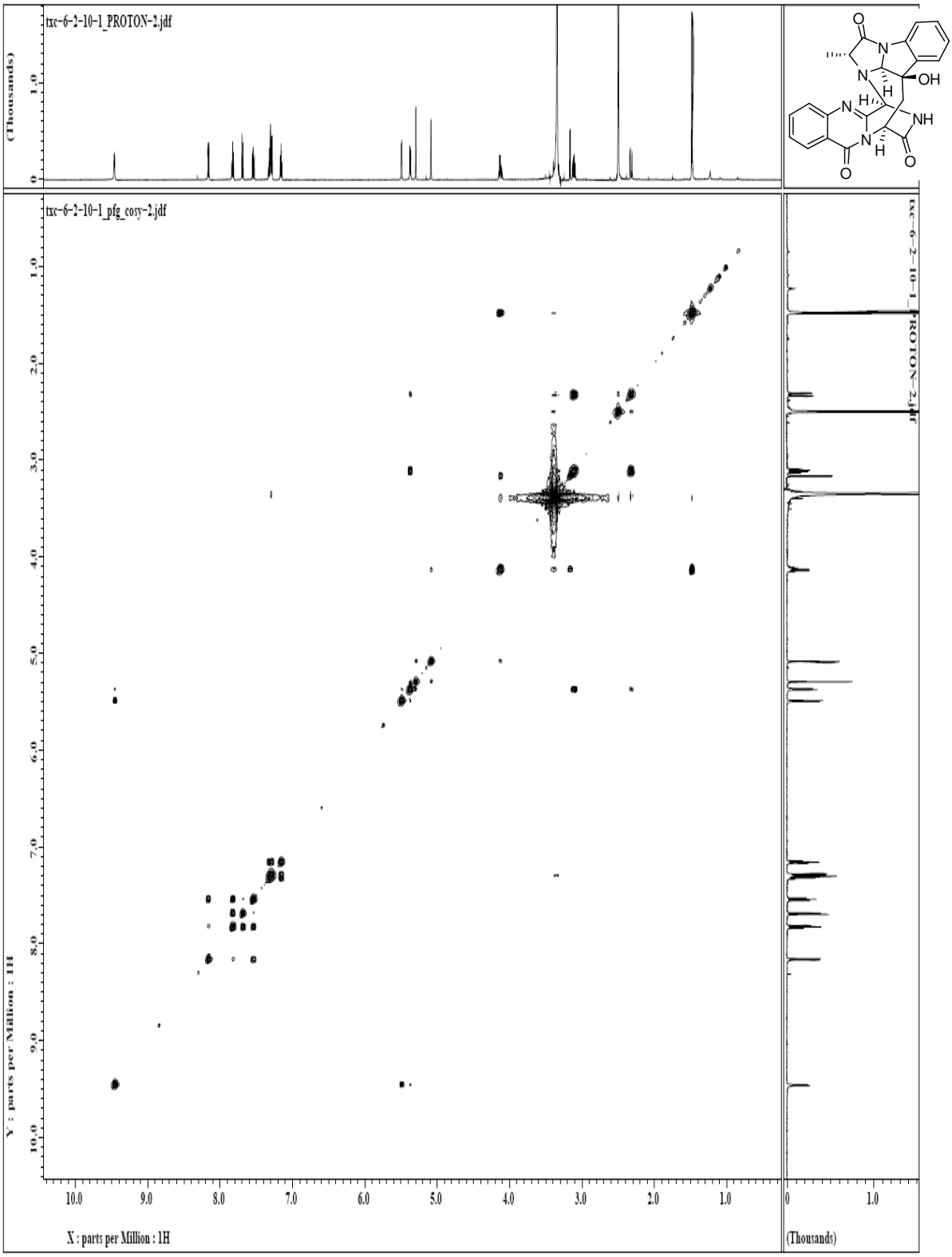

Figure S5. The HMBC spectrum of cottoquinazoline B (**1**) in DMSO-d_6

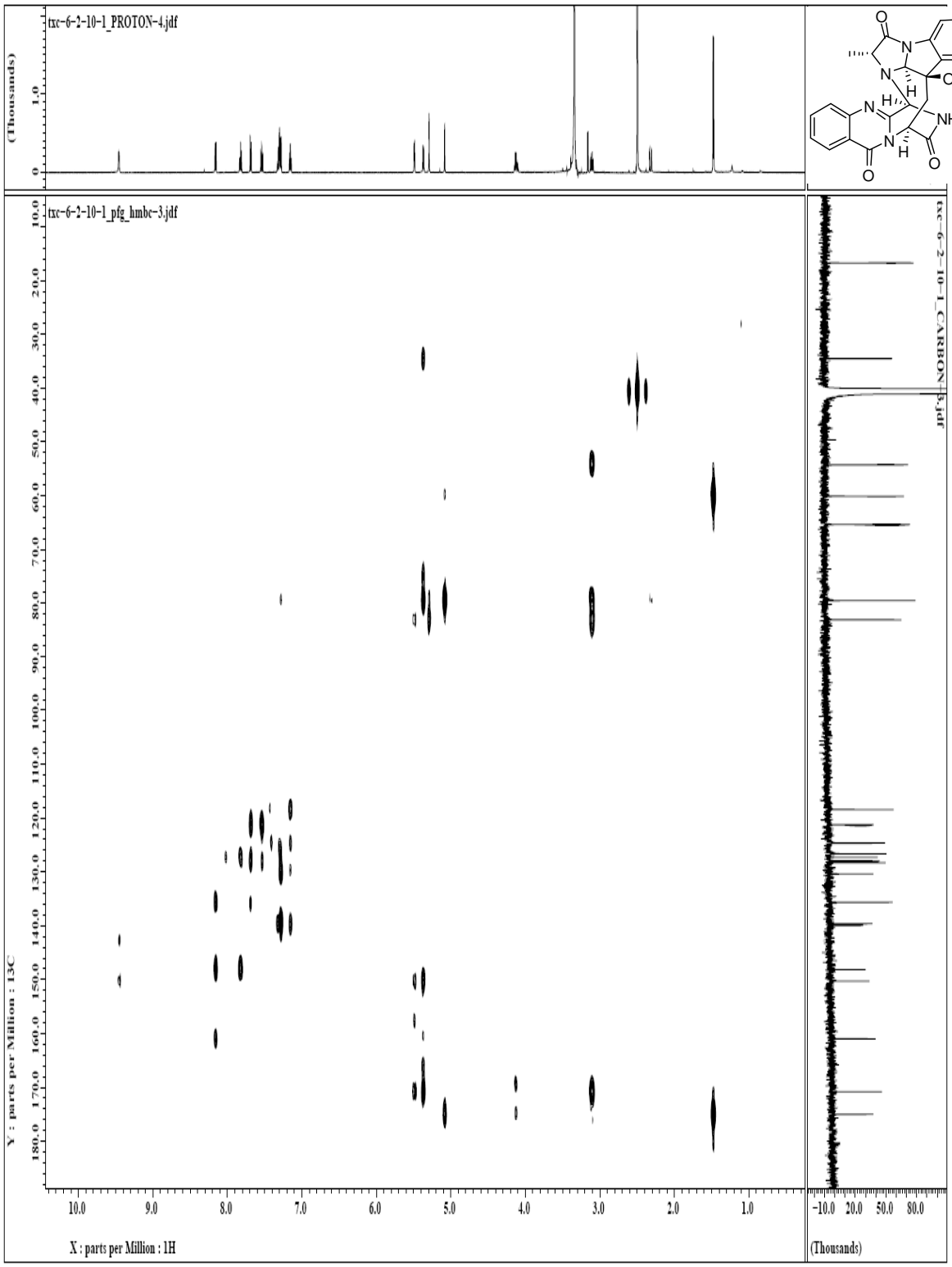

Figure S6. The ^{1}H-NMR spectrum of cottoquinazoline C (**2**) in DMSO-d_6

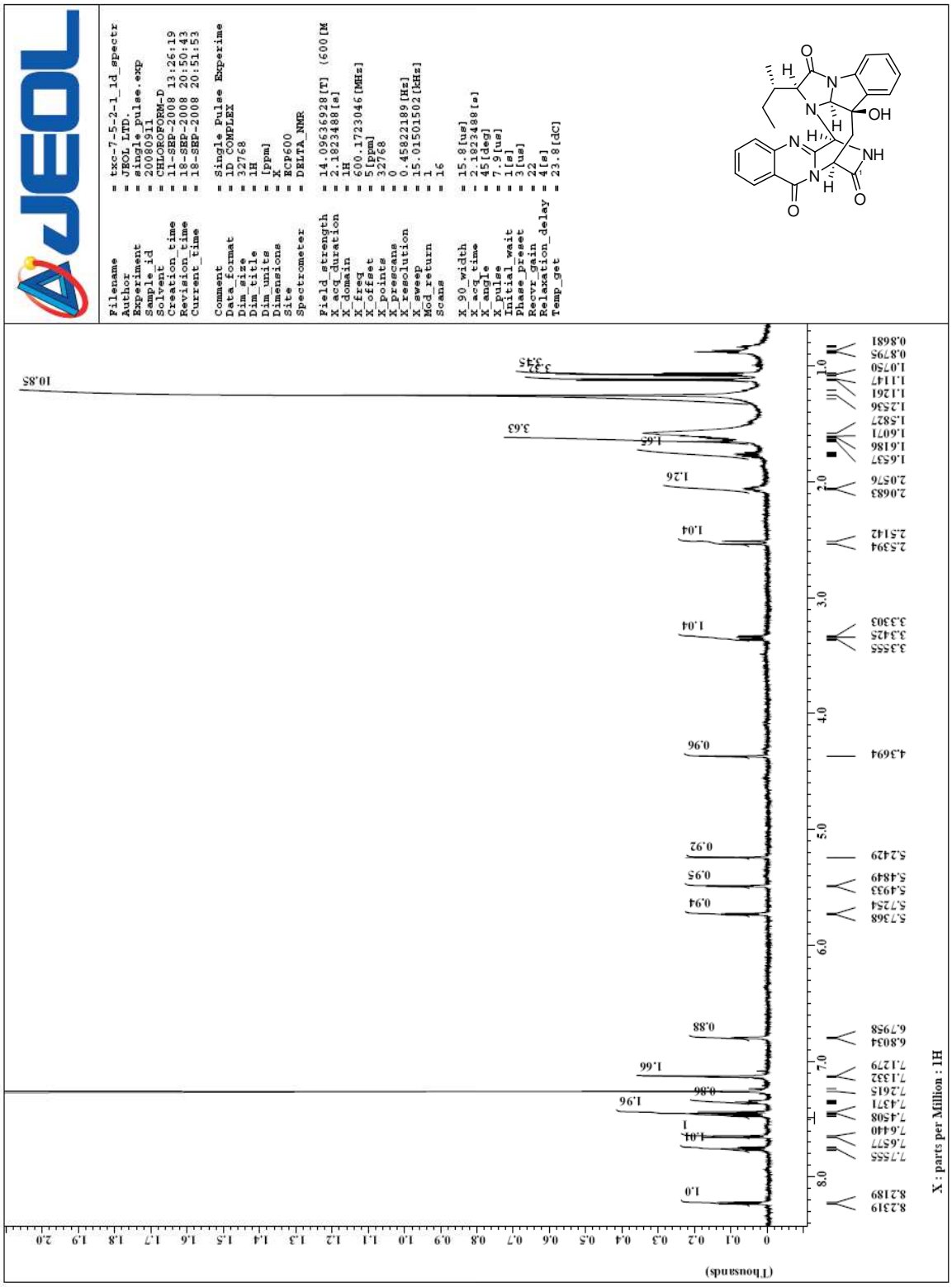

Figure S7. The ^{13}C-NMR spectrum of cottoquinazoline C (**2**) in DMSO-d_6

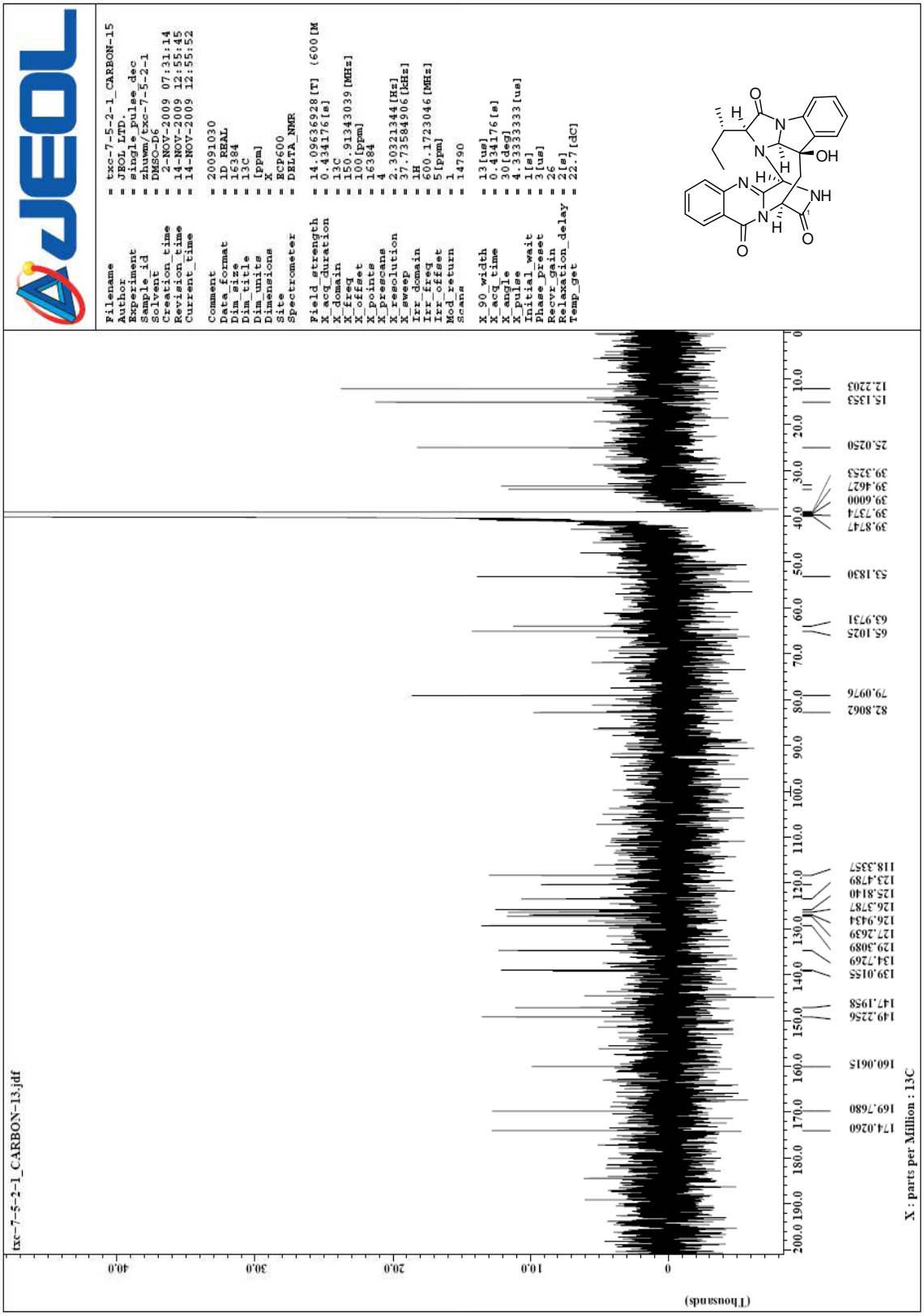

Figure S8. The HMQC spectrum of cottoquinazoline C (**2**) in DMSO-d_6

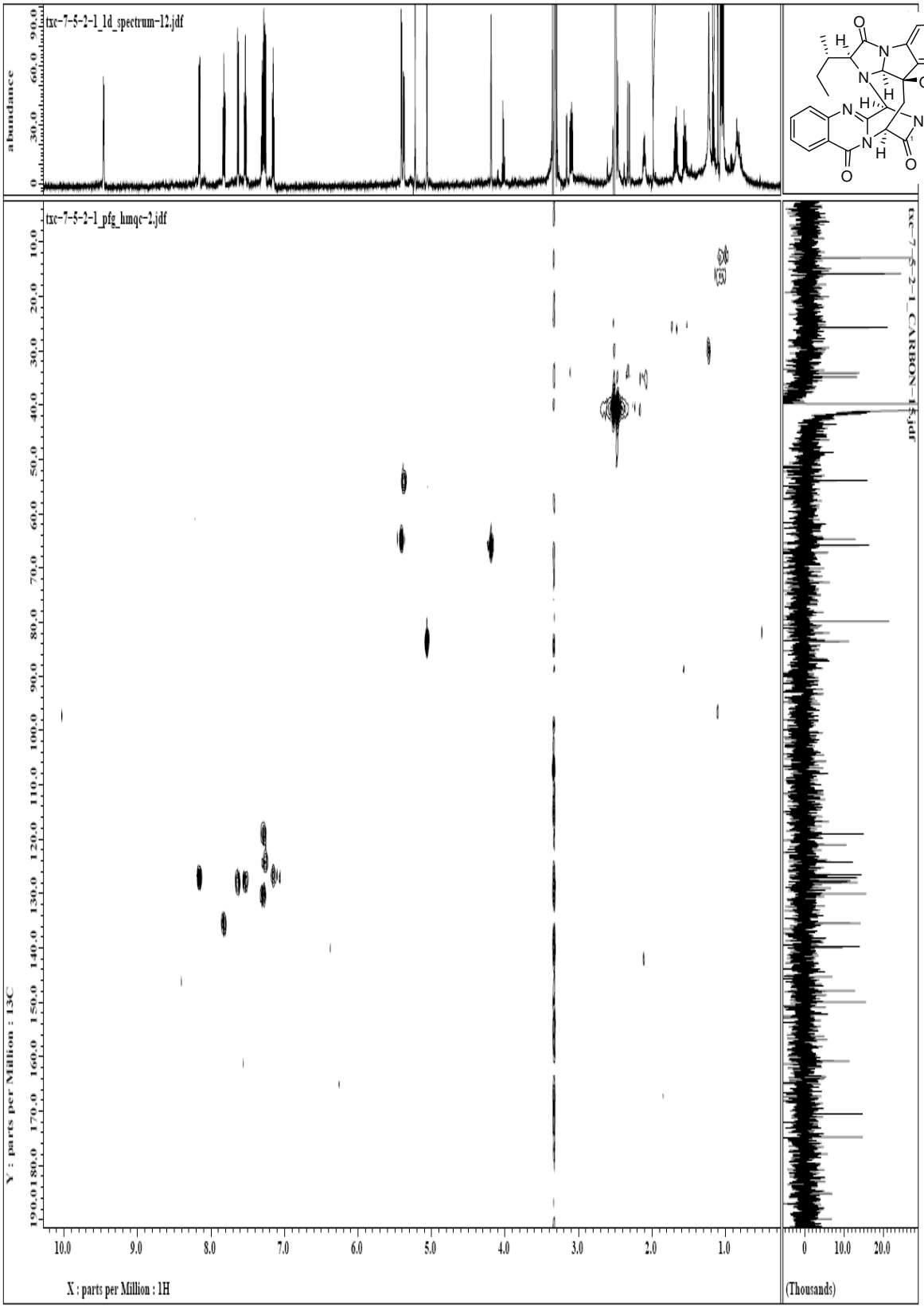

Figure S9. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline C (**2**) in DMSO-d_6

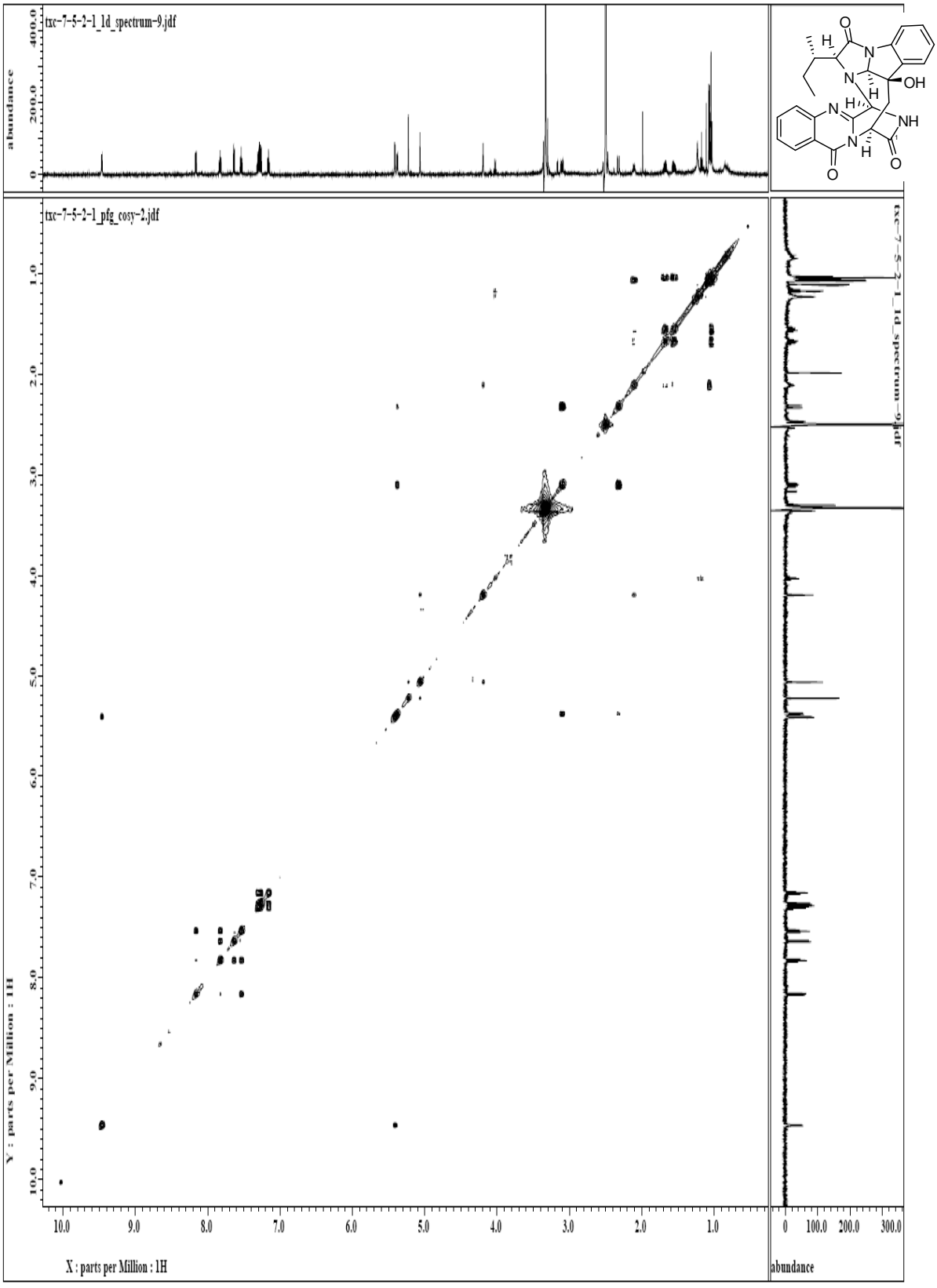

Figure S10. The HMBC spectrum of cottoquinazoline C (**2**) in DMSO-d_6

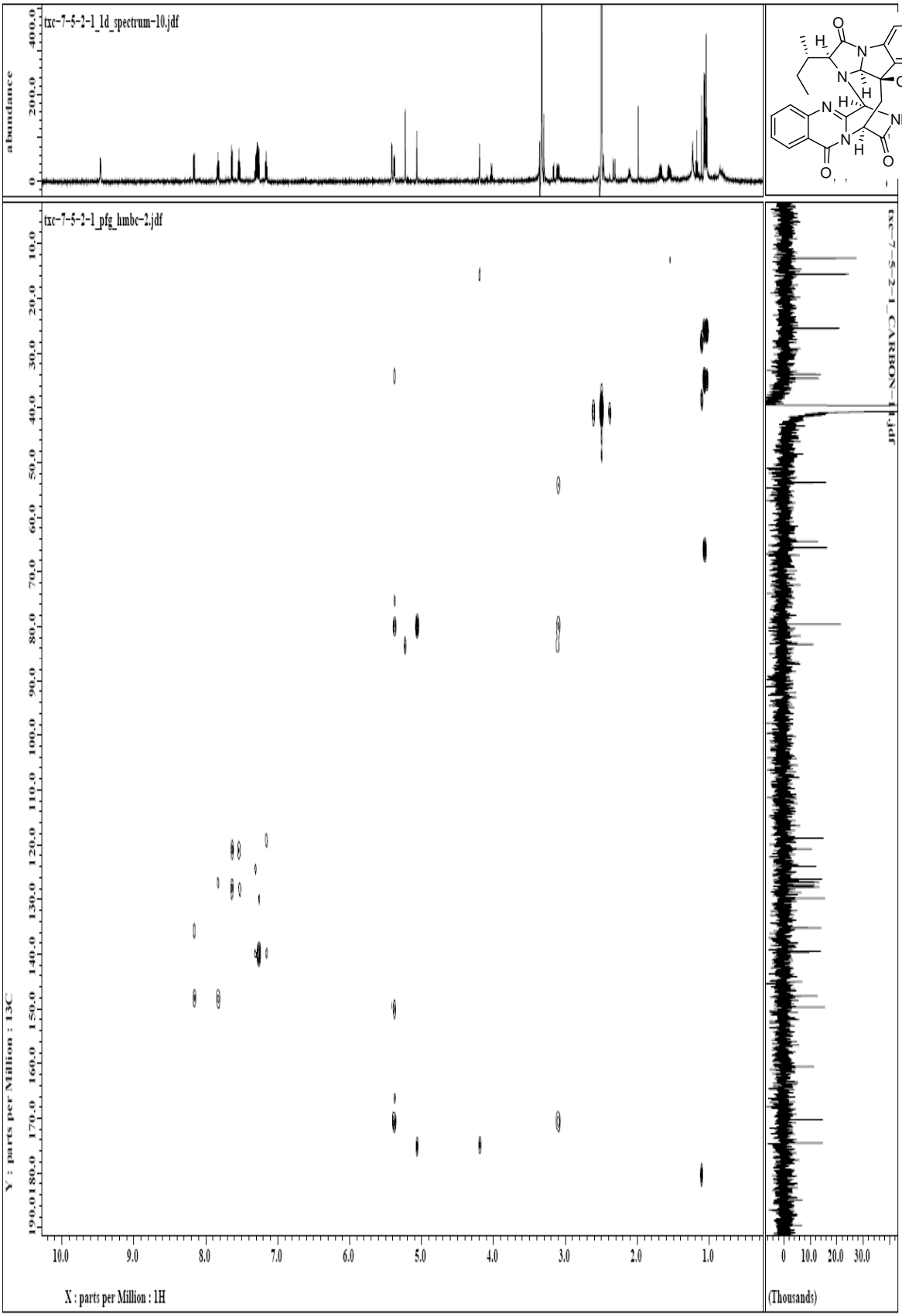

Figure S11. The ^{1}H-NMR spectrum of cottoquinazoline D (**3**) in DMSO-d_6

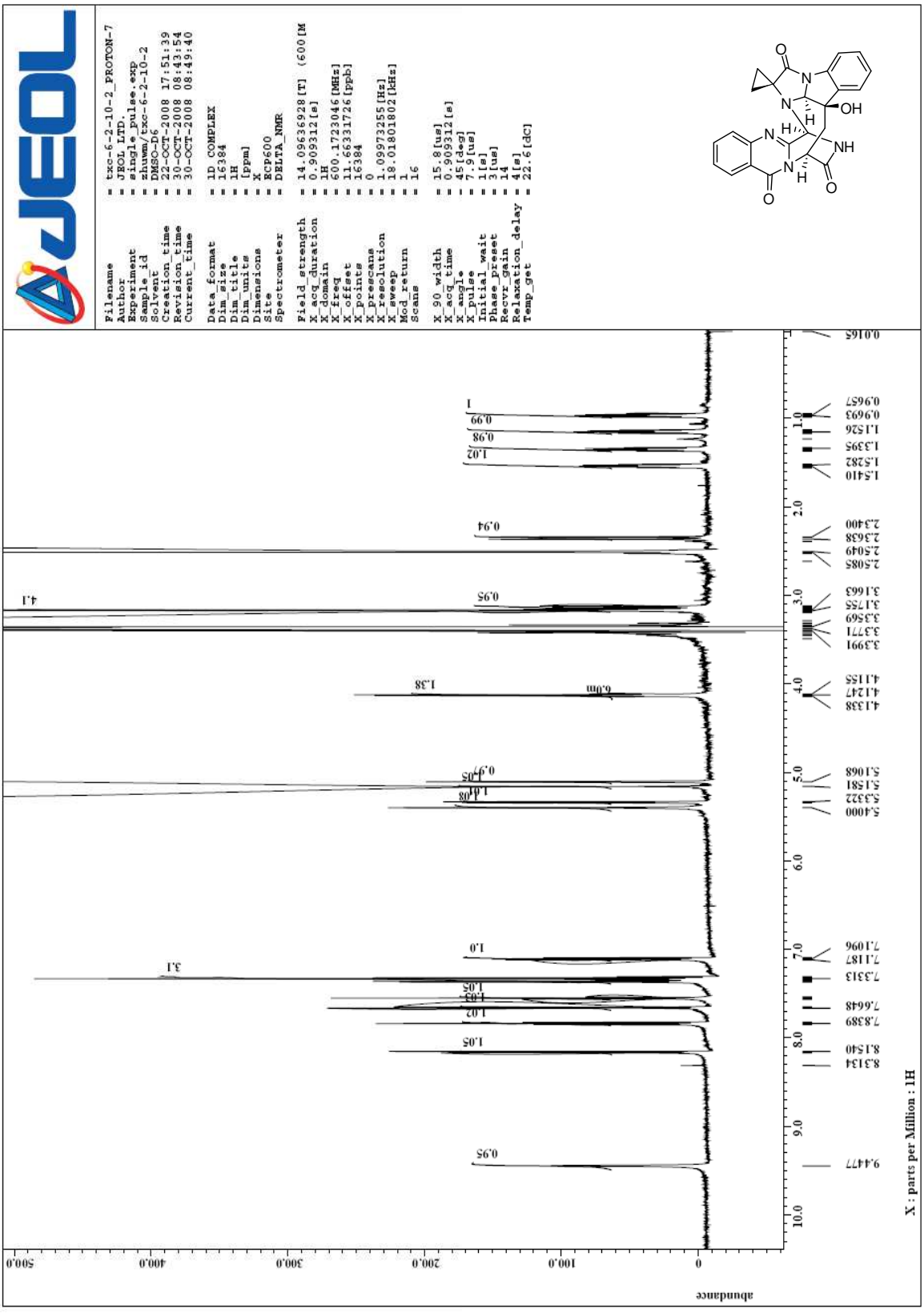

Figure S12. The ^{13}C-NMR spectrum of cottoquinazoline D (**3**) in DMSO-d_6

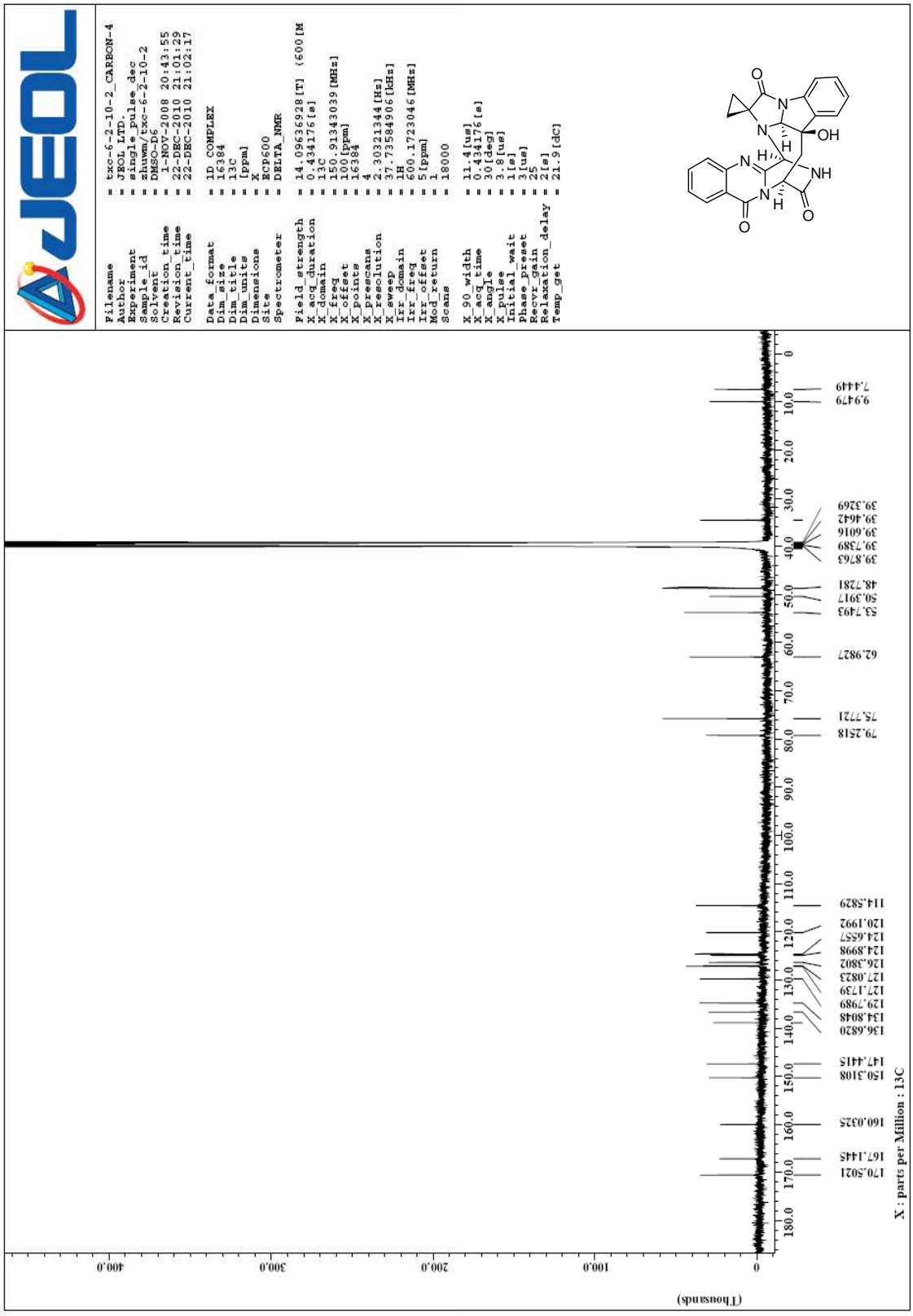

Figure S13. The HMQC spectrum of cottoquinazoline D (**3**) in DMSO-d_6

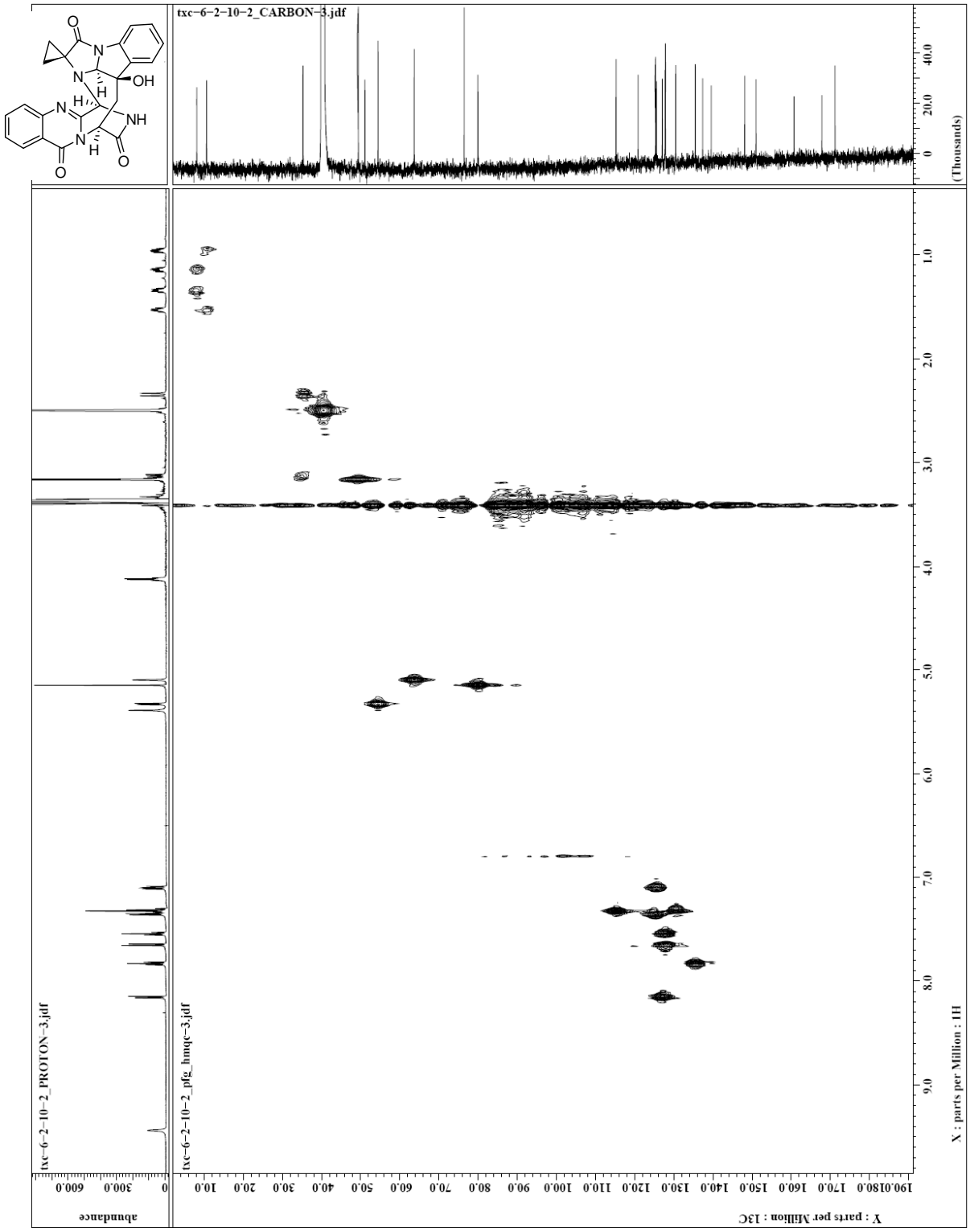

Figure S14. The ^{1}H-^{1}H COSY spectrum of cottoquinazoline D (**3**) in DMSO-d_6

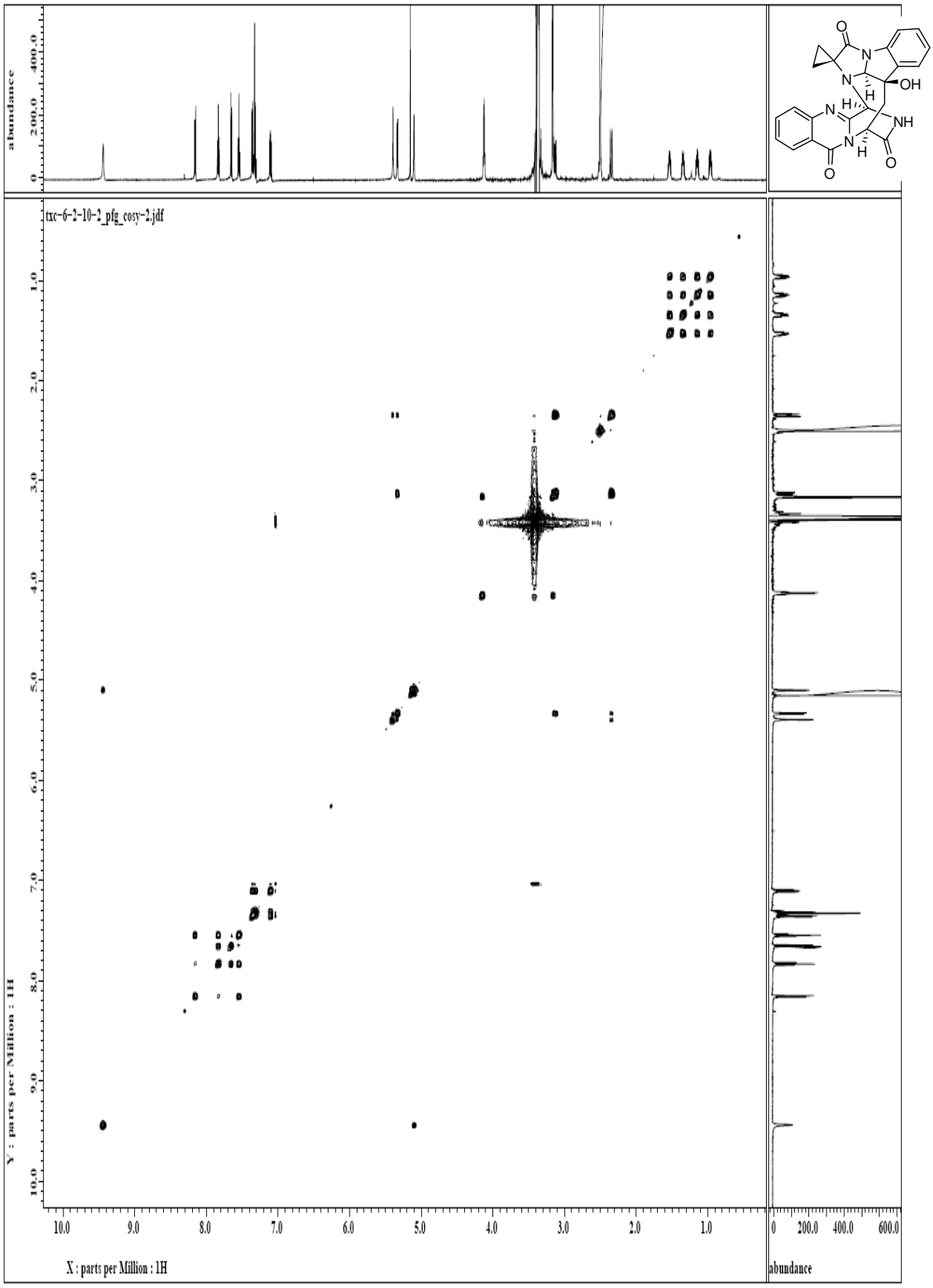

Figure S15. The HMBC spectrum of cottoquinazoline D (**3**) in DMSO-d_6

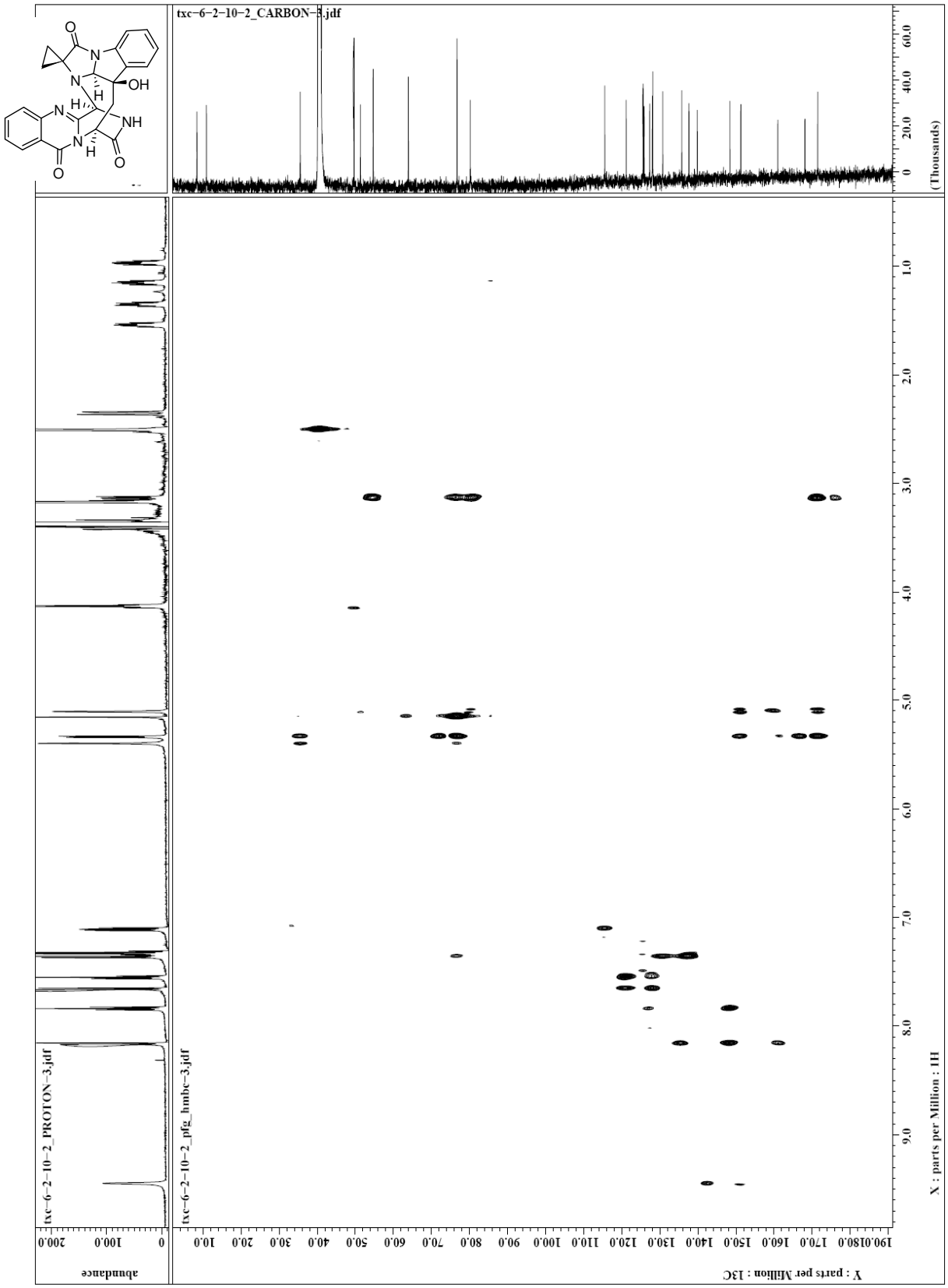

Figure S16. HPLC profiles of acidic hydrolysates of **1** co-elution with D/L-Ala on chiral Crownpak CR (+) column (flow rate: 0.5 mL/min; solvent: aqueous $HClO_4$ (pH = 1.5); detection: 201 nm; temperature: 0 °C)

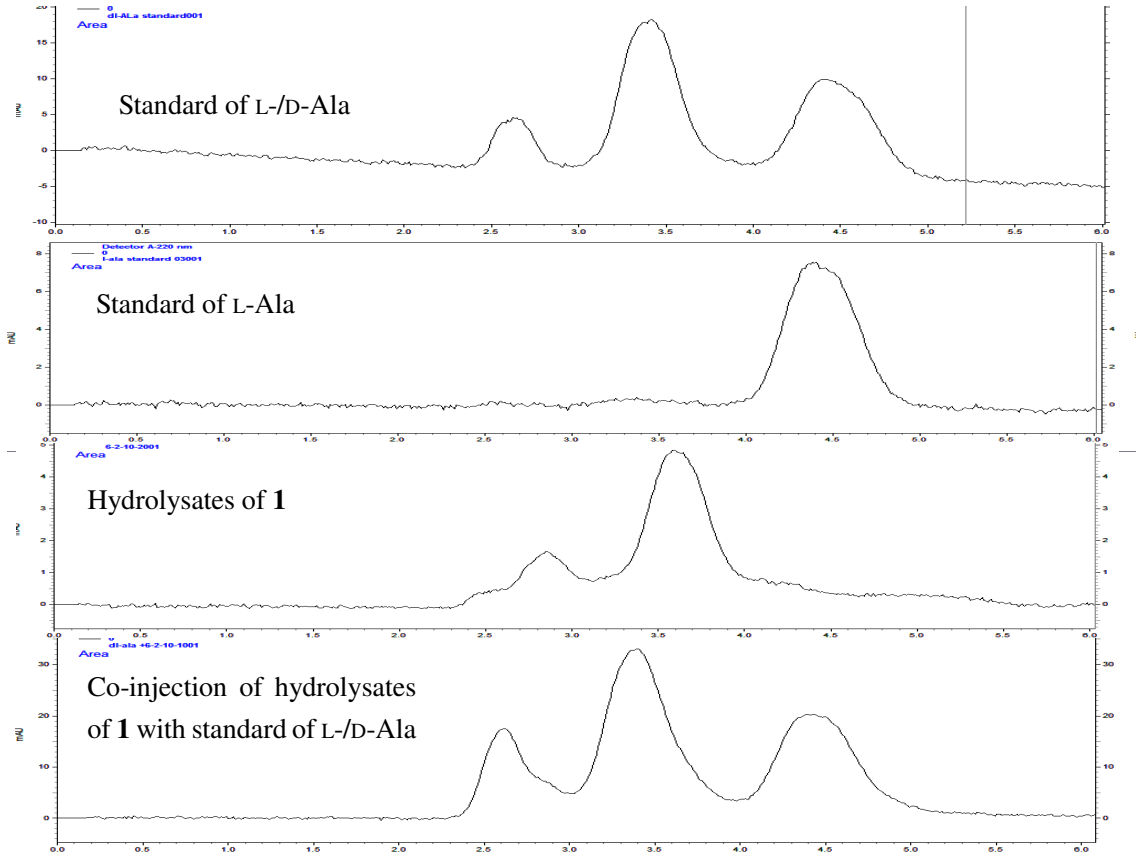

Figure S17. HPLC profiles of acidic hydrolysates of **2** co-elution with D/L-Ile on chiral Crownpak CR (+) column (flow rate: 0.5 mL/min; solvent: aqueous $HClO_4$ (pH = 1.5); detection: 201 nm; temperature: 0 °C)

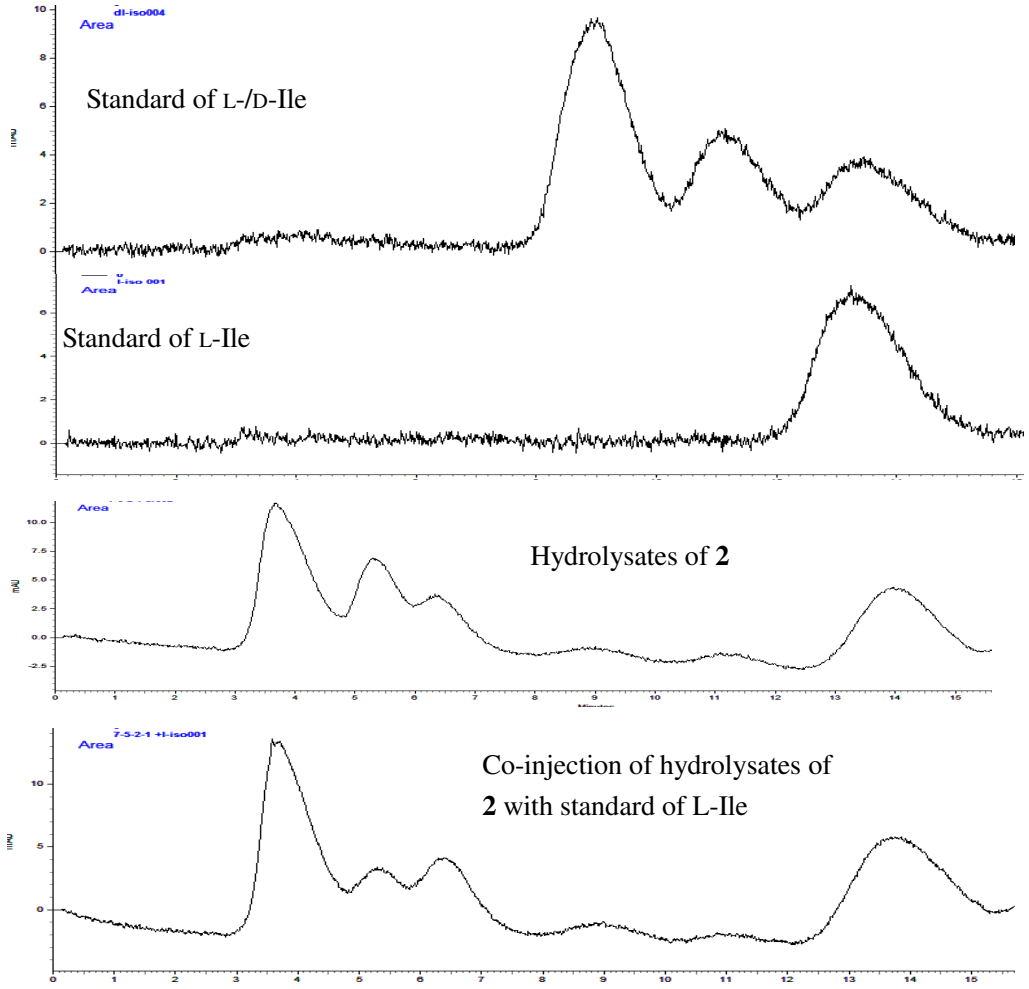

Figure S18. HPLC profiles of acidic hydrolysates of **3** co-elution with 1-aminocyclopropane-1-carboxylic acid on chiral Crownpak CR (+) column (flow rate: 0.5 mL/min; solvent: aqueous $HClO_4$ (pH = 1.5); detection: 201 nm; temperature: 0 °C)