Supplementary Material

Characterization and abolishment of the cyclopiazonic acids produced by *Aspergillus oryzae* HMP-F28

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[*] Equal contribution

Table S1 Nucleotide primers used in this study

Primer	Nucleotide Sequence (5'-3')	Function
cpaSup_F	TGCTCGCCGTTAGCCTTTCGTTTCAC	Left fragment amplification
cpaSup_R	ATCAAAAGAATAGCCCGAGATAGAGTTGAGAGCGGCAG AACTGGCAGCAGATAATAGAG	Left fragment amplification
cpaSdn_F	GTATTCAACATTTCCGTGTCGCCCTTATTAGCGACGAAA GCGTGTTAACCAAGGTATG	Right fragment amplification
cpaSdn_R	TTTGAGCGCAATCGGGATGAGTAATGTAG	Right fragment amplification
ptrA_F	CTCTATTATCTGCTGCCAGTTCTGCCGCTCTCAACTCTAT CTCGGGCTATTCTTTTGAT	*ptrA* fragment amplification
ptrA_R	CATACCTTCGTTAACACGCTTTCGTCGCTAATAAGGGCG ACACGGAAATGTTGAATAC	*ptrA* fragment amplification
IDup_F	CAGCTCTTCGTGGTCCAGT	Mutant confirmation
IDup_R	TGGCTGTGTCCCGTATGT	Mutant confirmation
IDdn_F	TACGGTCGCTGGAAGTATCG	Mutant confirmation
IDdn_R	GTGGTCACTGATACACGGC	Mutant confirmation
cpaS1_F	CACAGCAACGGCTCTTACT	*cpaS* amplification
cpaS1_R	TCGTCGCCATCTTTCAGT	*cpaS* amplification
cpaS2_F	GCCAAGTATTCTCTCAAATCGC	*cpaS* amplification
cpaS2_R	ATCTCCAGCCTGTGTTCCA	*cpaS* amplification
cpaS3_F	ACTGGAACACAGGCTGGAGATC	*cpaS* amplification
cpaS3_R	GTCTTCTTACGGTCGAGGAGT	*cpaS* amplification
cpaS4_F	ACTCCTCGACCGTAAGAAGAC	*cpaS* amplification
cpaS4_R	GTAAGATCGATGACCTTGGAAT	*cpaS* amplification
cpaS5_F	CACCGCAACCTATATTCCAAG	*cpaS* amplification
cpaS5_R	AGCCAAGCCTCATGCATA	*cpaS* amplification
cpaS6_F	GTATGCATGAGGCTTGGCT	*cpaS* amplification
cpaS6_R	GACATCTTCTCGATCCGTGTG	*cpaS* amplification
cpaS7_F	GTCATCCTGTGTCCTATGTCCTT	*cpaS* amplification
cpaS7_R	AGATATCGTACGGCGCATTG	*cpaS* amplification
cpaS8_F	GCAATGCGCCGTACGATAT	*cpaS* amplification
cpaS8_R	AGCCACGTCTTCGAGATCAAT	*cpaS* amplification
cpaS9_F	GAGGACTTCGGATTGATCTCG	*cpaS* amplification
cpaS9_R	AGCTCTTCACCGGAACCTTG	*cpaS* amplification

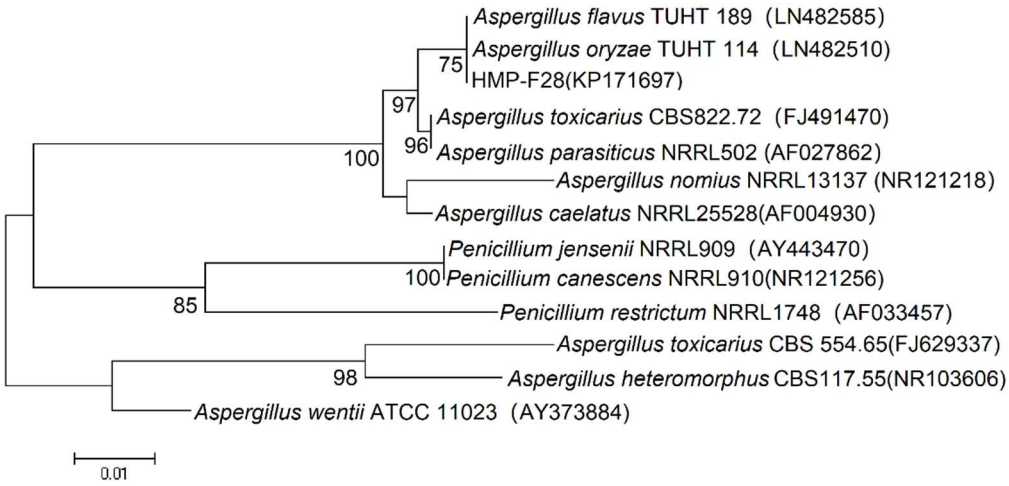


Figure S1 Phylogenetic tree of the rDNA HMP-F28 and selected fungi closely related with *Aspergillus flavus* and *A. oryzae* from NCBI database. HMP-F28 showed 99% similarity to both *A. oryzae* TUHT 189 and *A. flavus* TUHT 114.

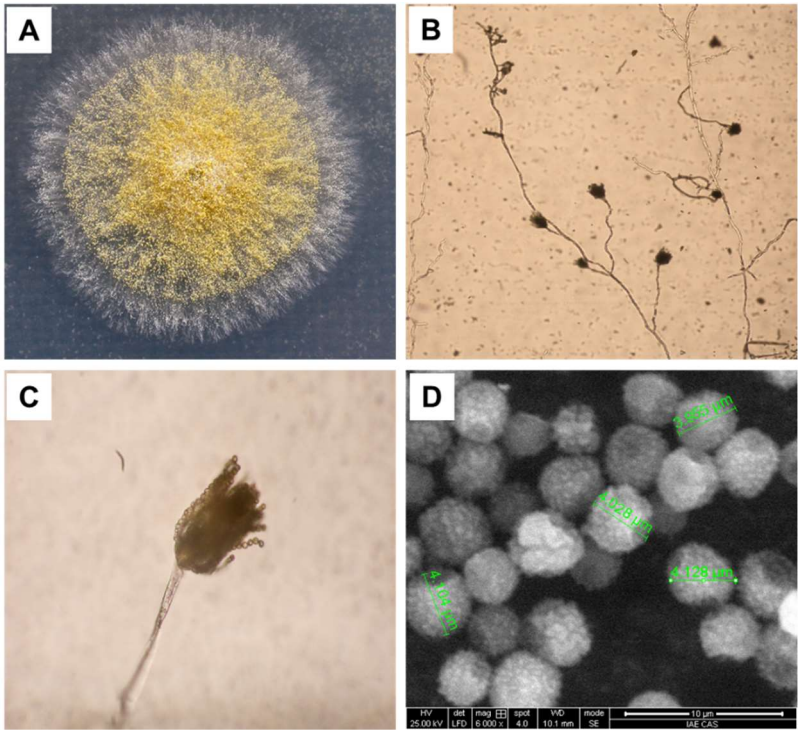


Figure S2 Morphological analysis of HMP-F28. A: A representative colony with yellow to olivaceous conidia and white floccose edge; B and C: Morphological features under an optical microscope; D: Conidia captured with an environmental scanning electron microscope.

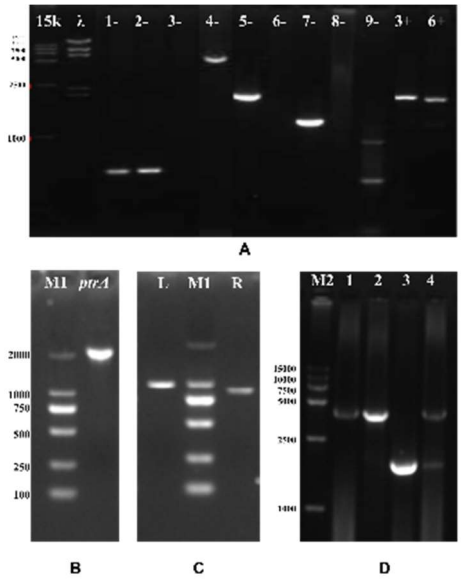


Figure S3 (A): DNA fragments of *cpaS* amplified from the genomic DNA of *A. oryzae* HMP-F28. 15k and λ are DL15, 000 DNA marker and λ-*Hin*d III digest DNA marker, respectively. Signs of + and – indicate PCR reactions with or without DMSO. Fragments 3, 6 gave amplicon bands only with the addition of DMSO. Fragment 8 failed to give amplicon bands with or without DMSO; (B): The *ptrA* gene fragment (2169 bp) amplified from the pPTRII plasmid. M1: DL2,000 DNA marker; (C): The left (L, 931 bp) and right (R, 831 bp) DNA fragments amplified from the *cpaS* gene of *A. oryzae* HMP-F28; (D): The DNA fragment (3931 bp) from fusion PCR of the left, right and *ptrA* fragments. 15K: DL15, 000 DNA marker; Lanes 1~4 are different fusion PCR reactions with different combinations of the left, *ptrA* and right fragments (*wt/wt/wt*): lane 1, 1:2:1; lane 2, 1:3:1; lane 3, 1:1:1; lane 4, 2:7:2. Lane 2 showed the best fusion efficiency, but lane 3 gave unspecific fusion band. The combination shown in lane 3 was chosen for the preparation of the DNA fragment for gene targeting.

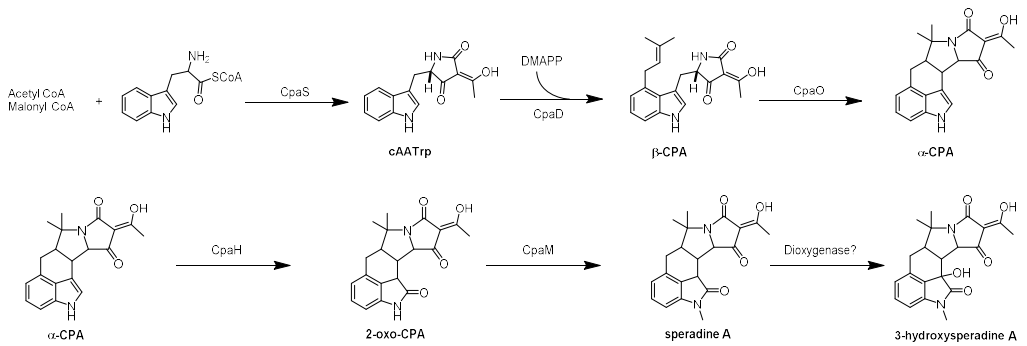
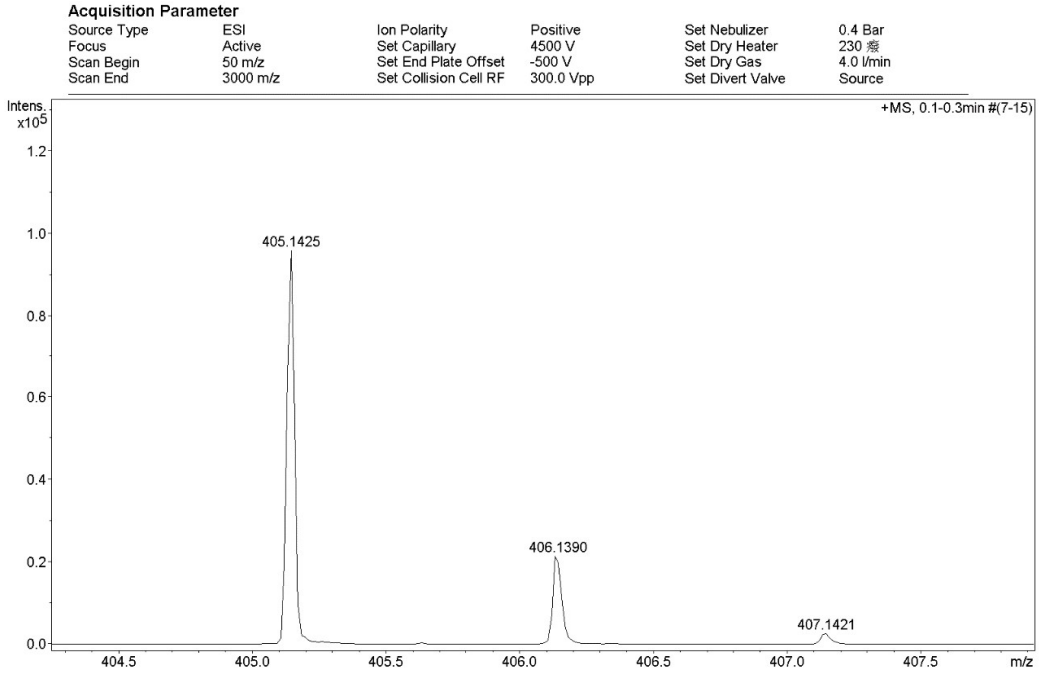
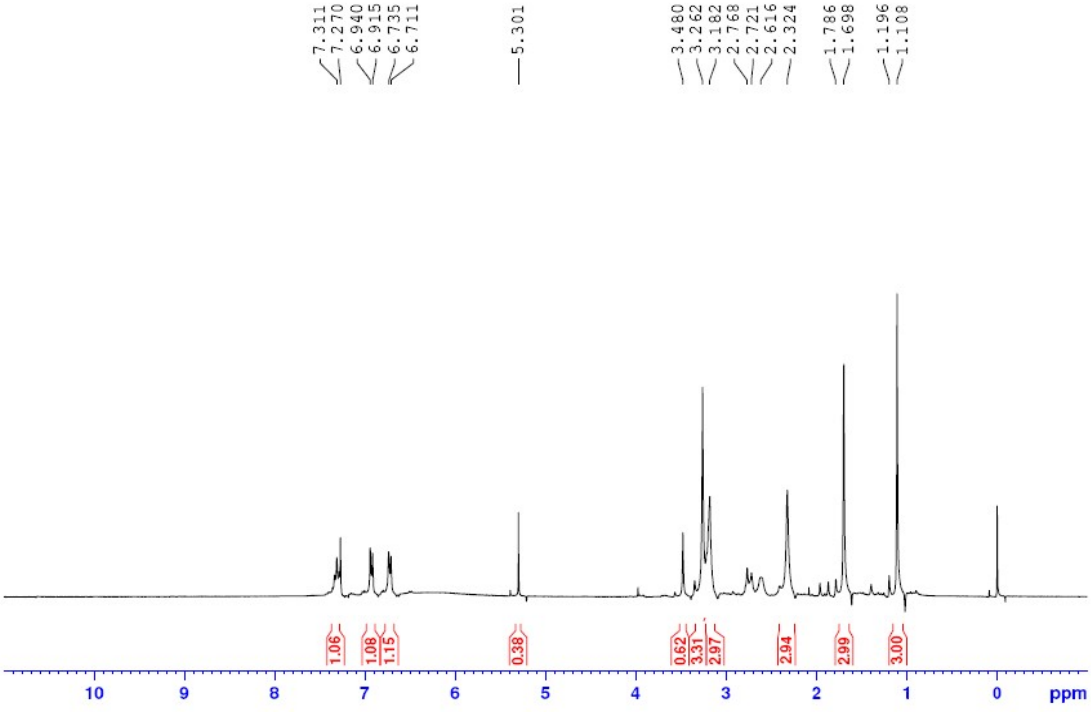


Figure S4. Proposed biosynthetic pathway of 3-hydroxysperadine A. CpaS: a hybrid PKS-NRPS; CpaD: a DMAPP transferase; DMAPP: dimethylallyl pyrophosphate; CpaO: a putative oxidoreductase; CpaH: a cytochrome P450; CpaM: a *N*-methyltransferase.

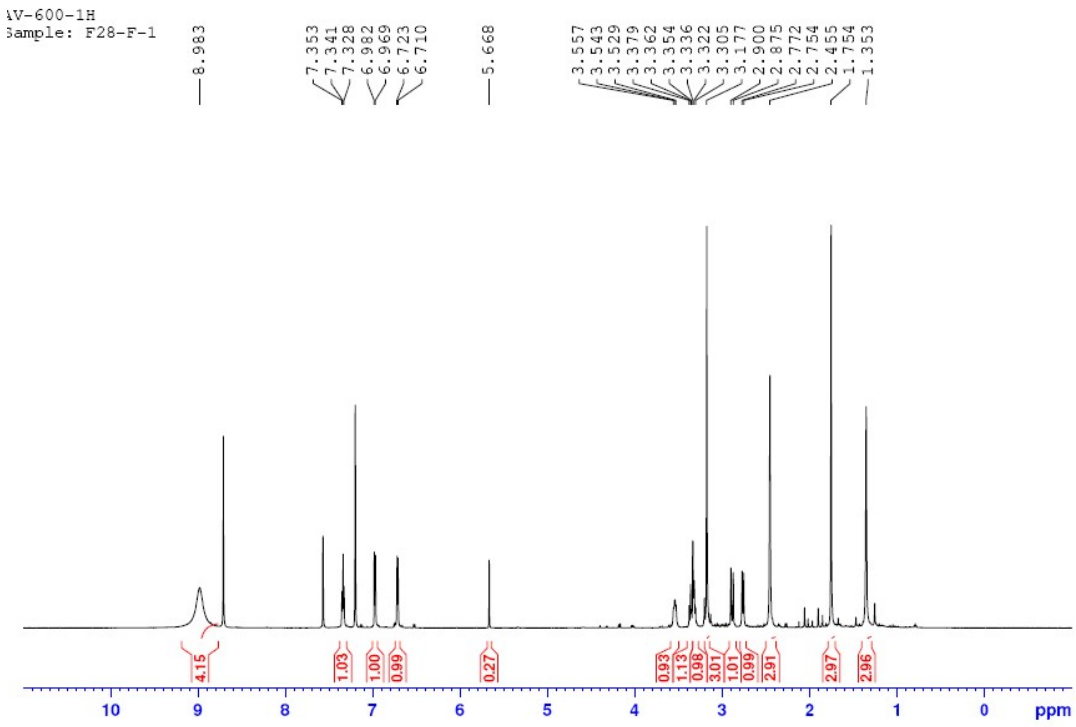
Appendix A: Spectra of compound 4.



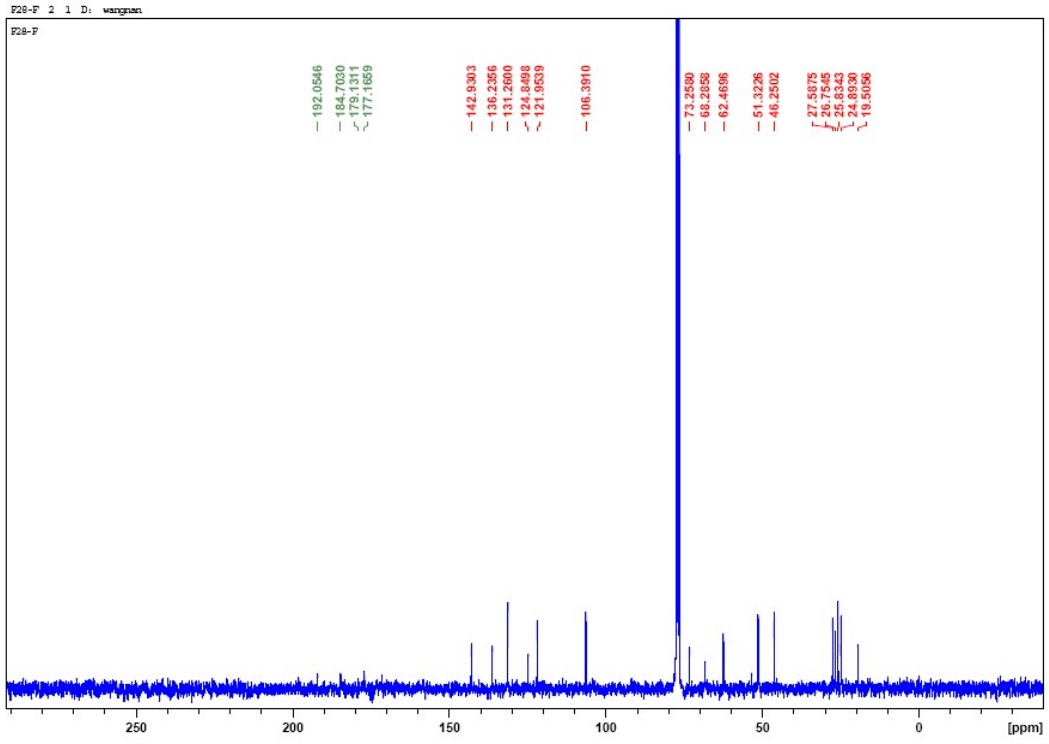
The HR-ESI-MS spectrum of compound **4**



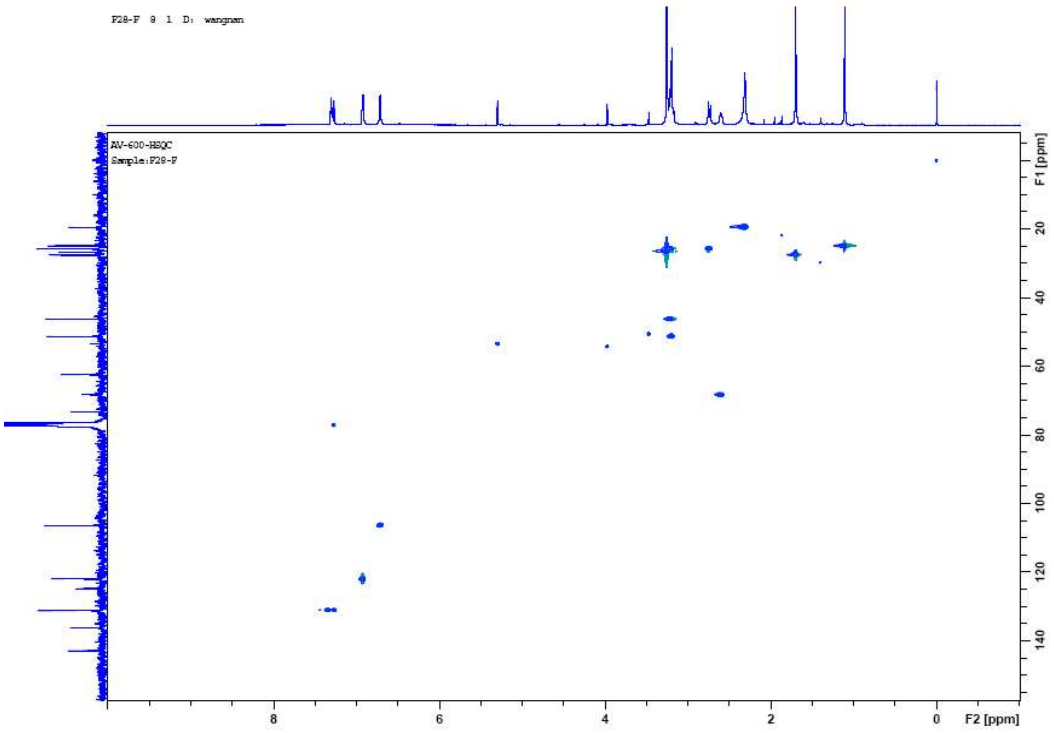
The ^{1}H-NMR spectrum of compound **4** in $CDCl_3$



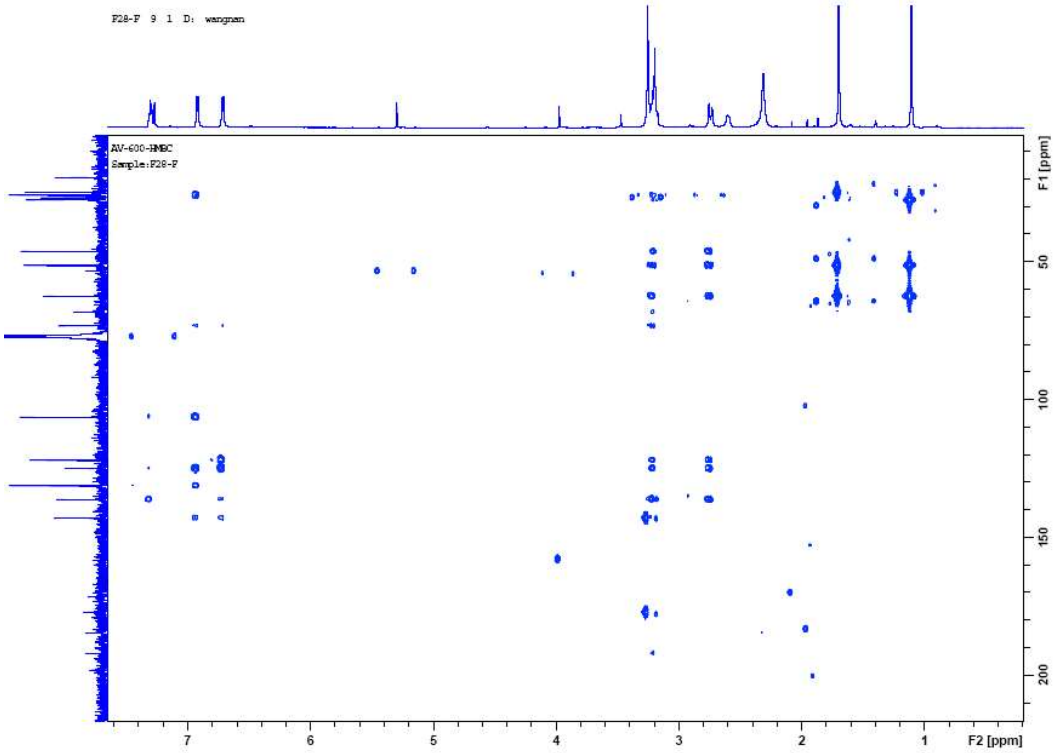
^{1}H NMR spectrum of compound **4** in pyridine-d_5



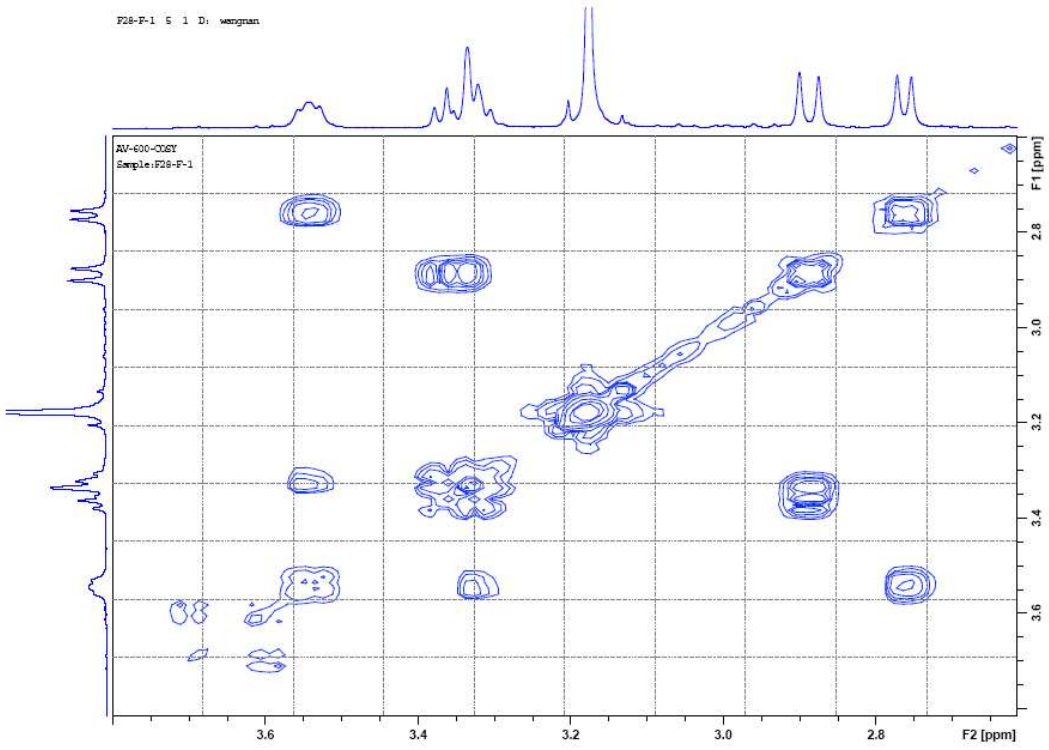
The ^{13}C-NMR spectrum of compound **4** in $CDCl_3$



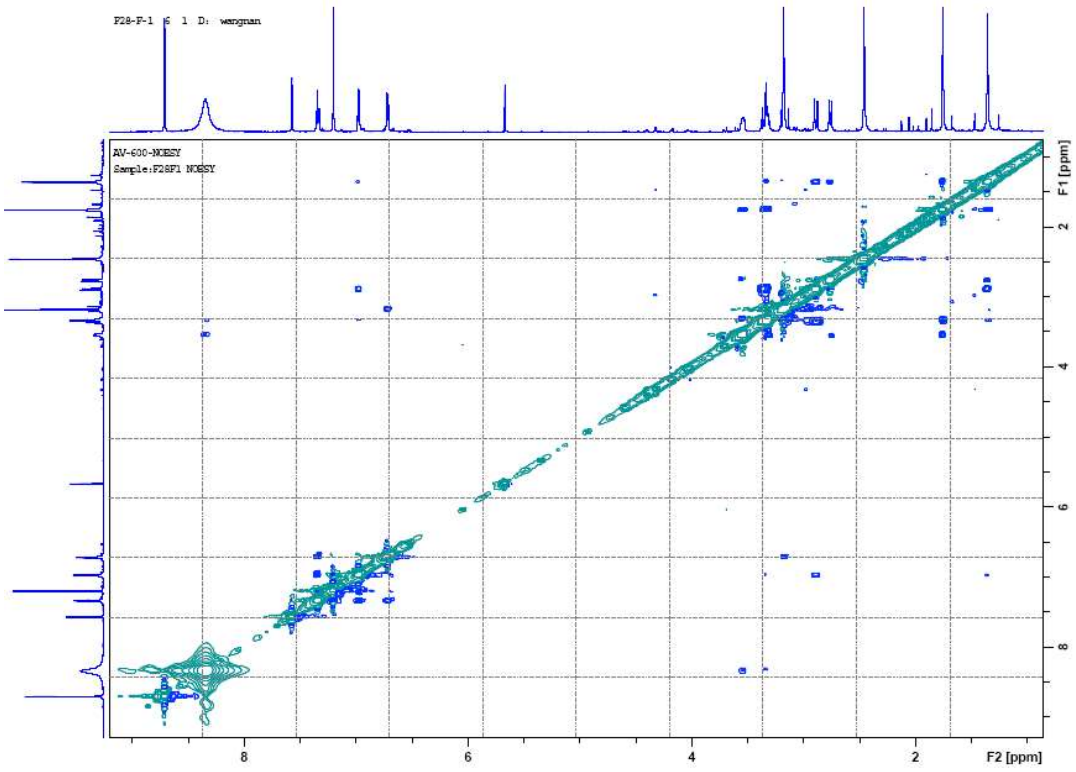
The HSQC spectrum of compound **4** in $CDCl_3$



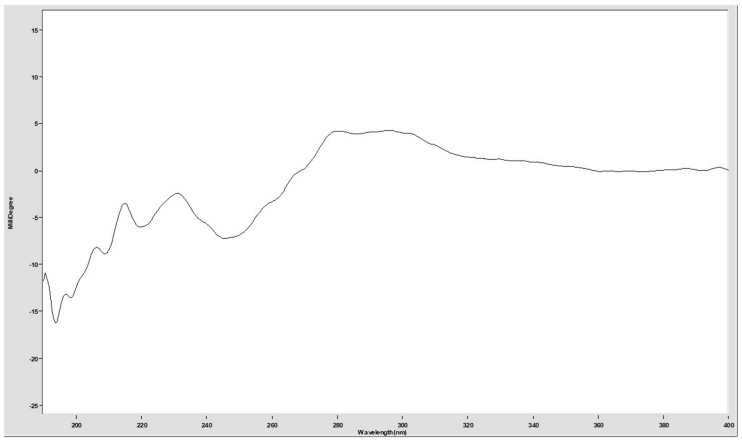
The HMBC spectrum of compound **4** in $CDCl_3$



The ^{1}H-^{1}H COSY spectrum of compound **4** in pyridine-d_5



The NOESY spectrum of compound **4** in pyridine-d_5



The CD spectrum of compound **4**.