Supporting Information

Talaramide A, an Unusual Alkaloid from the Mangrove Endophytic Fungus *Talaromyces* sp. (HZ-YX1) as Inhibitor of Mycobacterial PknG

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Fig. S1 HREIMS spectrum of **1**

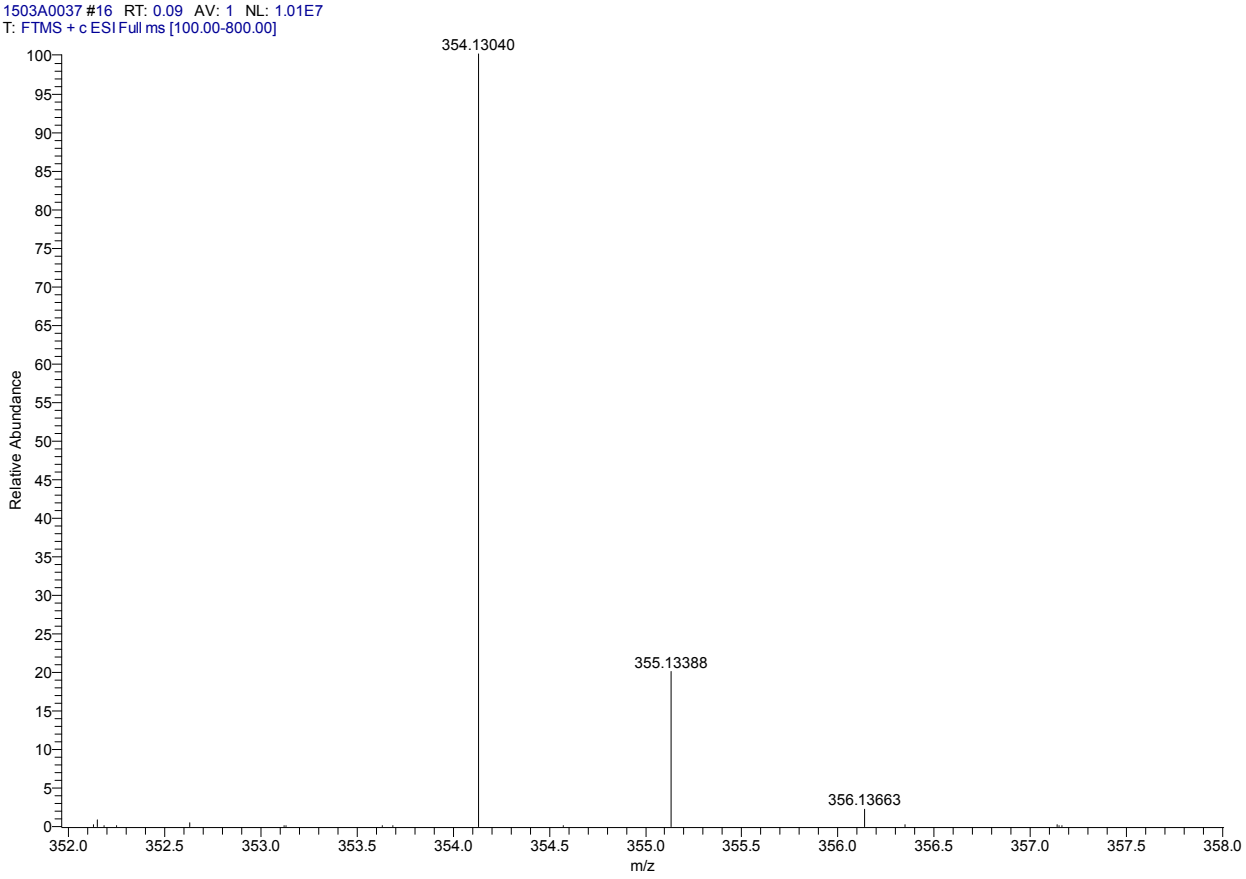


Fig. S2 ^{1}H NMR spectrum of **1** in DMSO-d_6

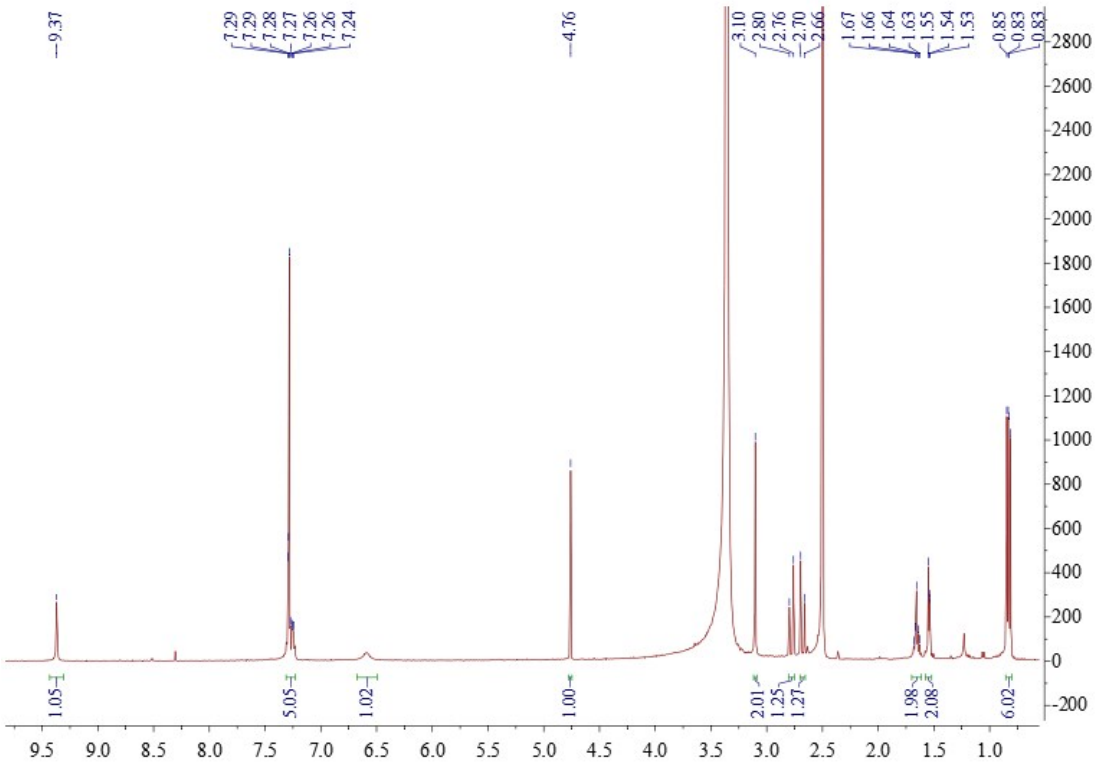


Fig. S3 ^{13}C NMR spectrum of **1** in DMSO-d_6

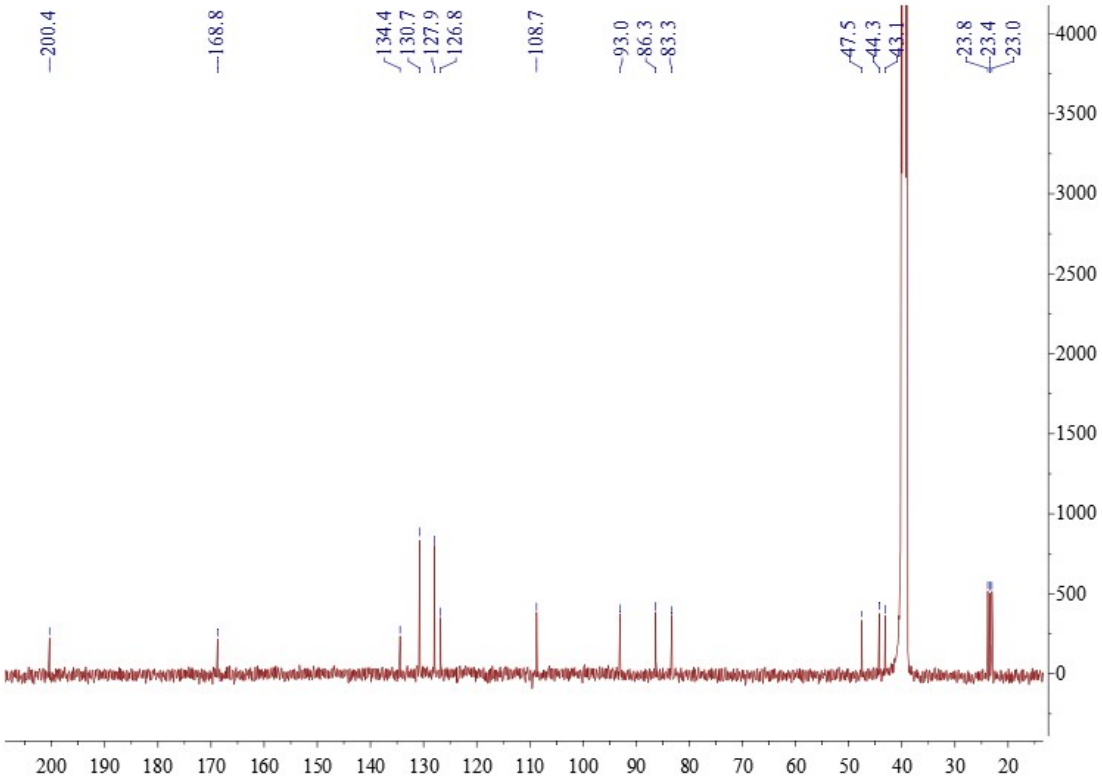


Fig. S4 HSQC spectrum of **1** in DMSO-d_6

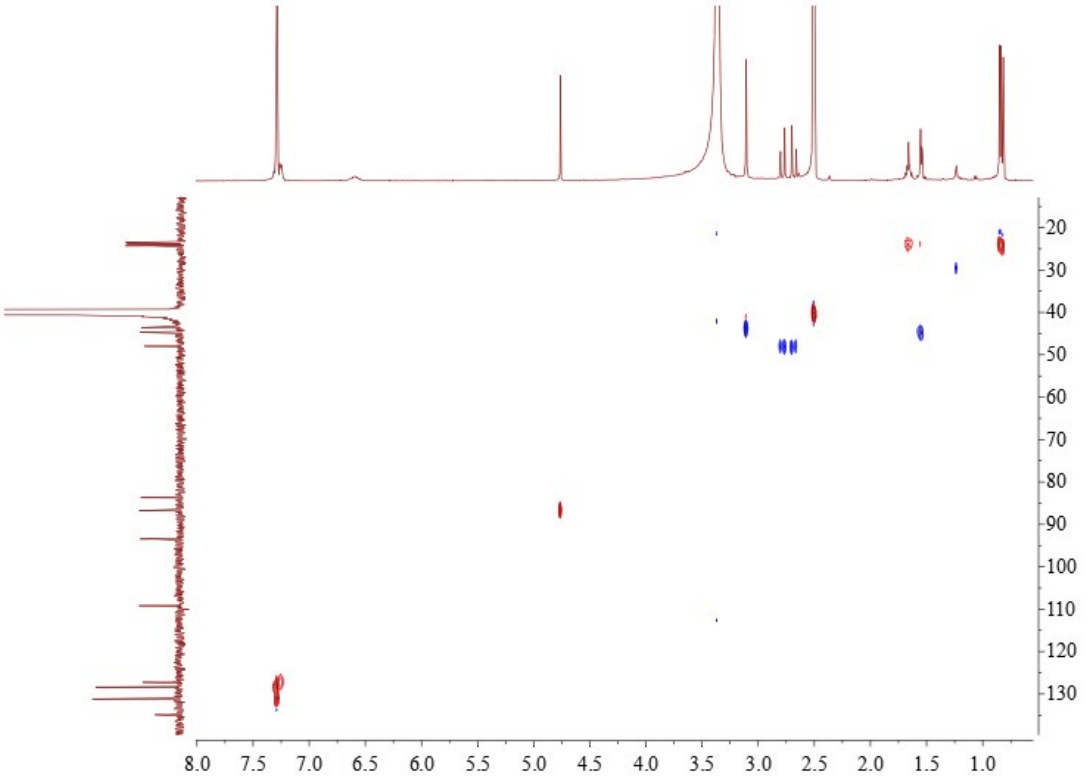


Fig. S5 ^{1}H-^{1}H COSY spectrum of **1** in DMSO-d_6

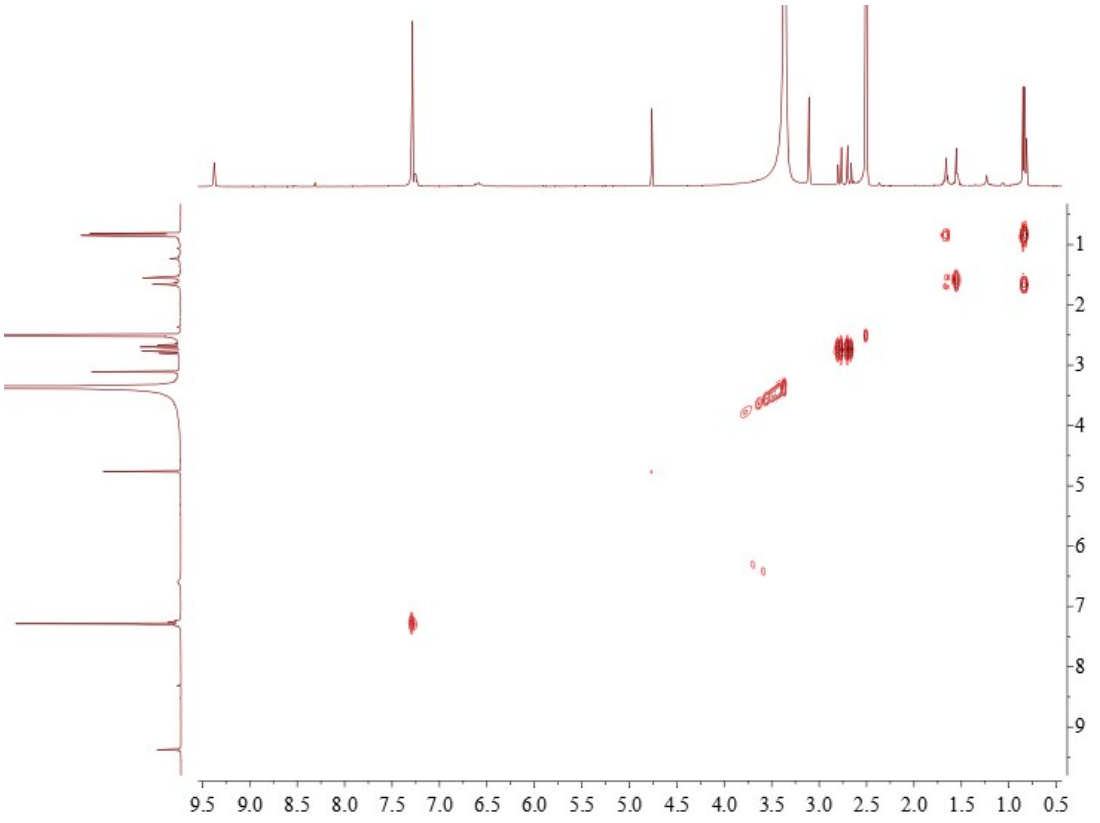


Fig. S6 HMBC spectrum of **1** in DMSO-d_6

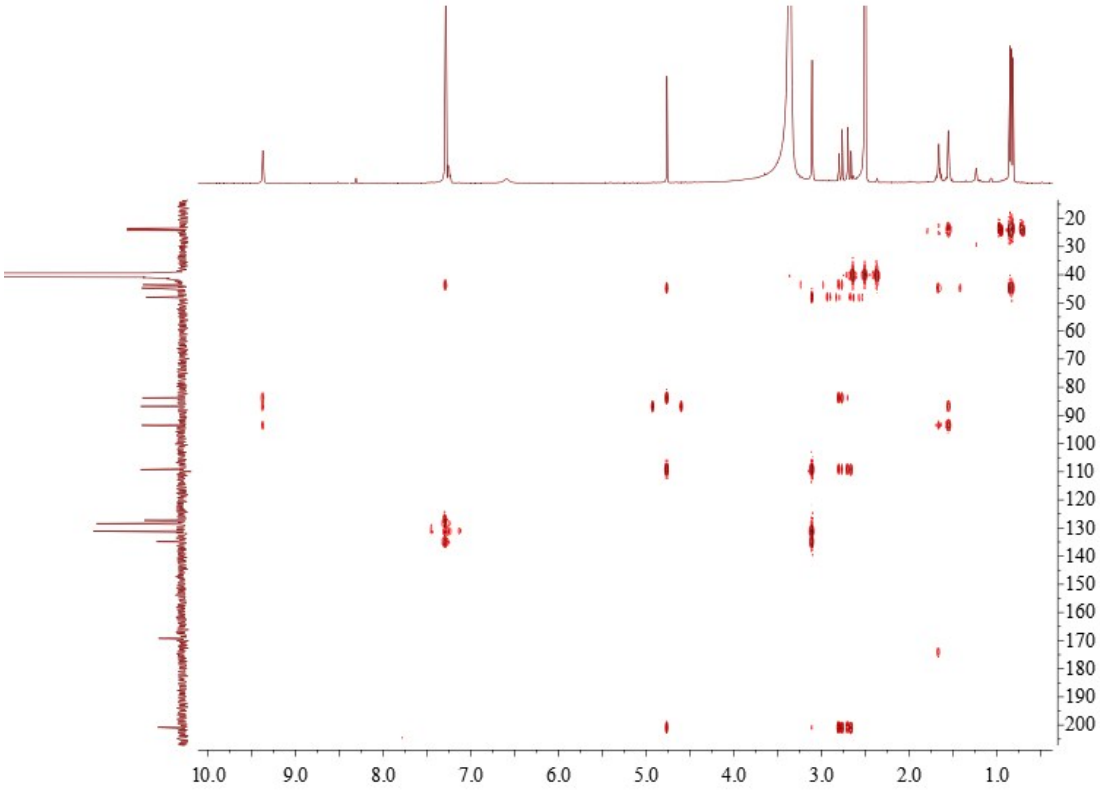
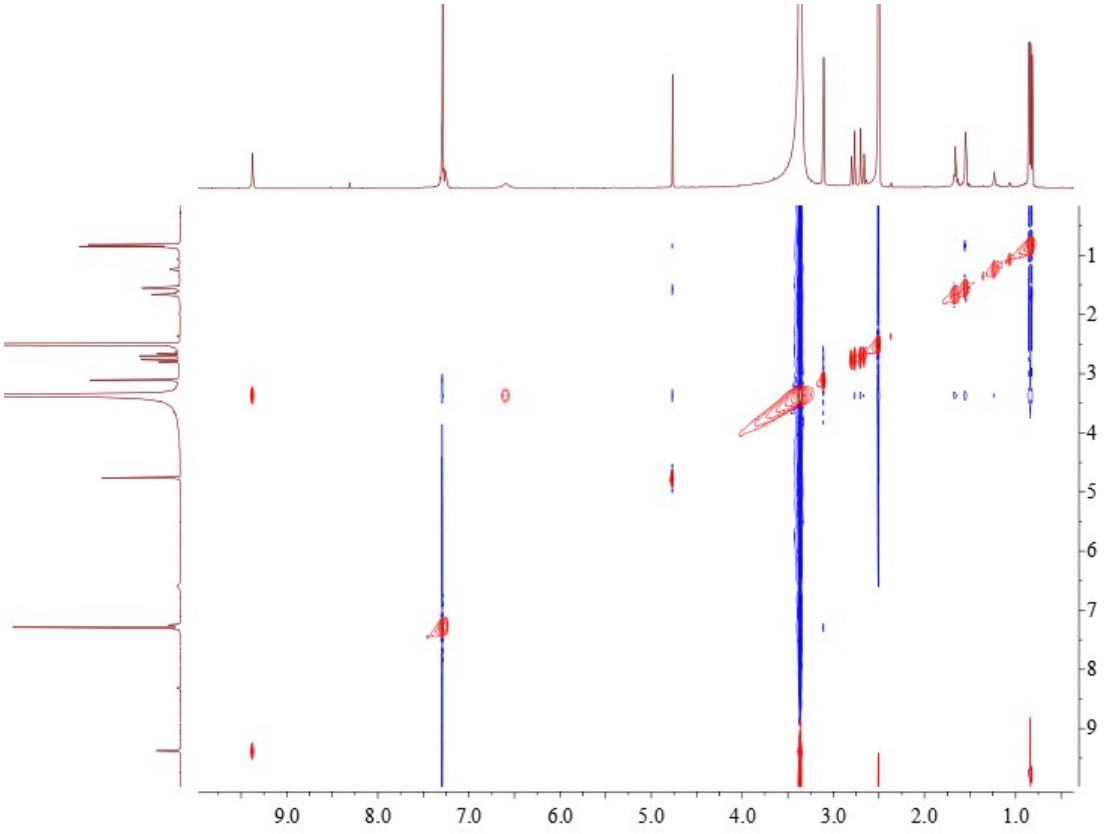


Fig. S7 NOESY spectrum of **1** in DMSO-d_6



Computational details

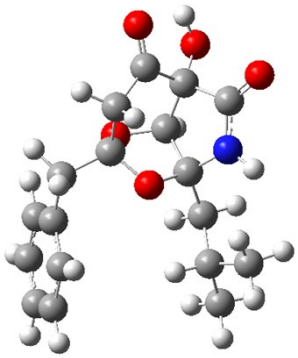
Molecular Merck force field (MMFF) and DFT/TD-DFT calculations were carried out with Spartan' 14 software (Wavefunction Inc., Irvine, CA, USA) and Gaussian 09 program, respectively. Conformers within 10 kcal/mol energy window were generated and optimized using DFT calculations at B3LYP/6-31G(d) level. Conformers with Bolzmann distribution over 1% were chosen for ECD calculations in methanol at B3lYP/6-311+g(2d,p) level. The IEF-PCM solvent model for MeOH was used. ECD spectra were generated using the program SpecDis 3.0 (University of Würzburg, Würzburg, Germany) and OriginPro 8.5 (OriginLab, Ltd., Northampton, MA, USA) from dipole-length rotational strengths by applying Gaussian band shapes with sigma = 0.30 ev. All calculations were performed by Tianhe-2 in National Super Computer Center in Guangzhou.

Table S1. Energy Analysis for the Conformers of (2*S*,5*R*,7*S*,8*S*)-**1**.

compound	Conformation	G (Hartree)	G (Kcal/mol)	ΔG (Kcal/mol)	Boltzmann Dist (%)
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-1**	-1129.44085768	-708925.9635	0	24.03
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-2**	-1129.44068257	-708727.3037	0.109882033	19.96
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-3**	-1129.44063478	-708727.2737	0.139870396	18.98
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-4**	-1129.44052035	-708727.2019	0.211675553	16.81
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-5**	-1129.44049151	-708727.1838	0.229772737	16.30
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-6**	-1129.44026162	-708727.0395	0.374029379	12.78
(2*S*,5*R*,7*S*,8*S*)-**1**	**1-7**	-1129.44012904	-708726.9563	0.457223713	11.10

Figure S8. B3LYP/6-31G(d) optimized low-energy conformers of **1**.

1-1



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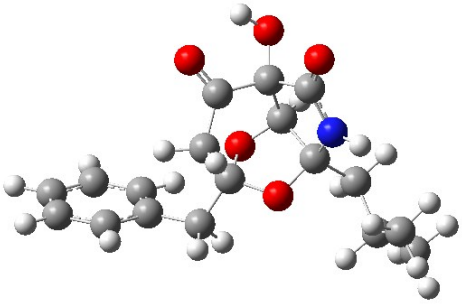
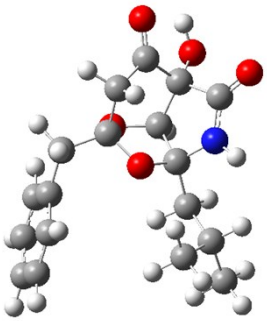


1-3

1-4



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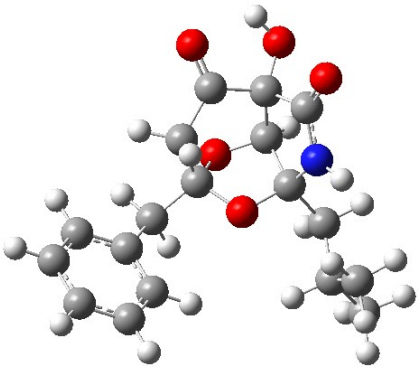

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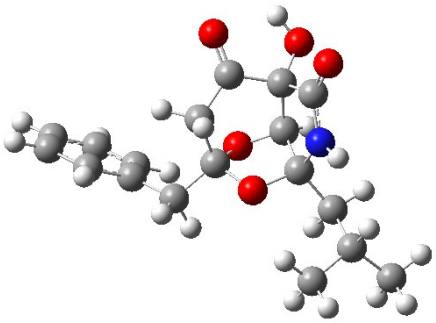

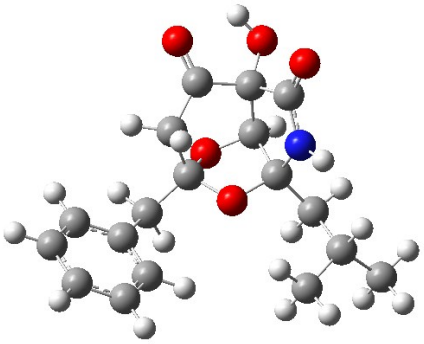

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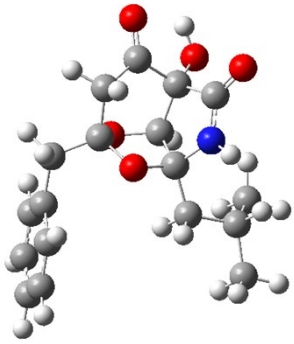

References.

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Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, revision C.01. Gaussian, Inc.: Wallingford CT, 2010.Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. SpecDis: Quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality 2013, 25, 243–249.

PknG Inhibition Assay.

Purification of PknG. The target enzyme PknG was prepared according to a modified literature procedure.[1-2] *E.coli* BL21(DE3)/pET28a(+)-PknG cells (Invitrogen) and grown in LB medium containing 50 μg/ml kanamycin at 37 °C till the OD_{600} of the solution were about 0.5. After addition of 0.1 mM IPTG, the culture was grown for another 16 h at 20 °C. The cells were harvested by centrifugation at 8,000 rpm for 3 min at 4 °C. The bacterial cell pellets were resuspended in the buffer containing 20 mM Tris, pH 7.9, 500 mM NaCl, 5 mM imidazole, and 1% TritonX-100, 10μLDTT(1M) were added. Cellular debris was removed by centrifugation at at 12,000 rpm for 30 min at 4 °C. The protein was purified from the supernantant using Ni SepharoseTM 6 FastFlow (GE Healthcare) according to the manufacturer's instructions. Protein concentration was measured using the Quick Start Bradford assay (Bio-Rad) according to the manufacturer's instructions with bovine serum albumin as standard. The purified PknG protein were stored in 20% glycerol at –20 °C.

PknG Inhibition Assay. PknG enzymatic activity was determined by quantification of the ADP generated by the kinase using the ADP–Glo luciferase reporter kit (Promega, USA).[2] PknG enzymatic activity was determined by quantification of the ADP generated by the kinase using the ADP–Glo luciferase reporter kit (Promega, USA). The fundamental principle as follow:

PknG+ATP ⟶ PknG-Pi+ADP

Enzyme solutions were prepared to give (0.6 μg/μL) in solution (25mM Tris-HCl (pH 7.8)). Buffer solutions [25mM Tris-HCl (pH7.8), 0.5mM $MgCl_2$, 20μM $MnCl_2$, 0.1mg/mL BSA] were prepared as reaction solutions. ATP solutions were prepared to give (0.6 μg/μL) in buffer solution. Diluted enzyme solution (3 μL), test samples (1 μL, in DMSO), and buffer solution (45 μL) were mixed in each well of a 96-well microtiter plate. After mixing five minutes, 1 μL ATP solutions was added in mixture solutions to react for 10 min at 37 °C. Move 5 μL reacted mixture solutions to a 384-well microtiter plate, then added 5μL ADP-Glo™ to incubate 40 minutes at 37 °C. 10 μL Kinase Detection Reagent was added to incubate 30 minutes at 37 °C. The absorbance were measured by a microtiter plate reader (infiniteM200：GE Healthcare).

Reference：

1. Walburger, A. Koul, G. Ferrari, L. Nguyen, C. Prescianotto-Baschong, K. Huygen, B. Klebl, C. Thompson, G. Bacher and J. Pieters, *Science*, 2004, **304**, 1800–1804.
2. N. Anand, P. Singh, A. Sharma, S. Tiwari, V. Singh, D. K. Singh, K. K. Srivastava, B. N. Singh and R. P. Tripathi, *Bioorg. Med. Chem.*, 2012, **20**, 5150–5163.