Supporting information

Exploration of Predicted Nitrogen-Containing Natural Products from Northern Canadian Bark Beetles-Associated Bacteria

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Experimental Conditions

General media procedures:

Isolation media consisted of Tryptone soy agar (TSA) 40 g in 1L of distilled water and Actinomycetes isolation agar (AIA) was prepared from 2 g sodium caseinate, 0.1 g L-asparagine, 4 g sodium propionate, 0.5 g dipotassium phosphate, 0.1 g magnesium sulfate, 0.001 g ferrous sulfate, and 15 g agar per L of distilled water at the pH of 8.1 ± 0.2 at 25 °C. Actinomycetes isolation agar (AIA) with antibiotics 2 g sodium caseinate, 0.1 g L-asparagine, 4 g sodium propionate, 0.5 g dipotassium phosphate, 0.1 g magnesium sulfate, 0.001 g ferrous sulfate, and 15 g agar per L of distilled water at the pH of 8.1 ± 0.2 at 25 °C. After the autoclave, add antibiotics (rifampicin 5 μg per mL and nystatin 100 μg per mL of distilled water and filter the using a 0.22-micron filter and inject into the media). Burkholderia Isolation Media 15g agar, 0.01g yeast extract, 4g KH_2PO_4, 4g K_2HPO_4, 0.2g $MgSO_4.7H_20$, 2g azelaic acid per L of distilled water, Burkholderia isolation media with antibiotics 15g agar, 0.01g yeast extract, 4g KH_2PO_4, 4g K_2HPO_4, 0.2g $MgSO_4.7H_20$, and 2g azelaic acid per L of distilled water after autoclave Add antibiotics (rifampicin 5μg per mL, nystatin 100μg per mL, and econazole 100 mg per mL of distilled water and filter the using a 0.22-micron filter and inject into the media), Insect frass agar 2g of insect frass and 12g of agar per L, Rohmer burkholderia medium II (RBM-II) 1.5 g agar, 1 g peptone, 1 g beef extract, 0.5 g NaCl, and 10 g yeast extract per L of distilled water, yeast extract, and malt extract medium (YEME) 4g yeast extract, 10g malt extract, 4g glucose, and 20g agar per L of distilled water and Chitin agar II 20 g agar, 4 g chitin, 0.77 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.37 g $KHPO_4$, 0.01 g $FeSO_4.7H_2O$, 0.001 g $MnCl_2.4H_2O$, and 0.001 g $ZnSO_4.7H_2O$ per L of distilled water.
Composition of a modified white solution 1 g $HgCl_2$, 6.5 g NaCl, 1.25 L HCl, 250 mL of 95% ethyl alcohol, and 750 mL of distilled water to make 1 liter of distilled water used in beetle wash.

Section A: List of Abbreviations Used in The Text:

General Abbreviations:

- NP – Natural Product
- BGC – Biosynthetic Gene Cluster
- GCF – Gene Cluster Family
- NRPS – Nonribosomal Peptide Synthetase
- PKS – Polyketide Synthase

- RiPP – Ribosomally Synthesized and Post-Translationally Modified Peptide
- NRP-PKS – Nonribosomal Peptide-Polyketide Hybrid
- GC – Guanine-Cytosine
- HSQC – Heteronuclear Single Quantum Coherence
- TOCSY – Total Correlation Spectroscopy
- NMR – Nuclear Magnetic Resonance
- ppm – Parts per million
- DNA – Deoxyribonucleic Acid
- RNA – Ribonucleic Acid
- DMSO – Dimethyl sulfoxide
- RPM – Revolutions per minute
- 1H – Proton NMR
- 15N – Nitrogen-15
- HMBC – Heteronuclear Multiple Bond Correlation

Bacterial and Sample-Related Abbreviations:

- NBB – Northern Bark Beetle
- MPB – Mountain Pine Beetle (Dendroctonus ponderosae)
- SBB – Spruce Bark Beetle (Dendroctonus rufipennis)
- DFB – Douglas Fir Beetle (Dendroctonus pseudotsugae)
- IPS – Ips perturbatus (a bark beetle species)
- UBCG – Universal Bacterial Core Gene
- EZbiome – A sequencing service provider
- Plasmidosaurus – A sequencing service provider

Analytical and Bioinformatics Tools:

- antiSMASH – Antibiotics and Secondary Metabolite Analysis Shell
- BiG-SCAPE – Biosynthetic Gene Cluster Scalable Analysis
- MIBiG – Minimum Information about a Biosynthetic Gene Cluster
- CORASON – Comparative Analysis of Gene Cluster Families
- cblaster – Comparative Genomic Analysis Tool for BGCs
- Clinker – Gene Cluster Visualization Tool
- Cytoscape – Network Analysis Software

Growth Media and Assays:

- TSB – Tryptic Soy Broth
- AIA – Actinomycete Isolation Agar

- RBM-II – Burkholderia Isolation Media
- CAII – Chitin Agar II
- IA – Insect Frass Agar
- YEME – Yeast Extract Malt Extract Agar
- YMS – Yeast Malt Starch Agar
- PDA – Potato Dextrose Agar
- TSA – Tryptic Soy Agar

Section B: Additional Gene Clusters of Low Sequence Homology

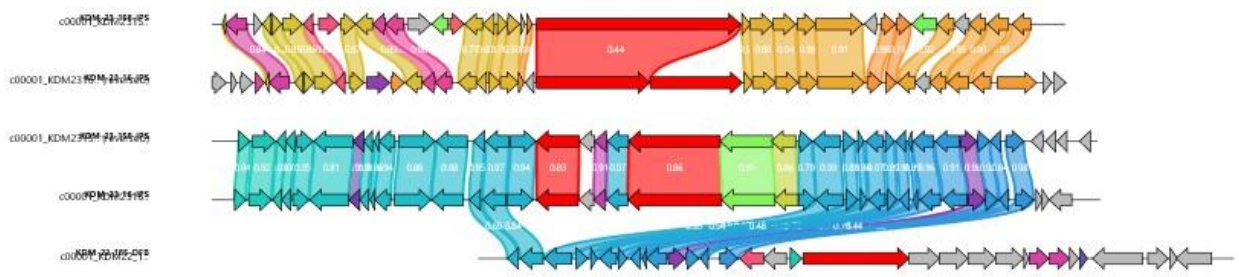


Figure S 1: Gene Clusters shared between isolates in this study that had low sequence homology to any characterized gene cluster

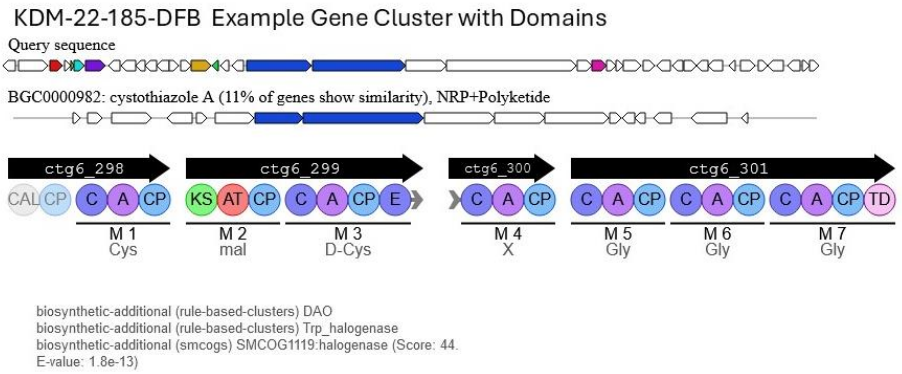


Figure S 2: AntiSMASH analysis revealed the top match to be the gene cluster producing cystothiazole with two similiar modules (CtaC and CtaD) bearing 45% sequence homology. Through MiBIG analysis, this gene cluster is seen to share the machinery to produce to produce typically peptide features from a typical peptide like a thalassospiramide-producer, thiazole producing-genes with a cystothiazole producer, and capacity for tryptophan rearrangement and production with the cladionamide producer.

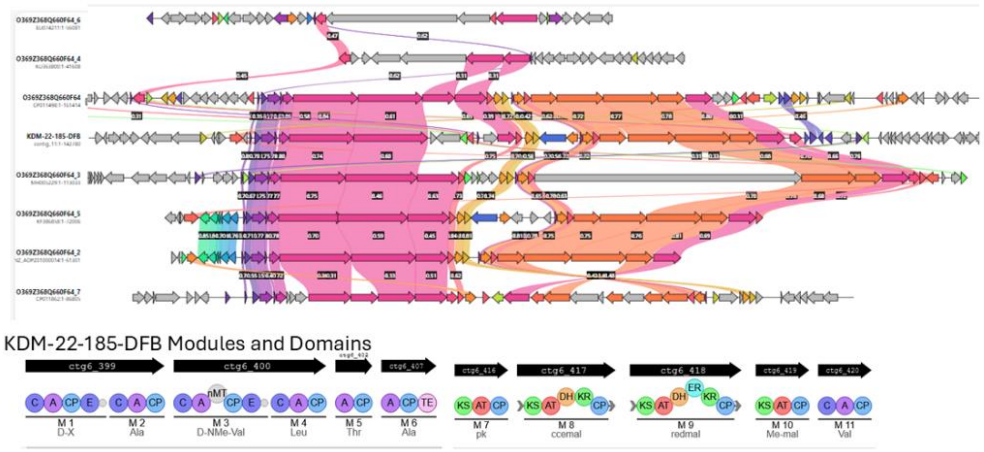


Figure S 3: KDM-22-185-DFB carries a BGC coding for an azinothricin natural product, four gene clusters from the top. Comparison to a suite of known azinothricin gene clusters, demonstrates that there is an unusual gene organization between the NRPS and PKS domains.

Section C: ^{15}N NMR Data of crude bacterial extracts

All 1H-15N HSQC NMR experiments were run with an F1 time domain (TD) of 32 as there are few nitrogens in natural products compared to protons and carbons. This broadens the nitrogen peaks, particularly along the F1 axis, but significantly decreases the length of time required to run the 2D spectra.

Natural Products Standards Runs:

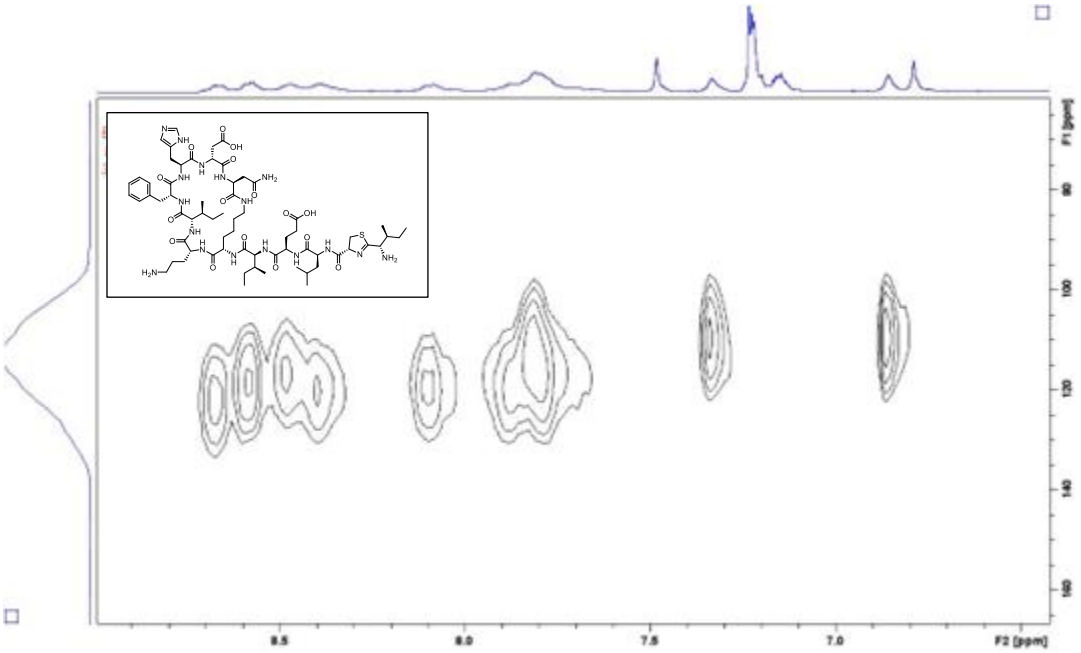


Figure S 4: Bacitracin NRP, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz. Bacitracin is a nonribosomal peptide that possesses multiple nitrogen functional groups. The proton-nitrogen signals are clear.

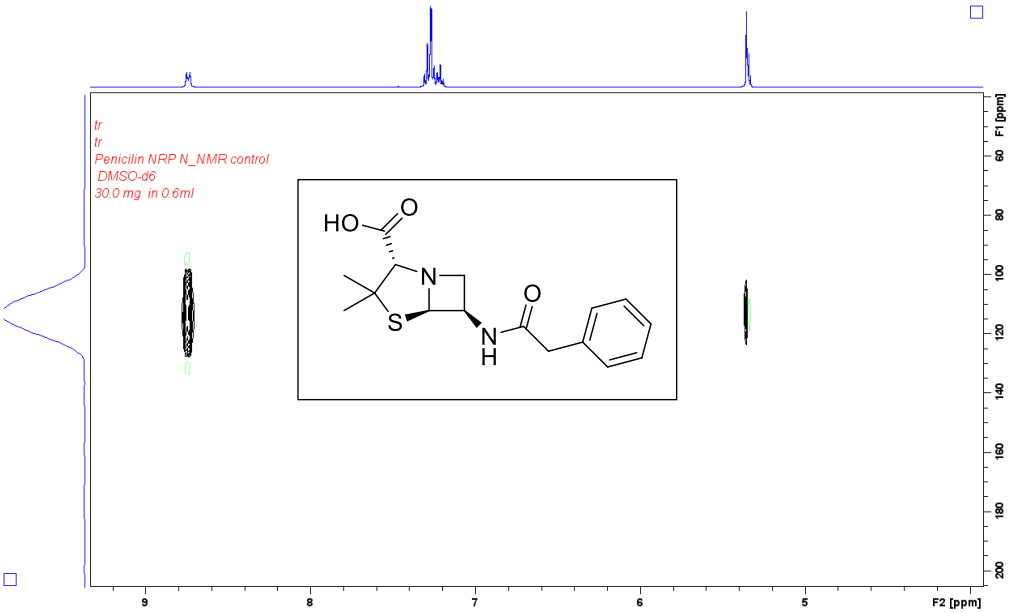


Figure S 5: Penicillin NRP, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz. Penicillin is a nonribosomal peptide that possesses multiple nitrogen functional groups. The proton-nitrogen signals are clear.

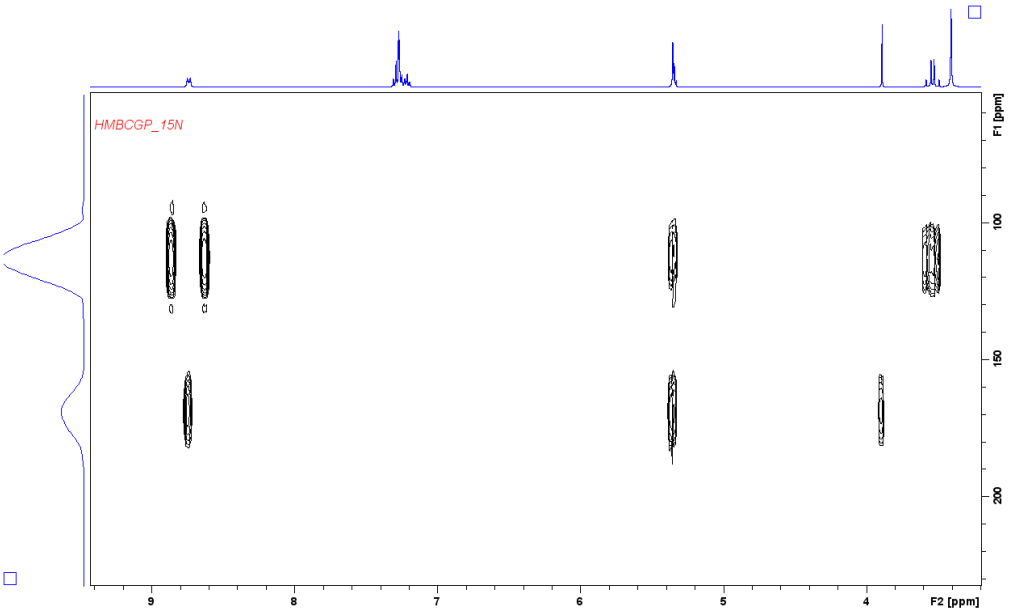


Figure S 6: Penicillin NRP, ^{1}H-^{15}N HMBC recorded in DMSO-d_6, at 400 MHz. Penicillin is a nonribosomal peptide that possesses multiple nitrogen functional groups. We see long range proton-nitrogen signals clearly.

Media Control

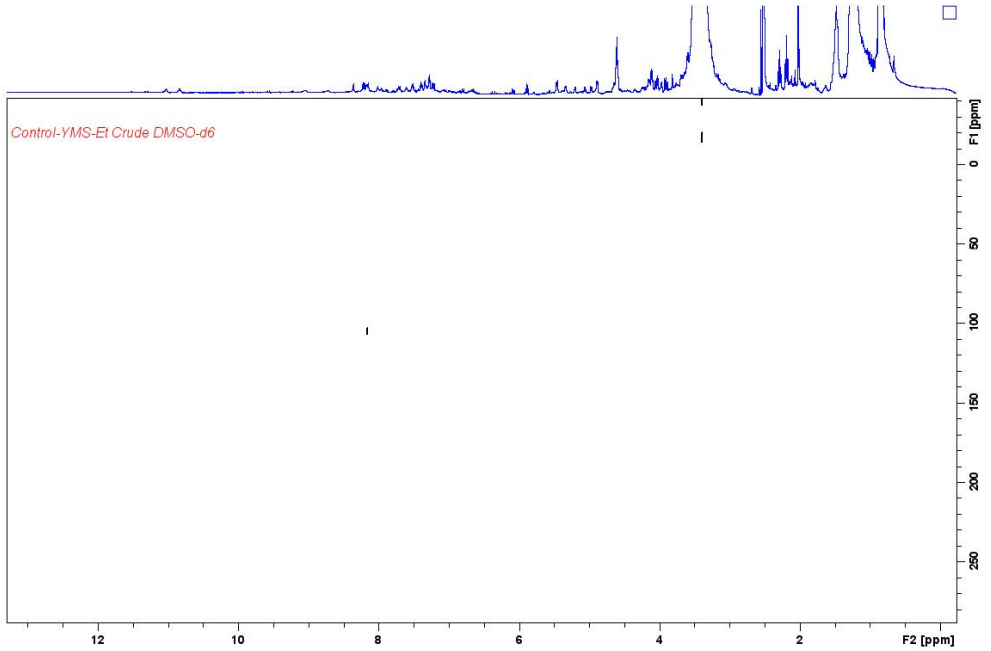


Figure S 7: Extract of plain YMS media as control. ^{1}H-^{15}N HSQC recorded in DMSO-d6, at 400 MHz.

Nitrogen NMR of Crude Extracts (Figure S8 – Figure S16)

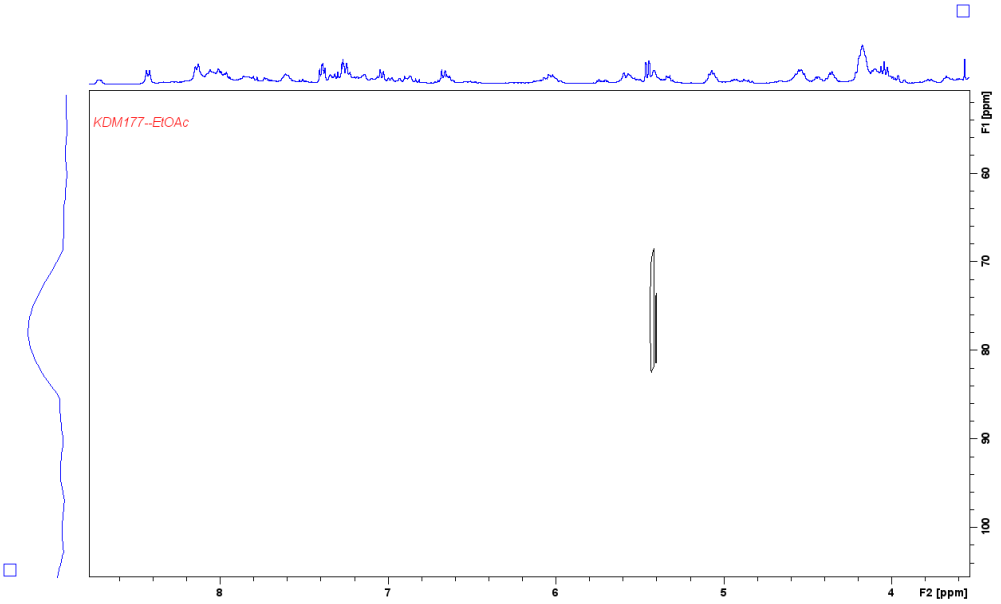


Figure S 8: KDM-22-177-SBBG, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

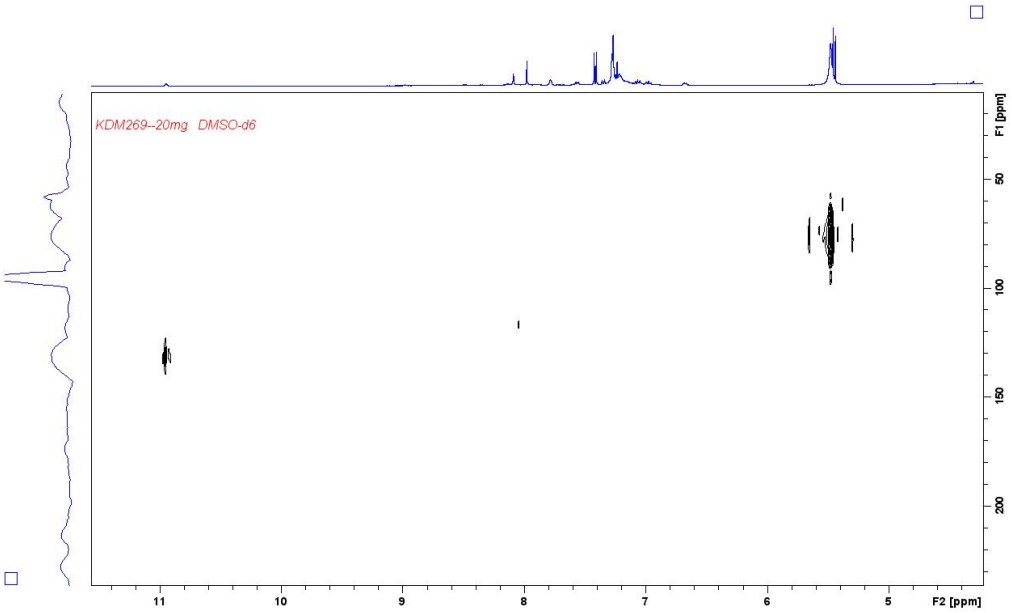


Figure S 9: KDM-22-269-MPB, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

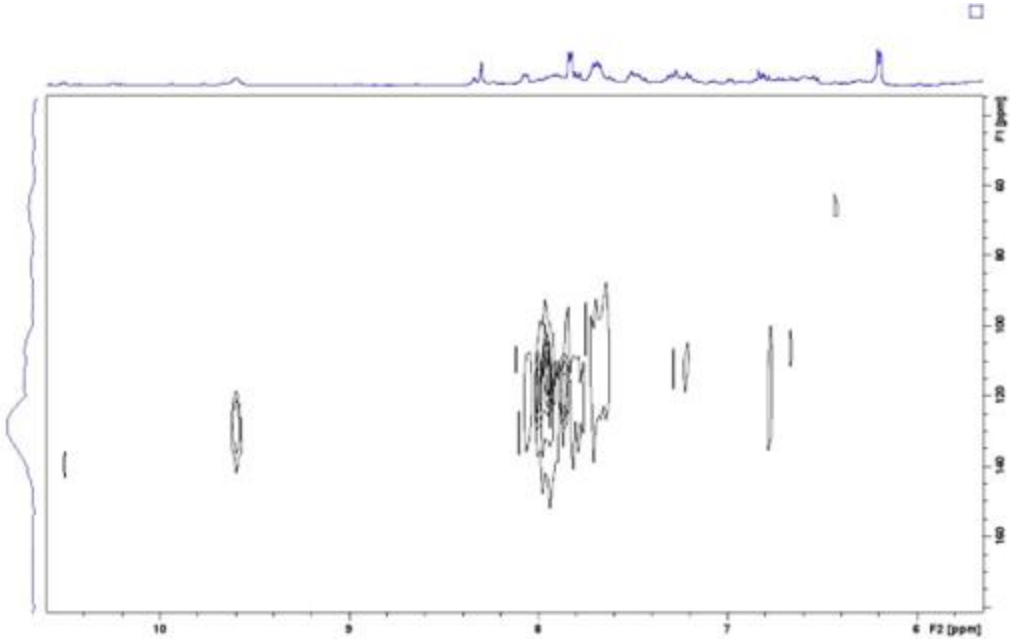


Figure S 10: KDM-22-148-DFB, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

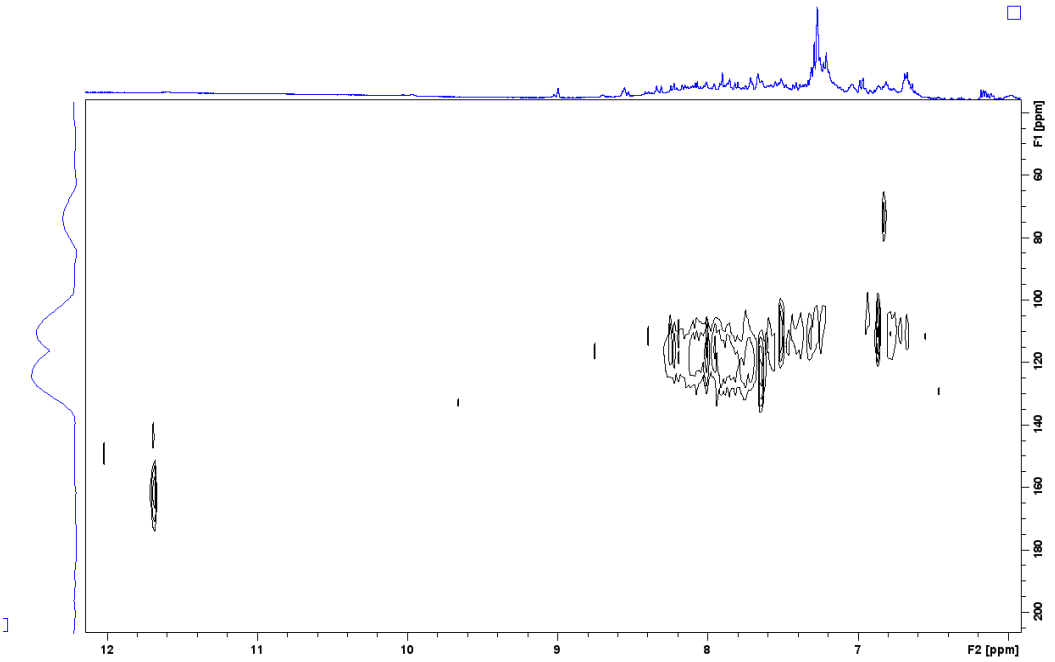


Figure S 11: KDM-22-161-SBBG, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

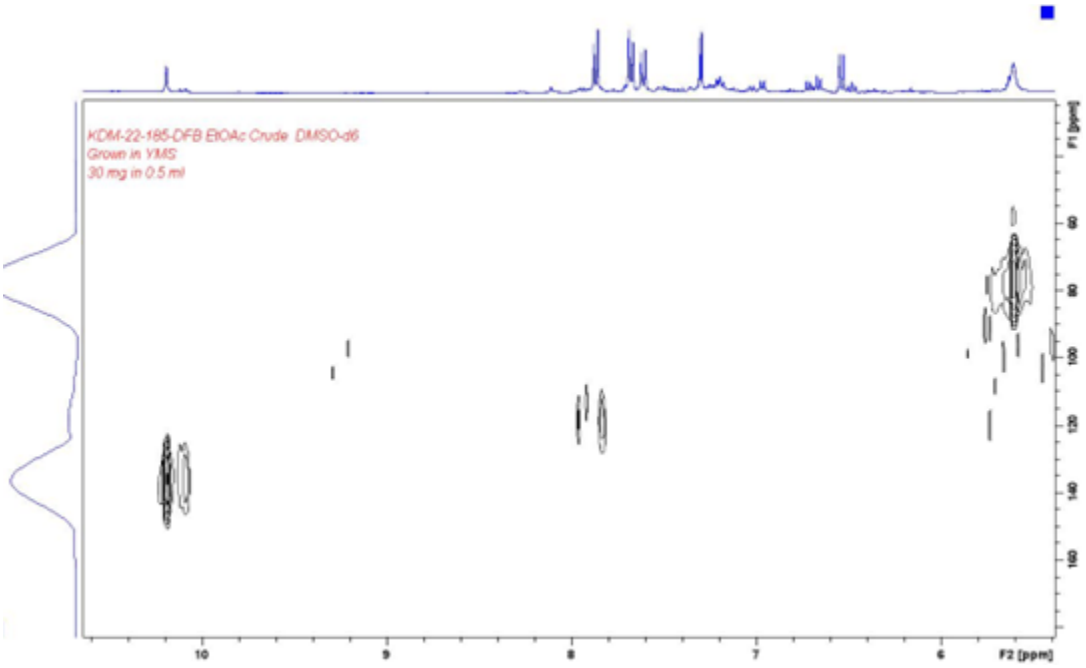


Figure S 12: KDM-22-185-DFB, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

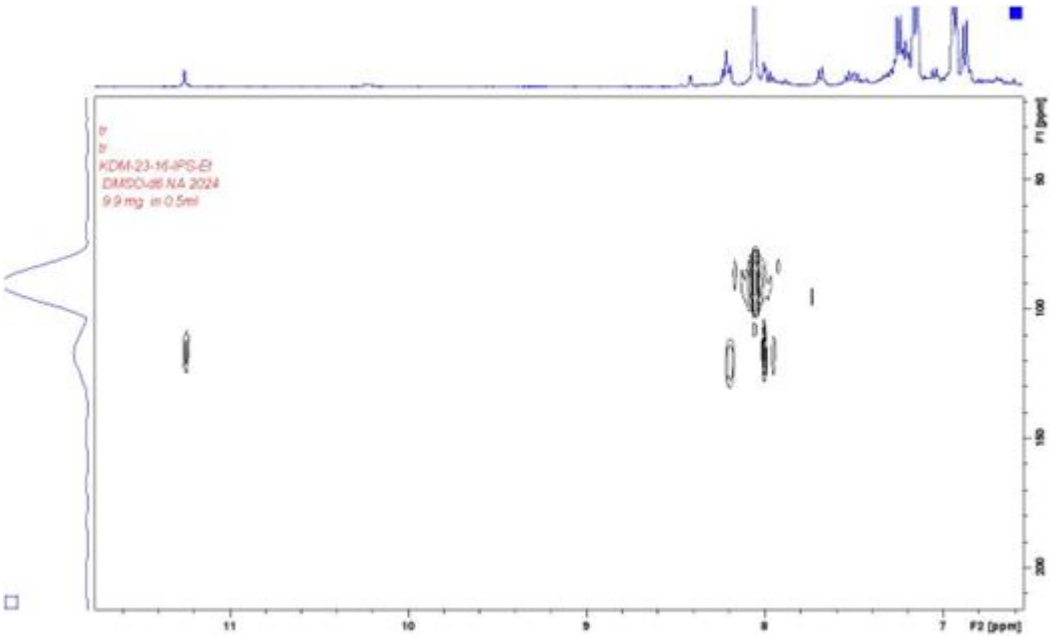


Figure S 13: KDM-23-16-IPS, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

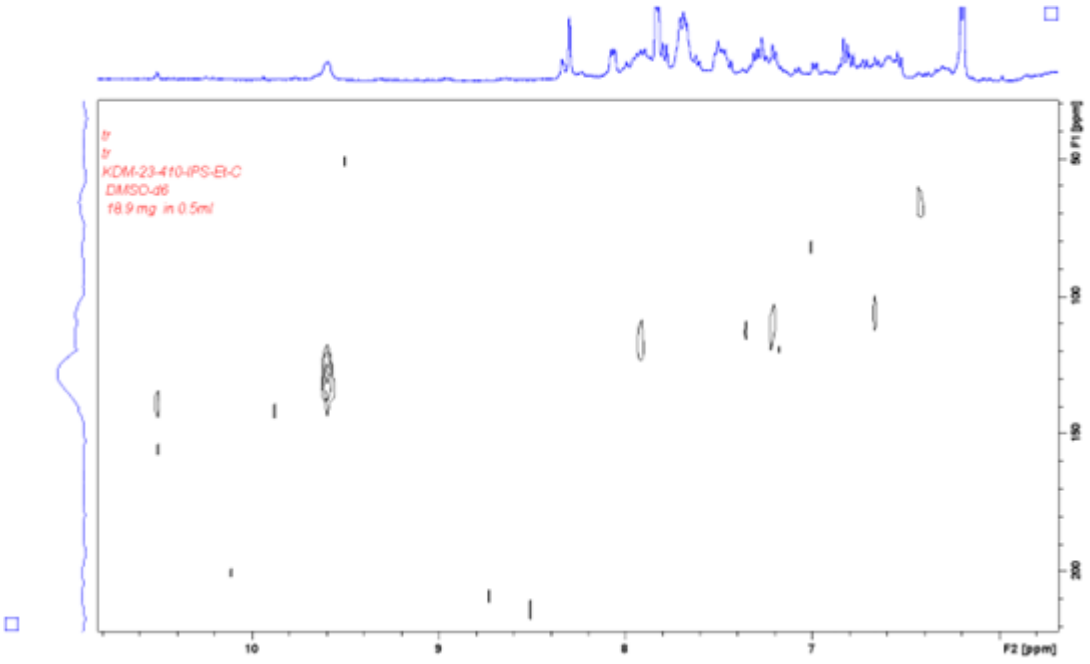


Figure S 14: KDM-23-410-IPS, 1H-15N HSQC recorded in DMSO-d6, at 400 MHz.

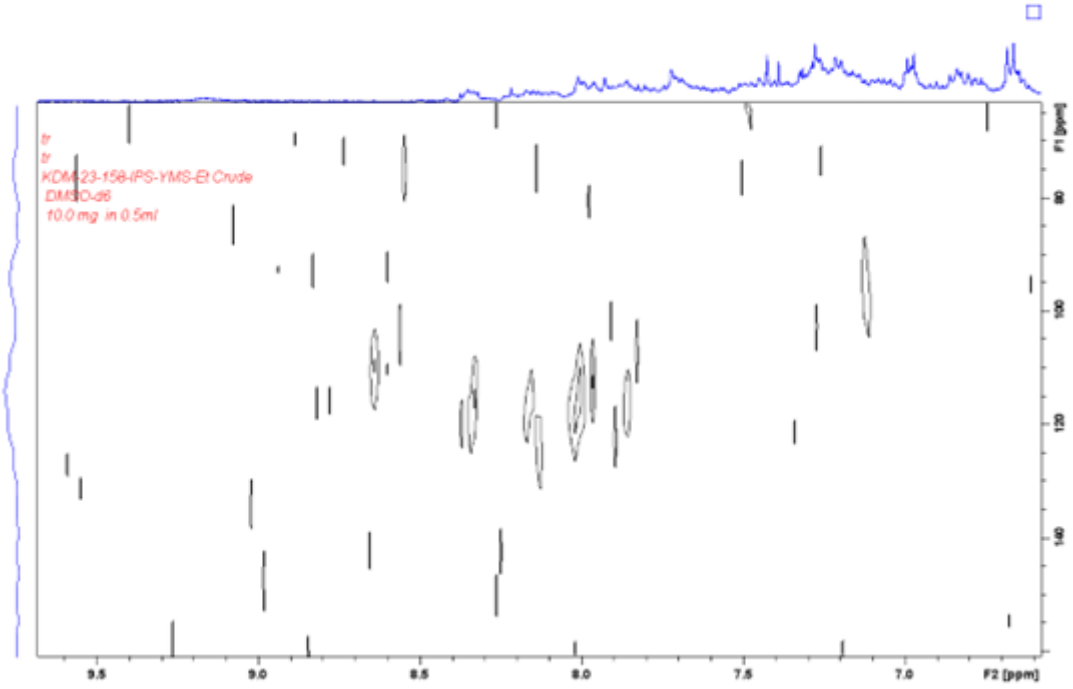


Figure S 15: KDM-23-158-IPS, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.

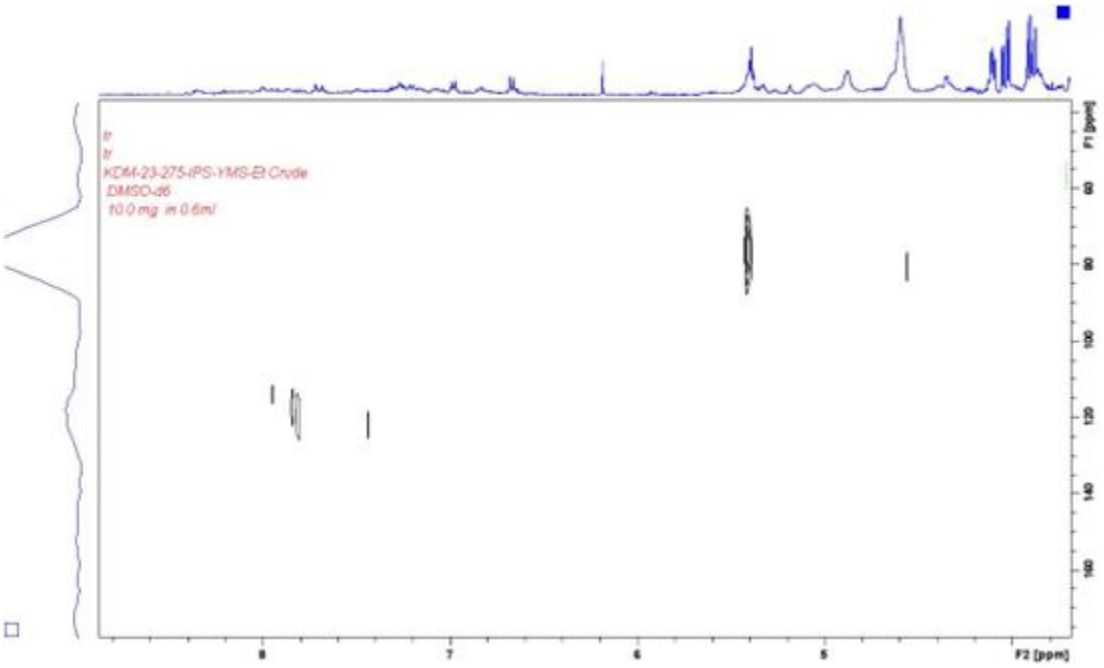


Figure S 16: KDM-23-275-IPS, ^{1}H-^{15}N HSQC recorded in DMSO-d_6, at 400 MHz.