Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Alkyl-Modified Nucleobases with 6/5/7/5 Ring Systems from the Insect *Cyclopelta parva*

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Contents of Supporting Information

Table S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of **2** (δ in ppm, J in Hz)

	2[a]		**2**[b]	
no	δ_H	δ_C	δ_H	δ_C
1		110.8 s		112.6, s
2		150.2 s		152.2, s
4	8.33 (s)	152.1 d	8.39 (s)	153.7, t
6		159.4 s		159.8, s
8	8.41 (s)	144.1 d	8.39 (s)	145.5, s
1′	Ha: 3.89 (dd, 9.3, 3.3)	73.1 t	Ha: 4.06 (dd, 9.6, 3.3)	74.9 t
	Hb: 3.76 (dd, 9.3, 1.8)		Hb: 3.92 (dd, 9.6, 1.9)	
2′	5.34 (dd, 5.4, 3.2)	57.6 d	5.38 (dd, 5.5, 3.3)	60.0 t
3′	Ha: 2.67 (dd, 14.1, 5.5)	42.0 t	Ha: 2.81 (dd, 14.4, 5.5)	43.3 t
	Hb: 2.29 (dd, 14.1, 1.8)		Hb: 2.47 (dd, 14.4, 1.8)	
4′		94.5 s		96.1 s
5′	Ha: 2.07 (m)	32.3 t	Ha: 2.17 (m)	33.9 t
	Hb: 1.87 (m)		Hb: 2.02 (m)	
6′	1.00 (t-like, 7.4)	8.7 q	1.14 (t-like, 7.5)	8.9 q
6-*N*H	8.46 (brs)			

[a] In DMSO-d_6; [b] In Methanol-d_4.

Figure S1. Optimized geometries of predominant conformers for (1′*R*,3′*R*,4′*S*)-**1** at the B3LYP/6-31G(d,p) level

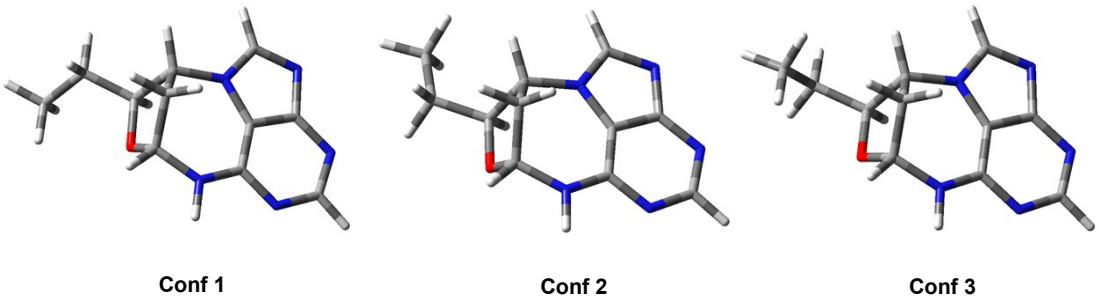


Figure S2. Optimized geometries of predominant conformers for (2′*R*,4′*R*)-**2** at the B3LYP/6-31G(d,p) level

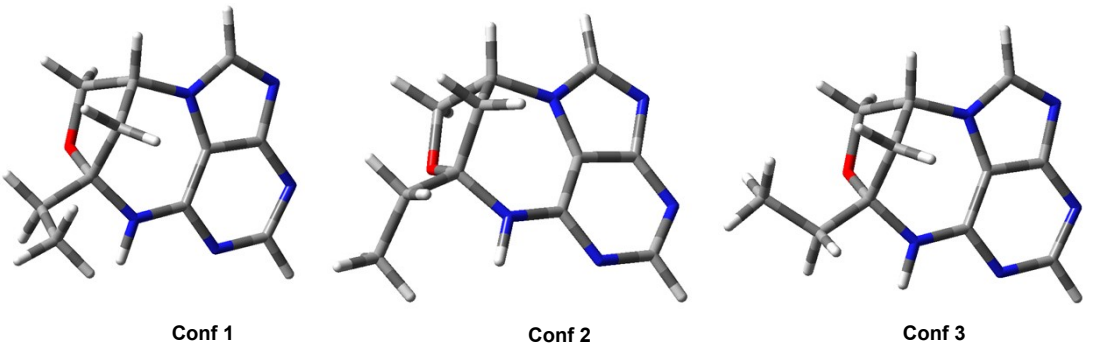


Table S2. The Cartesian coordinates of the lowest energy conformers for (1′*R*,3′*R*,4′*S*)-**1**

Conf 1	X axis(Å)	Y axis(Å)	Z axis(Å)	Conf 2	X axis(Å)	Y axis(Å)	Z axis(Å)
N	2. 429542	−1. 713392	0. 433504	N	2. 690173	−1. 410964	−0. 409405
C	3. 456236	−0. 883861	−0. 729585	C	3. 586876	−0. 412373	−0. 578331
N	3. 498531	0. 454275	−0. 648085	N	3. 405181	0. 907658	−0. 424493
C	2. 327336	0. 973514	−0. 215625	C	2. 137442	1. 206425	−0. 061567
C	1. 232746	0. 19741	0. 102032	C	1. 163923	0. 248274	0. 126511
C	1. 273087	−1. 178309	0. 001467	C	1. 434213	−1. 094403	−0. 041843
N	2. 053113	2. 304569	−0. 029854	N	1. 637634	2. 462863	0. 169201
C	0. 804867	2. 342469	0. 387822	C	0. 373175	2. 276038	0. 485897
N	0. 269297	1. 089384	0. 47695	N	0. 044118	0. 950709	0. 468046
C	−1. 12485	−0. 503626	1. 73324	C	−1. 159256	−0. 919893	1. 523518
C	−1. 089915	0. 739901	0. 862772	C	−1. 264753	0. 366632	0. 723604
C	−1. 094924	−1. 61204	0. 692962	C	−0. 874864	−1. 94164	0. 435538
C	−1. 912757	0. 309963	−0. 358433	C	−1. 914349	−0. 107456	−0. 583252
O	−1. 79979	−1. 12484	−0. 451453	O	−1. 570611	−1. 503033	−0. 733231
N	0. 234484	−2. 011584	0. 313715	N	0. 52502	−2. 099385	0. 141516

C	-3.395262	0.677421	-0.214541	C	-3.444706	-0.01213	-0.58981
C	-4.207318	0.275104	-1.438708	C	-3.955206	1.418994	-0.638289
H	4.365085	-1.366052	-1.07705	H	4.584512	-0.721596	-0.875847
H	0.253714	3.242461	0.629814	H	-0.33345	3.060061	0.727468
H	-2.081667	-0.535935	2.268834	H	-2.134572	-1.136485	1.976698
H	-0.322853	-0.567414	2.476084	H	-0.413342	-0.90264	2.325025
H	-1.505063	1.614269	1.37609	H	-1.844123	1.126367	1.256489
H	-1.625081	-2.494789	1.065063	H	-1.276981	-2.919414	0.720265
H	-1.524747	0.741103	-1.289293	H	-1.522271	0.422477	-1.459887
H	0.411068	-2.987314	0.116797	H	0.8714	-3.020305	-0.09102
H	-3.495047	1.758195	-0.061487	H	-3.863362	-0.526702	0.283604
H	-3.828281	0.17634	0.659109	H	-3.829888	-0.556038	-1.461786
H	-5.251831	0.577585	-1.313932	H	-5.047212	1.42533	-0.71516
H	-4.187101	-0.808383	-1.59195	H	-3.552708	1.948828	-1.507446
H	-3.820114	0.758207	-2.34144	H	-3.682965	1.977418	0.261534
Conf 3	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**				
N	2.484341	-1.704754	-0.252957				
C	3.53707	-0.871993	-0.424697				
N	3.56367	0.467183	-0.349337				
C	2.345269	0.983781	-0.072693				
C	1.223825	0.203709	0.111638				
C	1.280643	-1.172763	0.03138				
N	2.041561	2.315017	0.062915				
C	0.749293	2.348784	0.315973				
N	0.215067	1.093359	0.347832				
C	-1.29836	-0.479839	1.46093				
C	-1.181868	0.727196	0.545184				
C	-1.149665	-1.631047	0.481055				
C	-1.796531	0.228426	-0.771027				
O	-1.783516	-1.21243	-0.729657				
N	0.215546	-2.008964	0.221766				
C	-3.223783	0.71884	-1.045306				
C	-4.259319	0.320037	-0.003752				
H	4.484267	-1.352399	-0.651619				
H	0.16627	3.24735	0.474366				
H	-2.302465	-0.505761	1.898945				
H	-0.578052	-0.502559	2.285651				
H	-1.682824	1.612033	0.951856				
H	-1.683002	-2.513216	0.850295				
H	-1.186494	0.527167	-1.633188				

H	0.422062	-2.985435	0.059471				
H	-3.550674	0.306035	-2.007929				
H	-3.220079	1.810296	-1.146949				
H	-5.254719	0.644196	-0.324874				
H	-4.056471	0.792712	0.961462				
H	-4.290417	-0.764654	0.136129				

Table S3. The Cartesian coordinates of the lowest energy conformers for (2′*R*,4′*R*)-**2**

Conf 1	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**	**Conf 2**	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**
N	1.167325	2.27021	0.351902	N	1.091945	2.32032	0.013744
C	2.515252	2.21457	0.254515	C	2.439654	2.308397	0.128538
N	3.289762	1.151078	-0.00582	N	3.266295	1.252448	0.144764
C	2.563049	0.022989	-0.171764	C	2.596881	0.082726	0.035858
C	1.187204	-0.004896	-0.084835	C	1.224759	0.009366	-0.079491
C	0.460416	1.137917	0.174435	C	0.443506	1.14541	-0.096812
N	3.060969	-1.225576	-0.444022	N	3.156161	-1.169498	0.019944
C	2.003936	-2.007751	-0.5135	C	2.138798	-1.997178	-0.097444
N	0.85005	-1.31008	-0.296158	N	0.951288	-1.325077	-0.156624
C	-1.502856	-0.906045	-0.920054	C	-1.289954	-1.097953	-1.168291
C	-0.501073	-1.838958	-0.265325	C	-0.376985	-1.900492	-0.259228
C	-1.819685	0.074564	0.213013	C	-1.795089	0.003847	-0.226565
C	-3.261419	0.610524	0.135164	C	-3.218184	0.442592	-0.62687
C	-3.588087	1.361838	-1.14895	C	-3.898905	1.370203	0.376659
C	-1.038605	-1.916517	1.156361	C	-1.12083	-1.821842	1.06578
O	-1.705105	-0.67387	1.433476	O	-1.833343	-0.574632	1.084492
N	-0.902872	1.191688	0.265824	N	-0.918804	1.155078	-0.222053
H	3.036993	3.154861	0.405788	H	2.912854	3.28176	0.217281
H	2.025875	-3.071952	-0.711873	H	2.212918	-3.076515	-0.139375
H	-2.396471	-1.489246	-1.178194	H	-2.118412	-1.747451	-1.479438
H	-1.140657	-0.425086	-1.83531	H	-0.808152	-0.717924	-2.075916
H	-0.471647	-2.822012	-0.747212	H	-0.253715	-2.933708	-0.600662
H	-3.966613	-0.2233	0.249415	H	-3.199783	0.928476	-1.610269
H	-3.454661	1.269149	0.99261	H	-3.857505	-0.445081	-0.716859
H	-4.621881	1.721146	-1.118015	H	-4.914215	1.601794	0.038223
H	-2.936793	2.230853	-1.282115	H	-3.976079	0.902539	1.362986
H	-3.487254	0.716749	-2.026856	H	-3.36324	2.317368	0.484388
H	-1.791766	-2.70766	1.24058	H	-1.869733	-2.618571	1.135051
H	-0.270002	-2.091405	1.915799	H	-0.473139	-1.881201	1.946236
H	-1.249715	2.098196	0.551128	H	-1.315157	2.08221	-0.148827

Conf 3	X axis(Å)	Y axis(Å)	Z axis(Å)				
N	-1.519886	-2.228511	0.10732				
C	-2.850781	-1.989014	0.144627				
N	-3.48923	-0.811923	0.070761				
C	-2.629095	0.22483	-0.046333				
C	-1.260218	0.065038	-0.087321				
C	-0.679812	-1.183395	-0.016538				
N	-2.969076	1.550181	-0.141777				
C	-1.822704	2.191884	-0.231796				
N	-0.763356	1.330543	-0.197305				
C	1.455394	0.691634	-1.073852				
C	0.646386	1.671559	-0.242185				
C	1.727084	-0.434011	-0.068543				
C	3.052252	-1.177375	-0.339525				
C	4.314572	-0.337564	-0.1743				
C	1.298731	1.525699	1.125275				
O	1.793097	0.180042	1.225201				
N	0.666236	-1.421819	-0.062252				
H	-3.483595	-2.865617	0.246334				
H	-1.712503	3.265493	-0.318193				
H	2.381993	1.191864	-1.373819				
H	0.955776	0.353626	-1.988524				
H	0.716116	2.696138	-0.622851				
H	3.14004	-2.016647	0.364209				
H	3.036388	-1.614621	-1.345762				
H	5.199169	-0.969771	-0.305485				
H	4.373463	0.461819	-0.917323				
H	4.37139	0.109265	0.822862				
H	2.165046	2.190986	1.211252				
H	0.626181	1.726618	1.965023				
H	0.902087	-2.3945	0.086372				

Figure S3. Compound **1** was analyzed and isolated by chiral HPLC

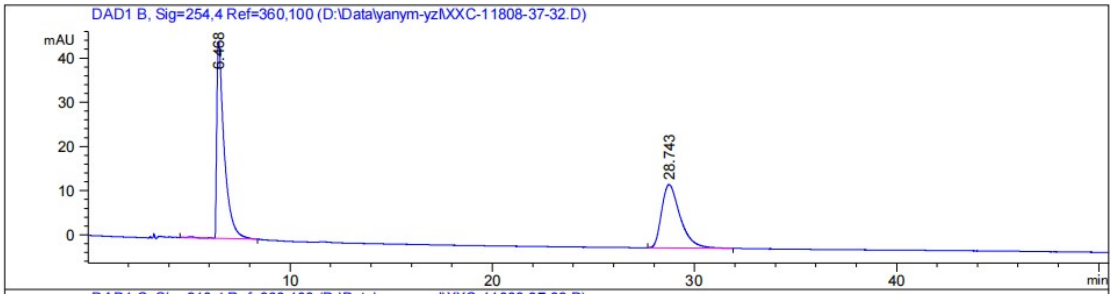


Figure S4. Compound **2** was analyzed and isolated by chiral HPLC

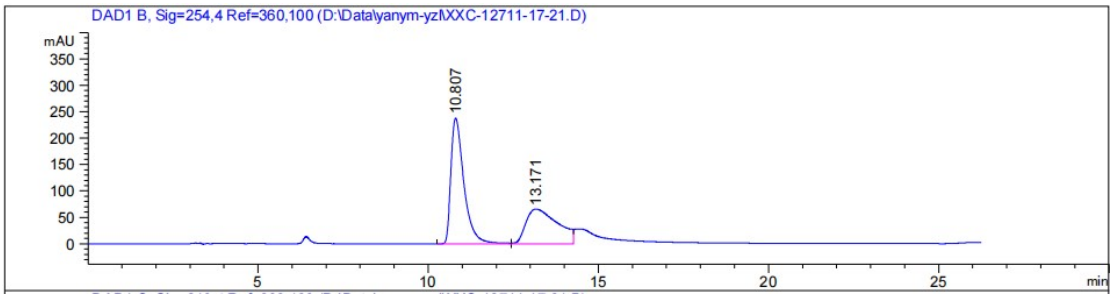


Figure S5. The UV spectrum of **1**

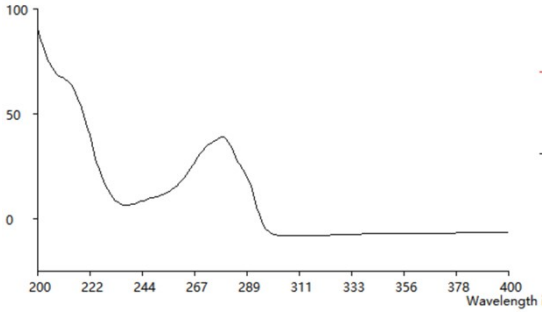


Figure S6. The UV spectrum of **2**

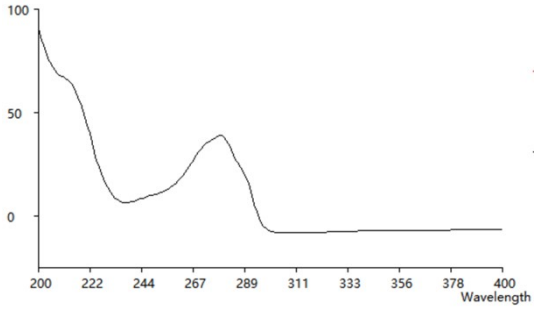


Figure S7. ^{1}H NMR (600 MHz) spectrum of **1** in DMSO-d_6

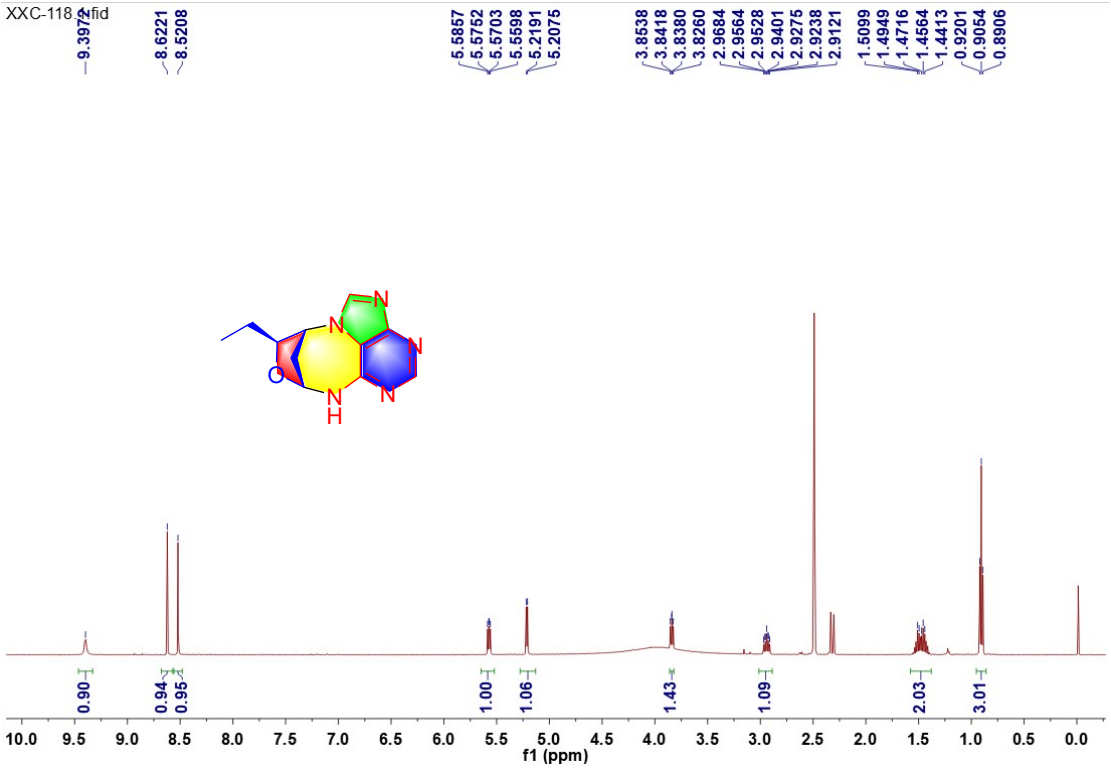


Figure S8. ^{13}C NMR and DEPT (150 MHz) spectra of **1** in DMSO-d_6

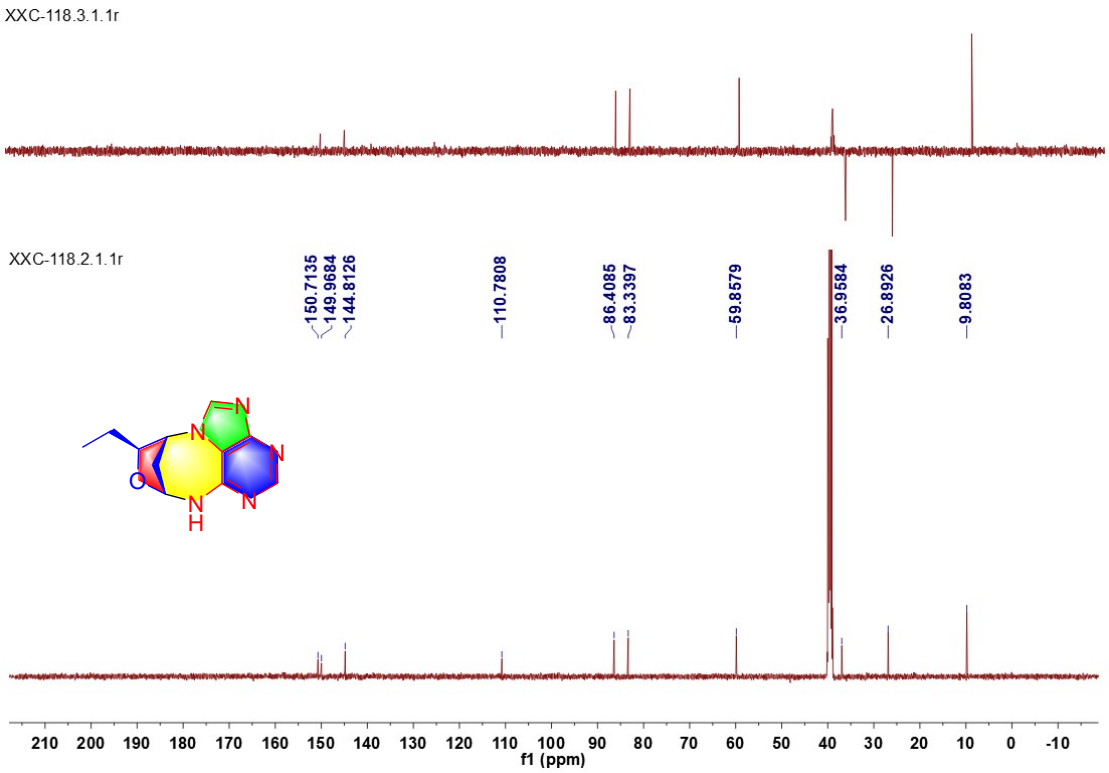


Figure S9. ^{1}H-^{1}H COSY (600 MHz) spectrum of **1** in DMSO-d_6

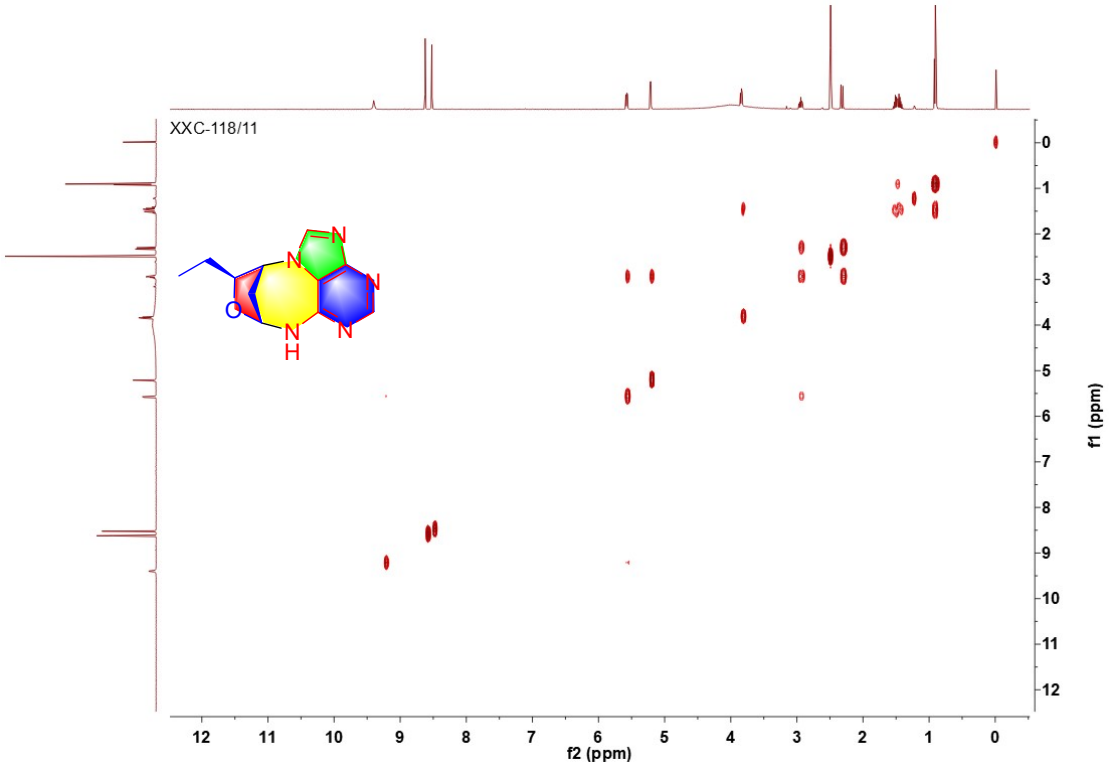


Figure S10. HSQC (600 MHz) spectrum of **1** in DMSO-d_6

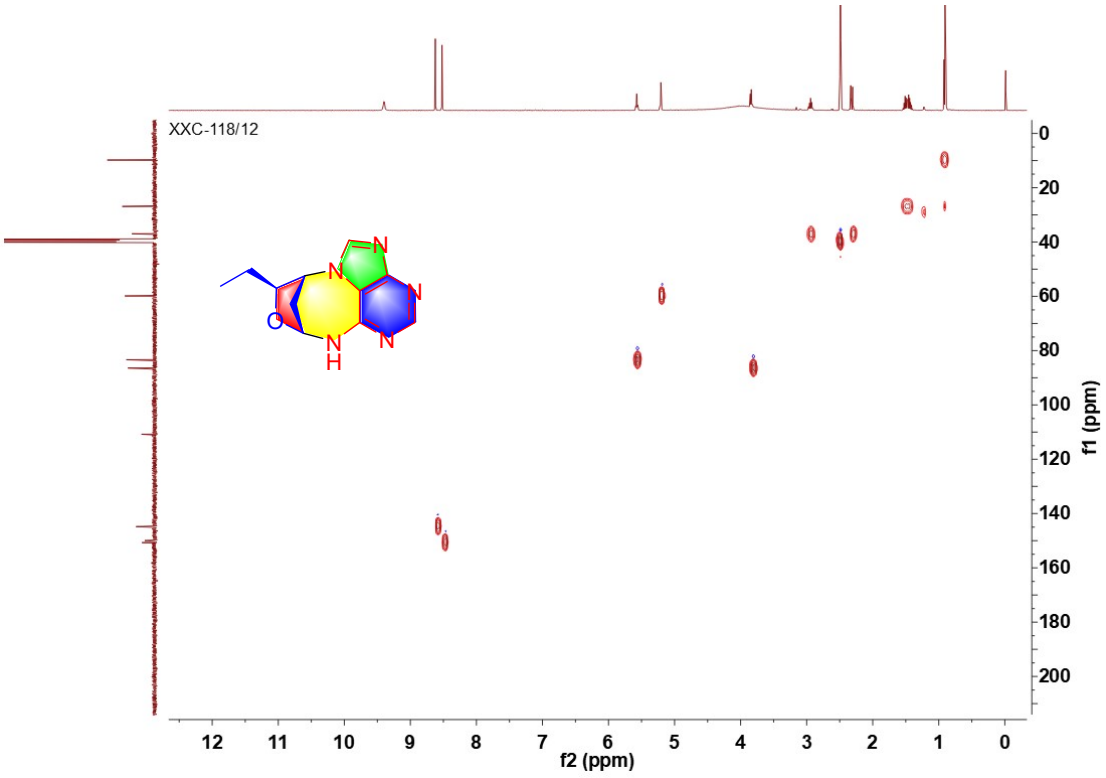


Figure S11. HMBC (600 MHz) spectrum of **1** in DMSO-d_6

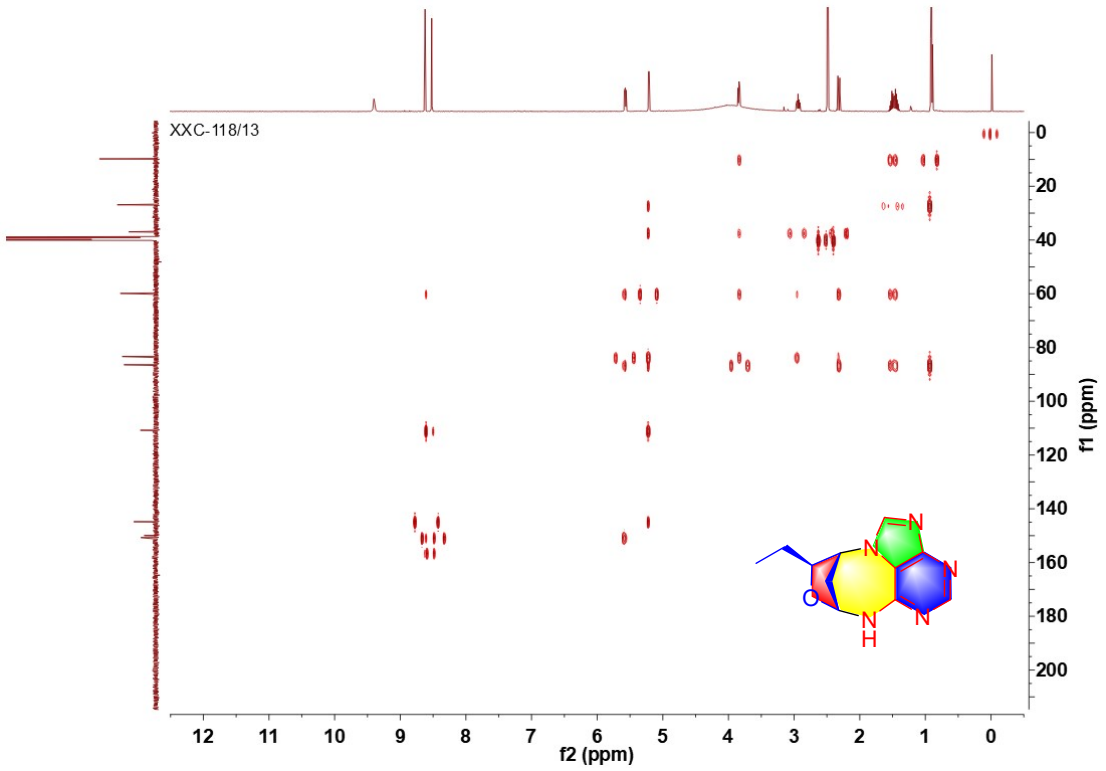


Figure S12. ROESY (600 MHz) spectrum of **1** in DMSO-d_6

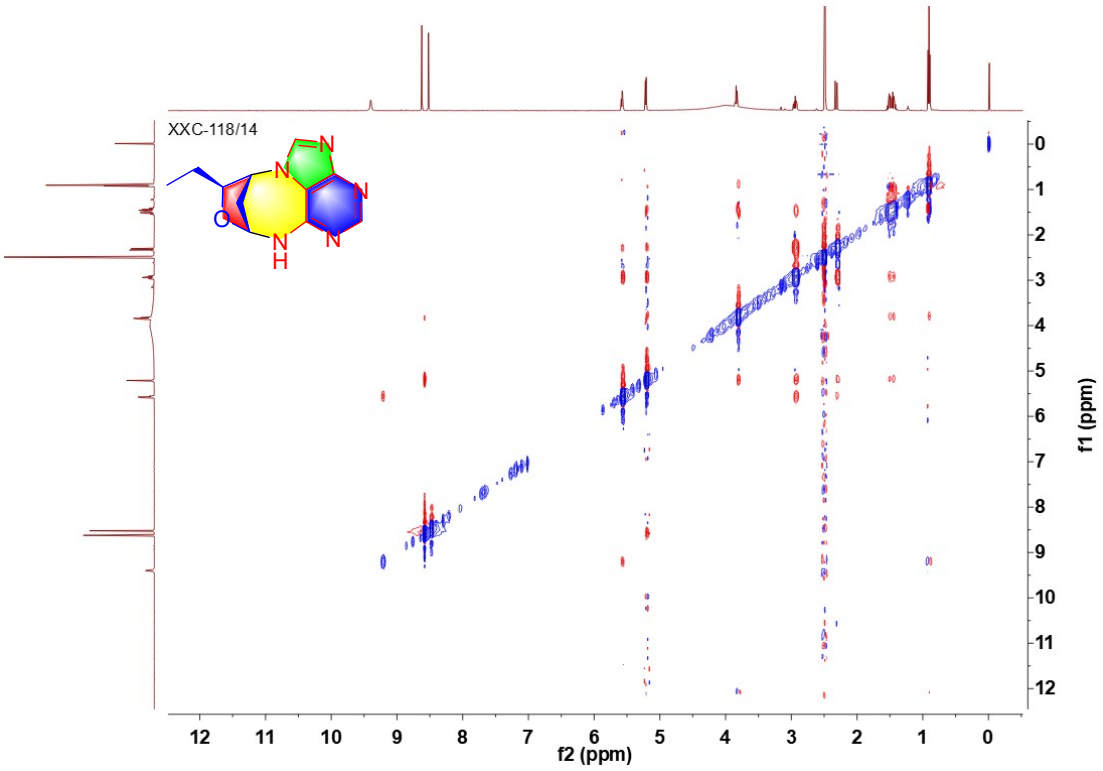


Figure S13. HRESIMS of **1**

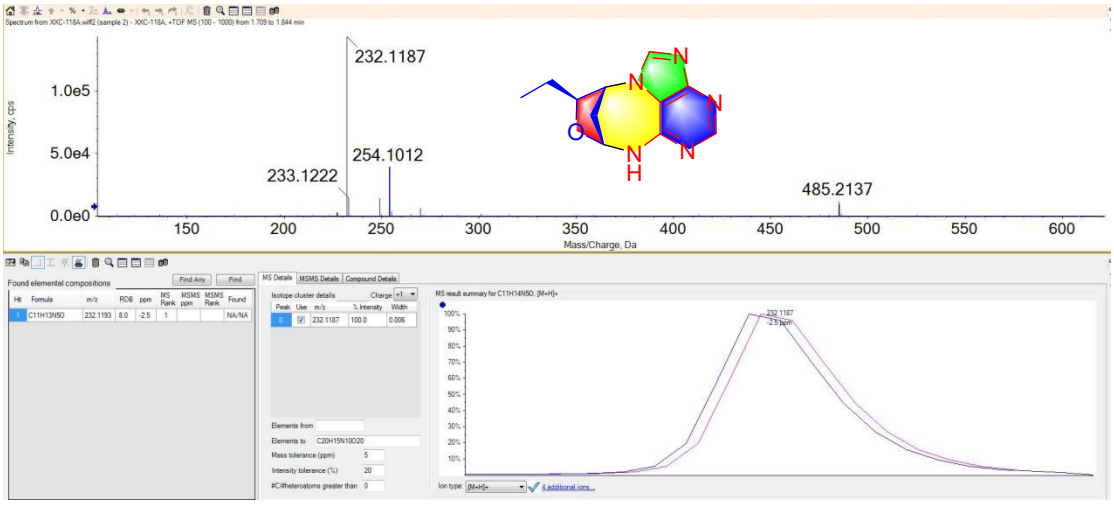


Figure S14. ^{1}H NMR (600 MHz) spectrum of **2** in methanol-d_4

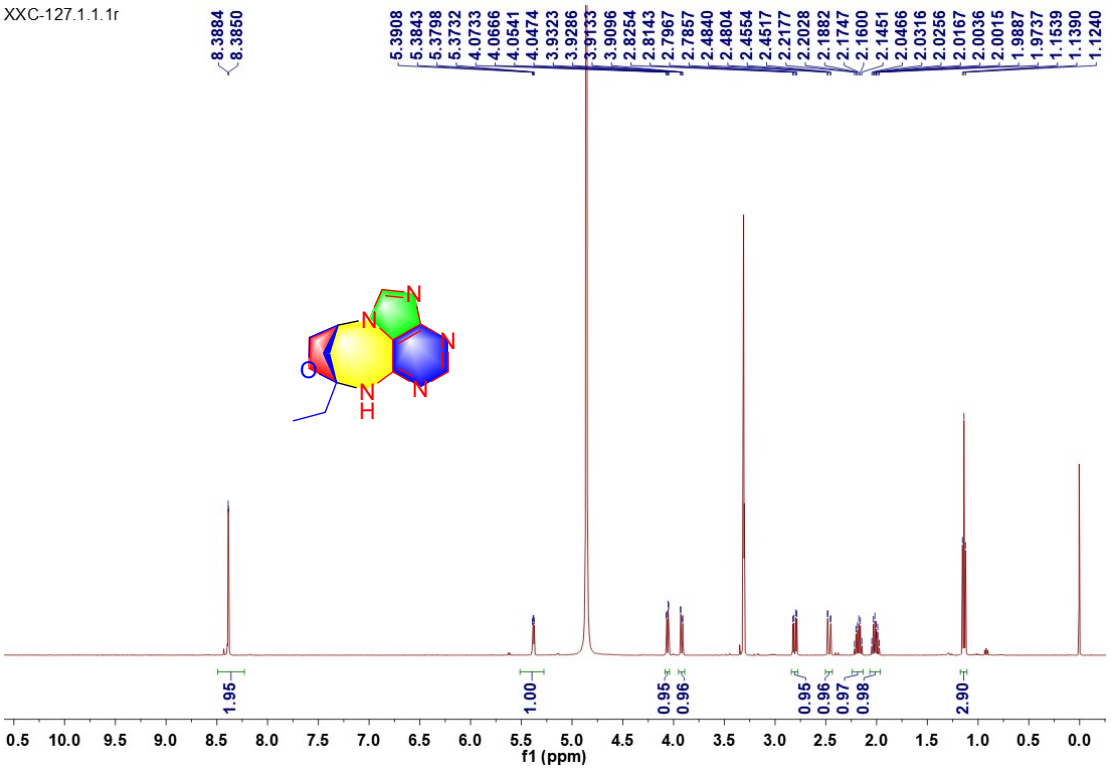


Figure S15. ^{13}C NMR and DEPT (150 MHz) spectra of **2** in methanol-d_4

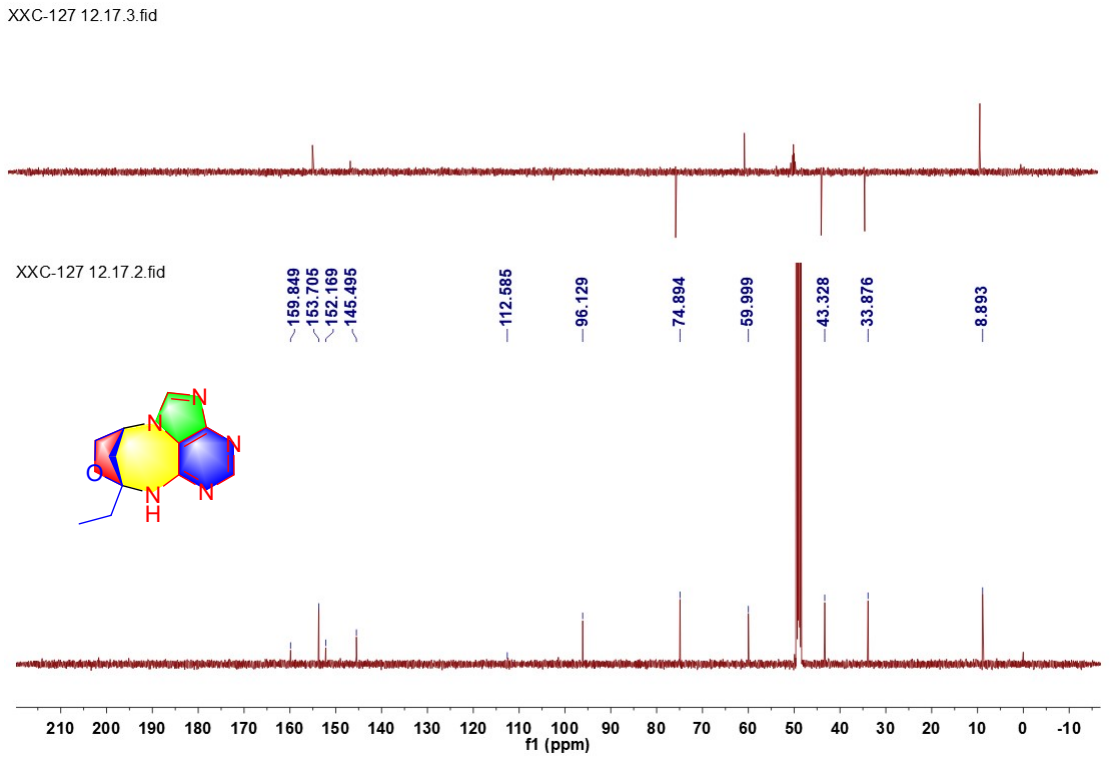


Figure S16. ^{1}H-^{1}H COSY (600 MHz) spectrum of **2** in methanol-d_4

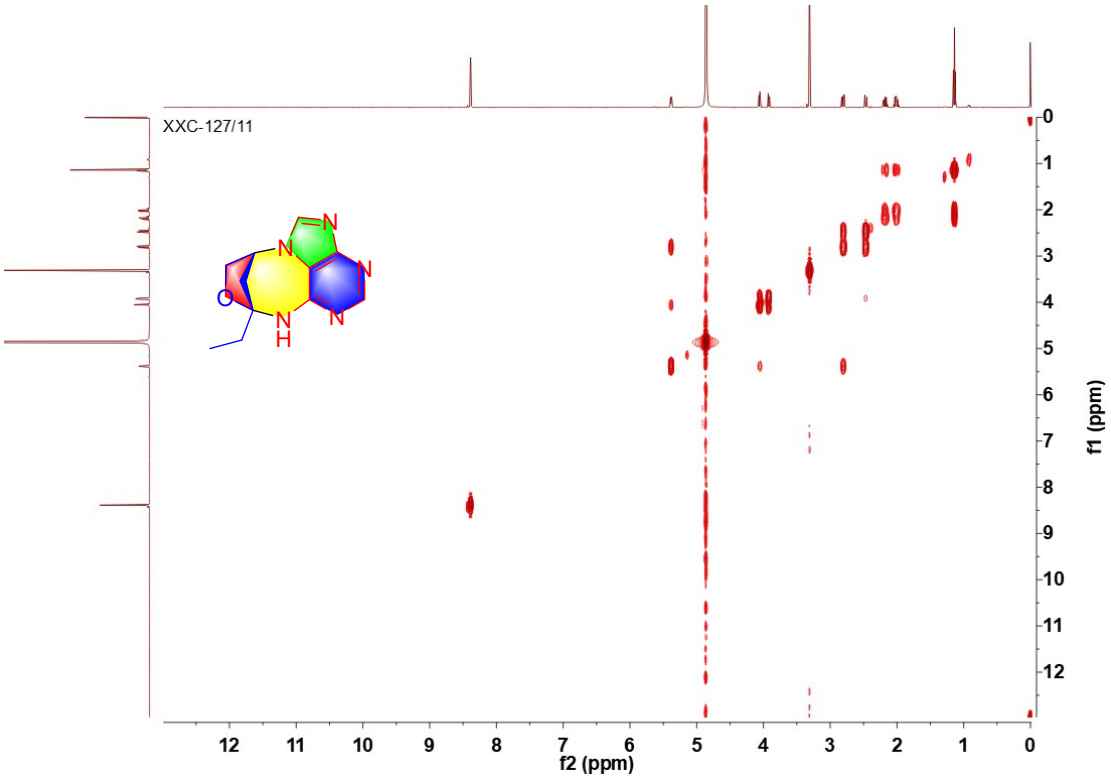


Figure S17. HSQC (600 MHz) spectrum of **2** in methanol-d_4

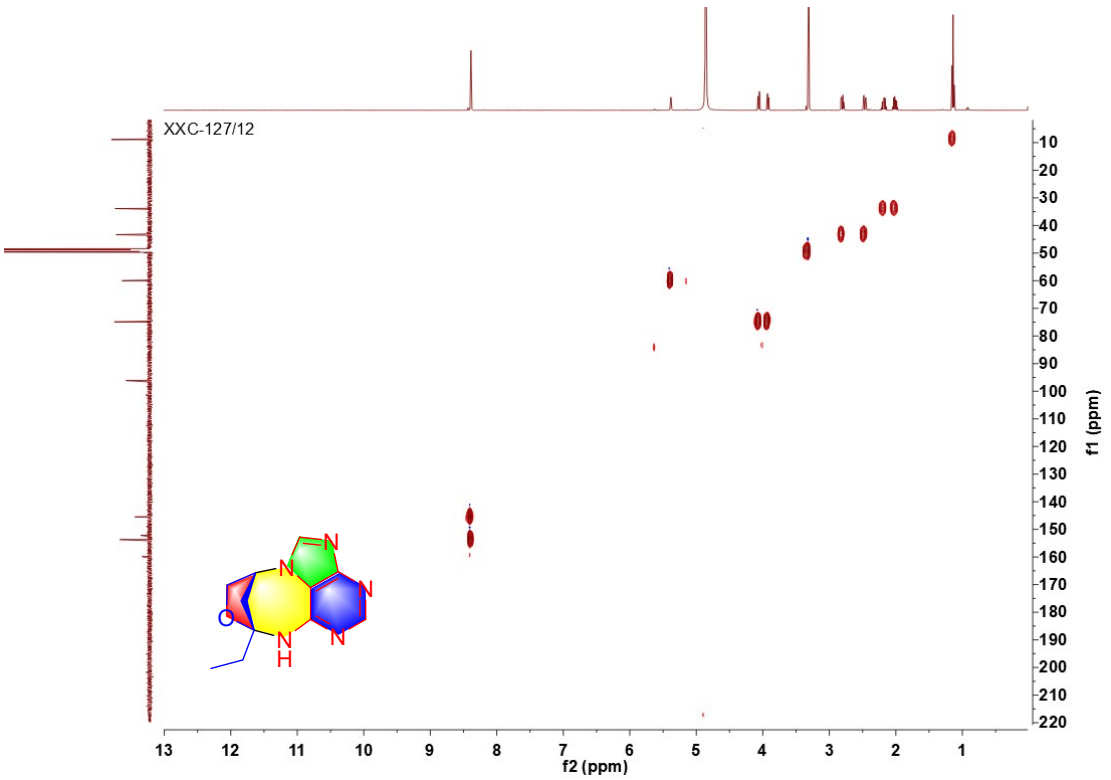


Figure S18. HMBC (600 MHz) spectrum of **2** in methanol-d_4

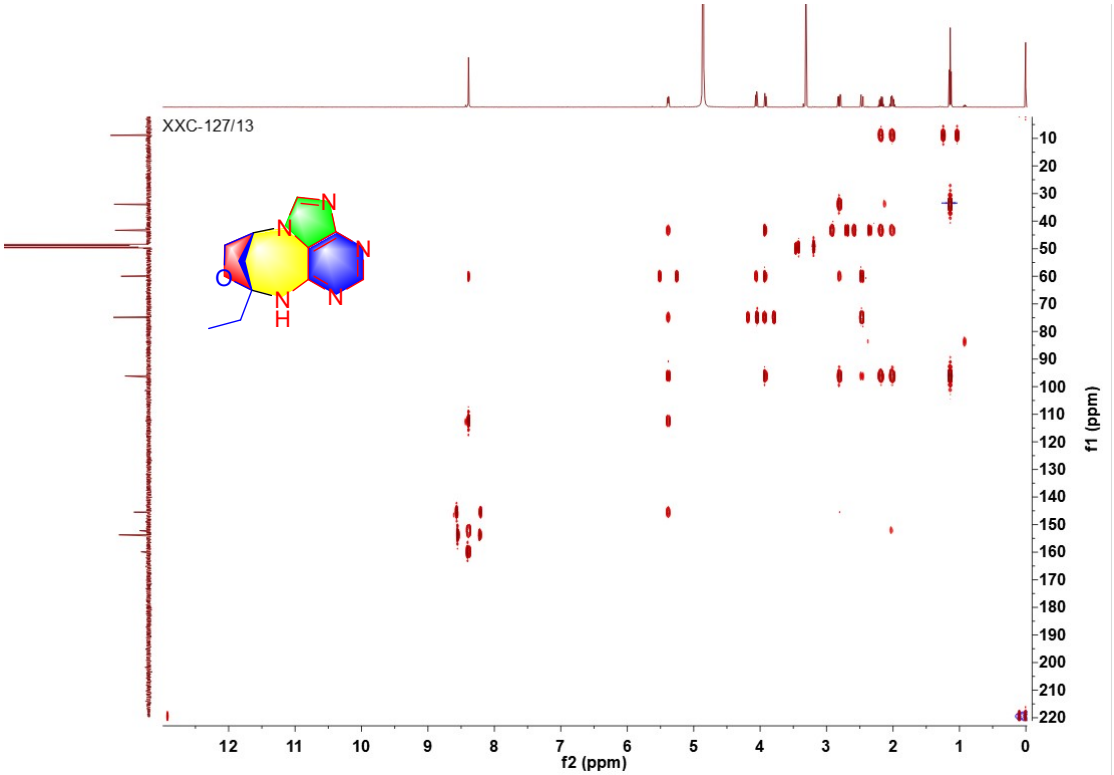


Figure S19. ROESY (600 MHz) spectrum of **2** in methanol-d_4

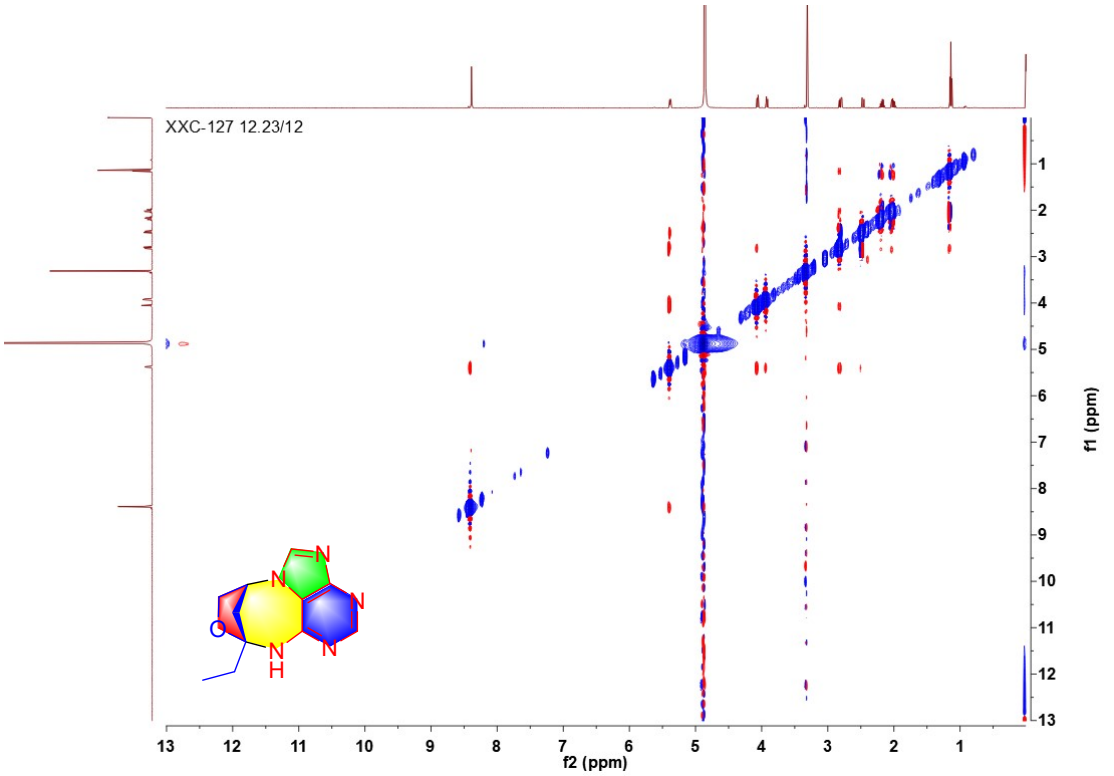


Figure S20. HRESIMS of **2**

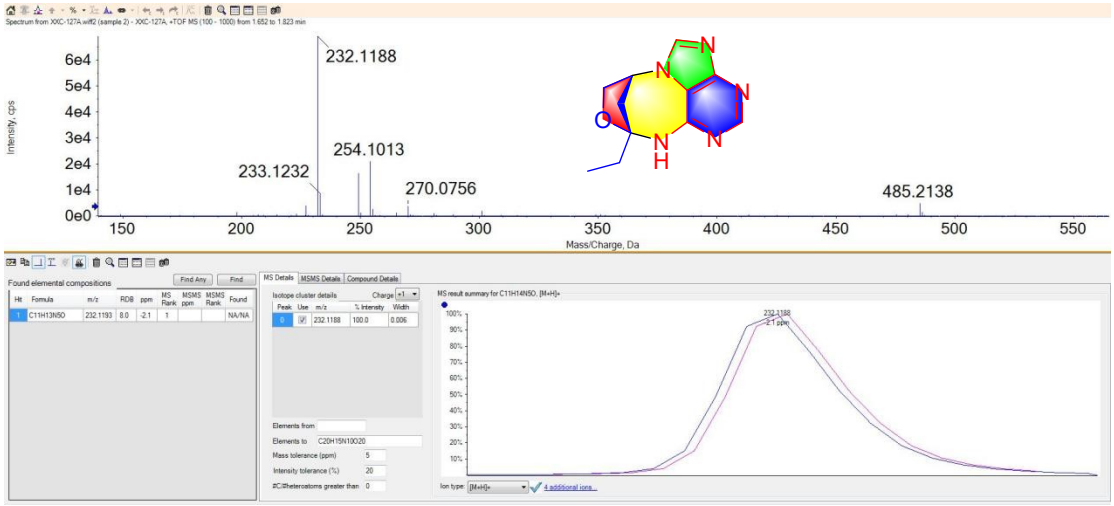


Figure S21. ^{1}H NMR (600 MHz) spectrum of **2** in DMSO-d_6

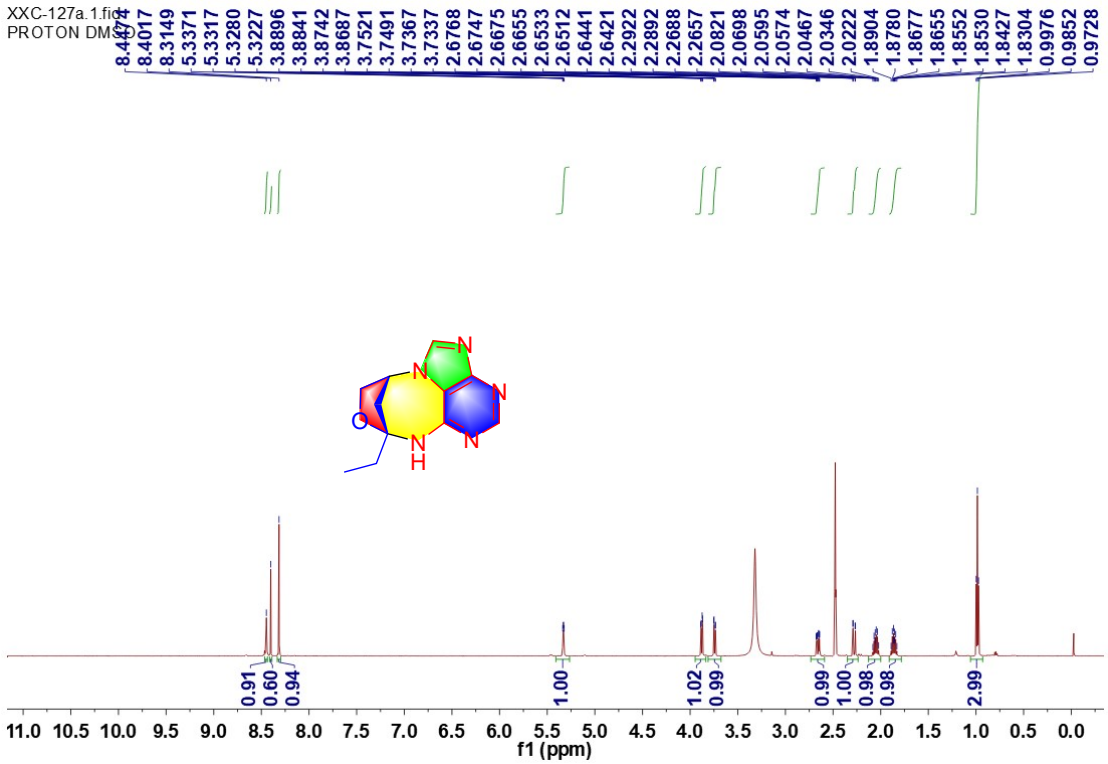


Figure S22. ^{13}C NMR spectrum of **2** in DMSO-d_6

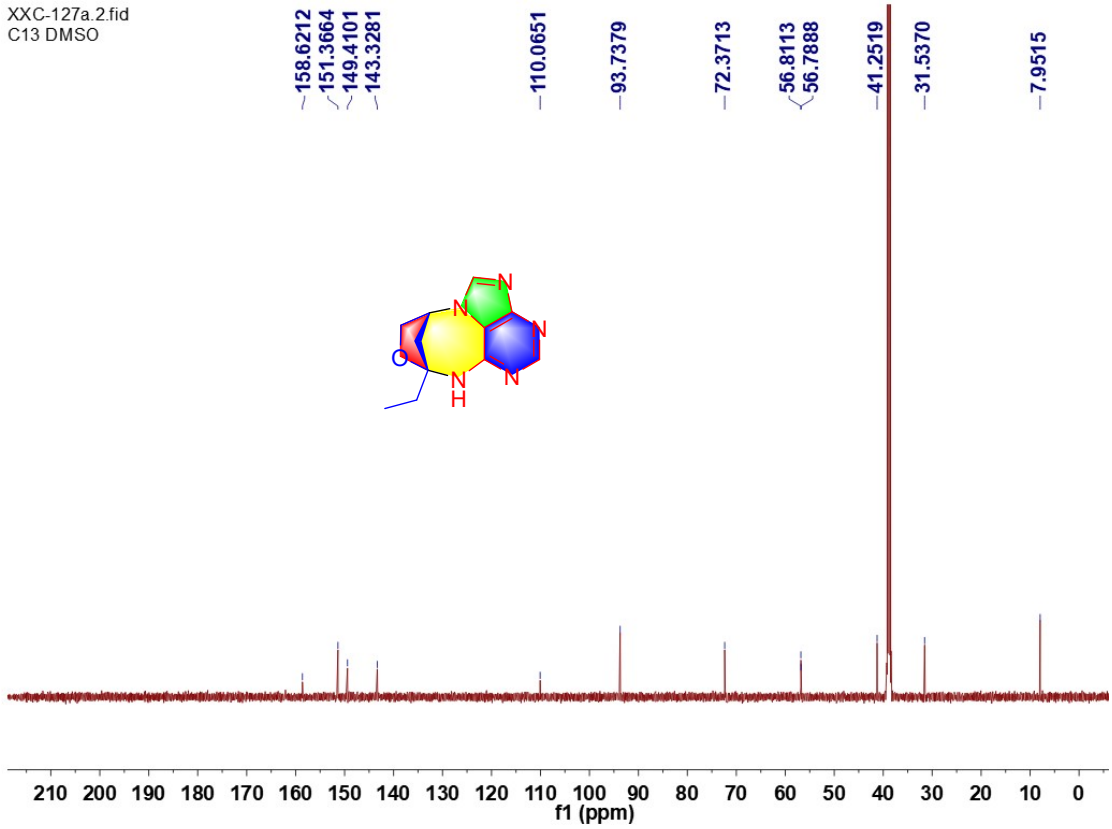


Figure S23. ^{1}H-^{1}H COSY (600 MHz) spectrum of **2** in DMSO-d_6

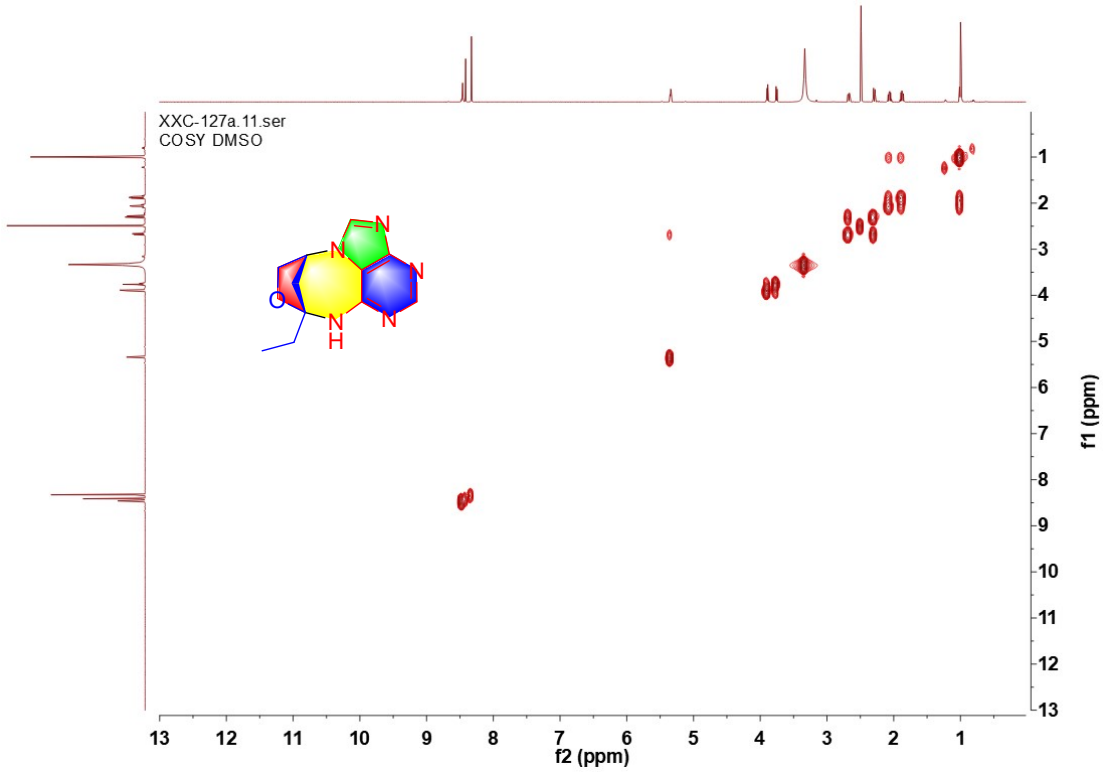


Figure S24. HSQC (600 MHz) spectrum of **2** in DMSO-d_6

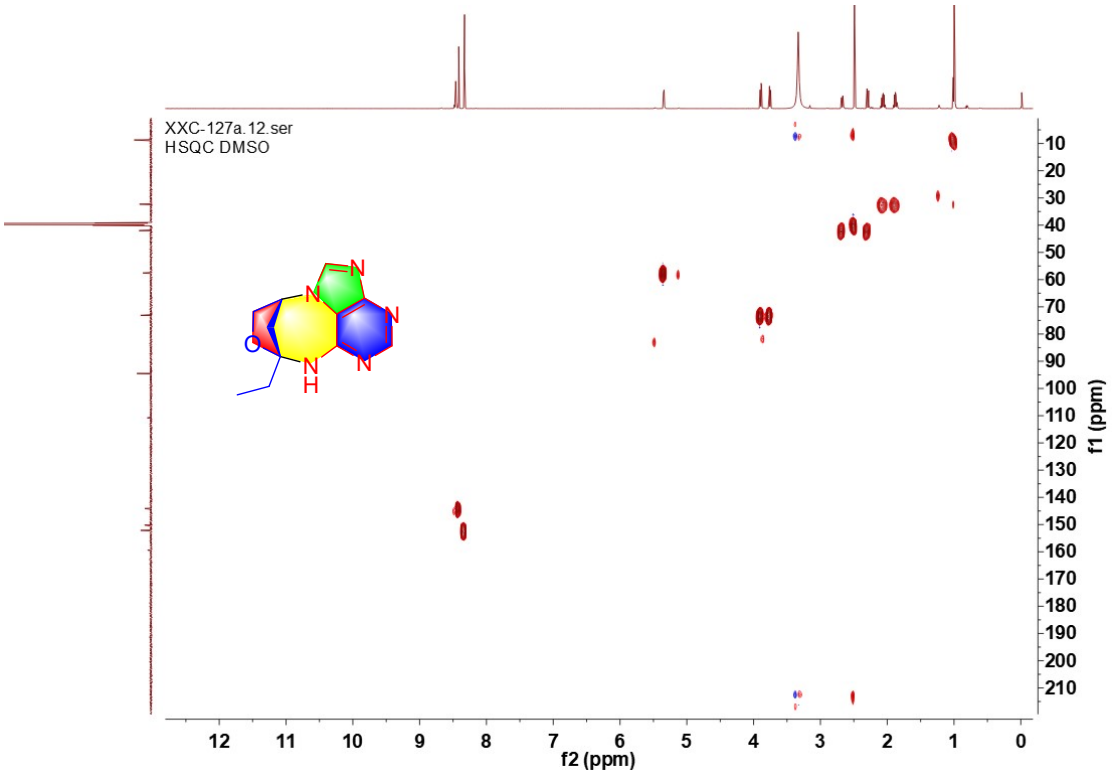


Figure S25. HMBC (600 MHz) spectrum of **2** in DMSO-d_6

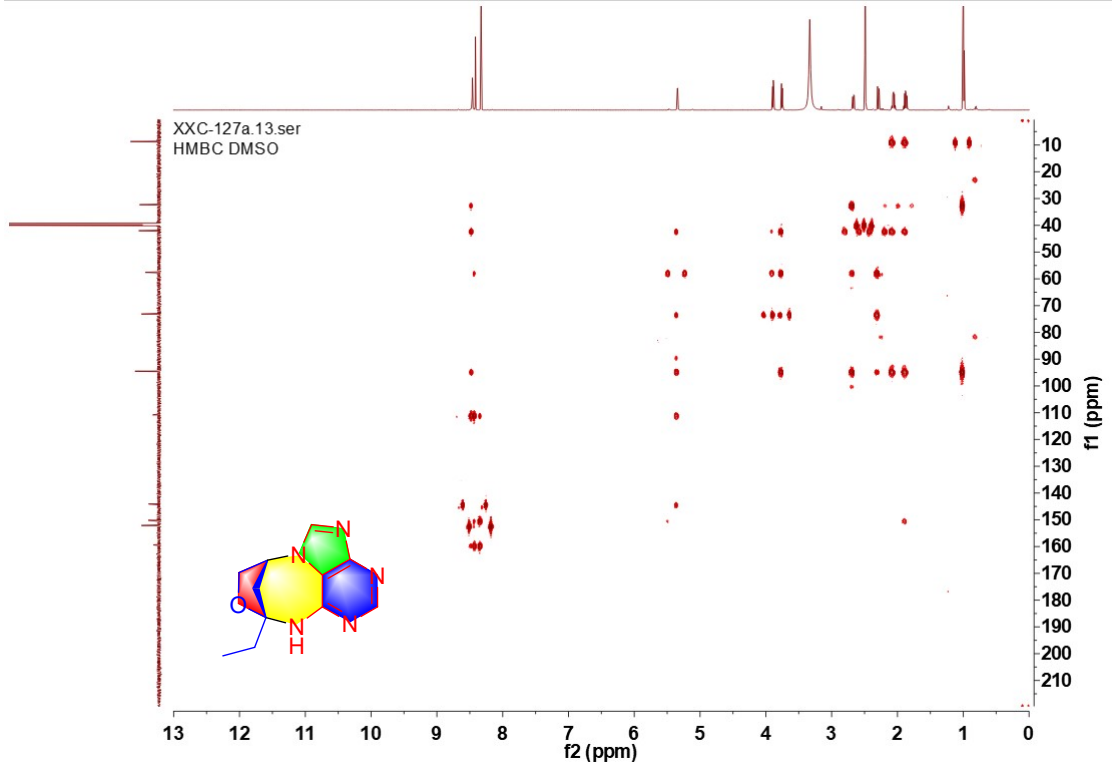
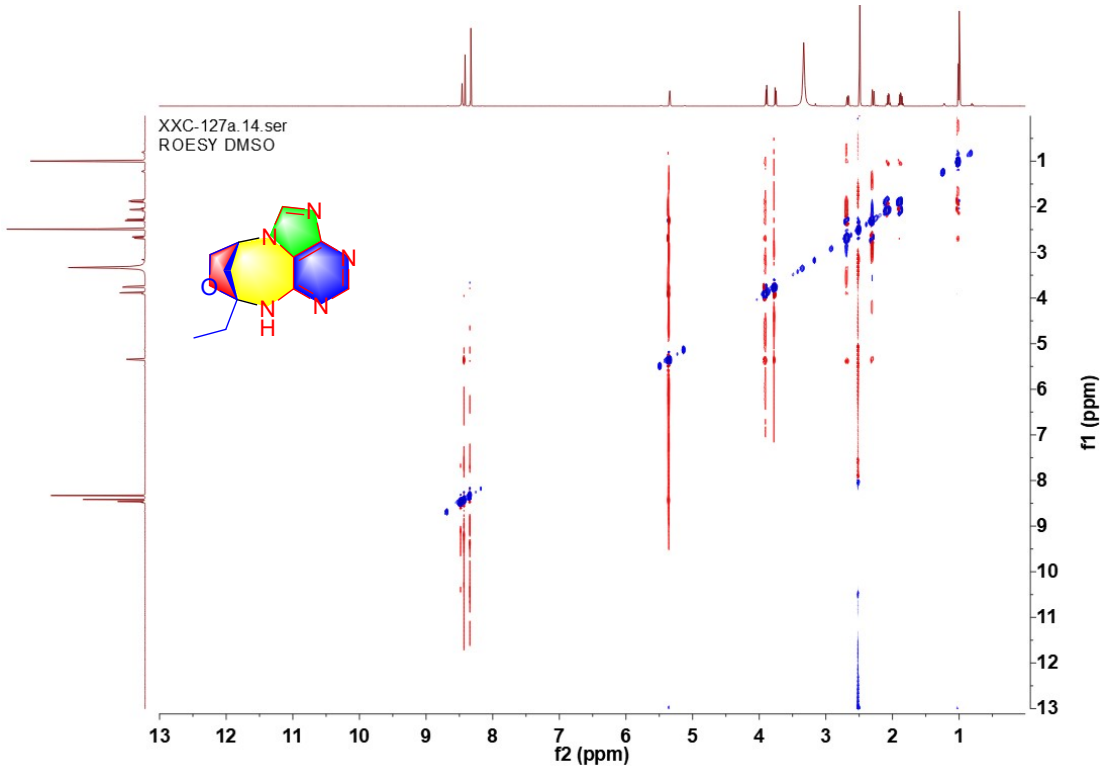


Figure S26. ROESY (600 MHz) spectrum of **2** in DMSO-d_6



Biological Assays

1. Cell culture

RAW264.7, a mouse macrophage cell line (Procell Life Science & Technology Co., Wuhan, P.R. China), was cultured in high-glucose DMEM (C11995500BT, Gibco) supplemented with 10% fetal bovine serum (FBS) (2094468CP, Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified environment containing 5% CO_2.

2. Cell viability assay

RAW264.7 (2×10^4 cells/mL) were seeded into 96-well plates with completed DMEM. After overnight culture, cells were treated with various concentrations of compound or DMSO for 24 h. Then Cell Count Kit-8 (CCK-8, Beyotime, Shanghai, P.R. China) was added into each well for 1 h at 37 °C. The absorbance of each well was recorded at 450 nm using a microplate reader (BioTek, USA).

3. ELISA of TNF-α and IL-6

The culture supernatants were collected and centrifuged from treated cells. The concentrations of TNF-α and IL-6 were measured using the ELISA Kit (Proteintech, USA) according to the manufacturer's instructions.

4. Immunofluorescence staining

Cells were plated on coverslips for 12 h in 24-well plates, then washed three times with PBS and fixed in 4% paraformaldehyde (PFA) for 15 min at 37 °C. The sections were blocked with 3% BSA in PBST (PBST (PBS with 0.1% Triton X-100), then incubated with primary antibody (COX-2, 1:500) (Cat. No: 12282, CST) for 2 h at room temperature, and stained with secondary antibody (Alexa Fluor 594 goat anti-rabbit IgG, 1:500) for 1 h at room temperature. The sections or cells were counter stained with DAPI. The images were visualized by confocal laser scanning microscope (ZEISS, Germany).

5. Western blot

After lipopolysaccharide (LPS) treatment, total protein was extracted from the cell lines using radio immunoprecipitation assay (RIPA) buffer (Beyotime, P.R. China) containing protease cocktail (Roche, Germany) and quantified protein samples using the BCA assay (Thermo Scientific, USA). Equal amounts of protein extracts were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% BSA, then with the indicated antibodies overnight at 4 °C, and followed the incubation with horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature. The bands were visualized and measured via the ECL kit (Pierce, USA) and b analysis system (Bio-Rad, CA, USA). The densitometry analysis of the immunoblots results was performed using ImageJ software (NIH, USA).

6. Statistical analysis

All experimental data obtained from this study were performed in triplicate. The results represent mean± SD. Statistical analyses were performed by Graphpad prism 6 (GraphPad Software, San Diego, CA, USA) and Excel (Microsoft) with Student's *t*-test, one-way ANOVA test. Differences were considered significant when $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ and $****P \leq 0.0001$.

Figure S27. Compounds suppress LPS-induced TNF-α and IL-6 expression in RAW

264.7 cells.

A-B, Compounds suppress LPS-induced TNF-α and IL-6 expression in RAW 264.7 cells. The cells were pretreated with compounds for 2 h and then stimulated with 1 μg/mL LPS for 18 h. Culture media were collected in order to measure TNF-α and IL-6 concentrations using ELISA kit. **C-D**, RAW264.7 cell proliferation in response to compound (–)-**1** (C) and compound (+)-**2** (D) at different concentrations assayed by CCK-8 assay. Data represent mean ± SD values of three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with LPS alone. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with DMSO alone.

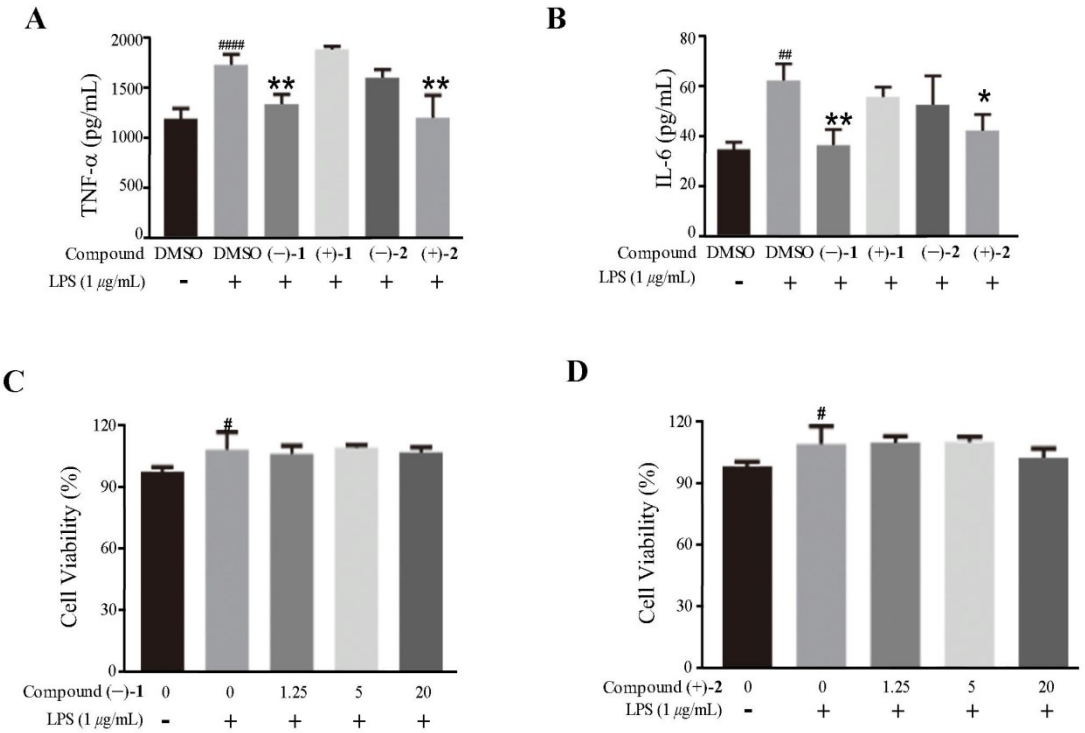


Figure S28. The full raw data (complete uncropped images) for the western blot experiments

Fig.4. COX-2 (lane 1–7)

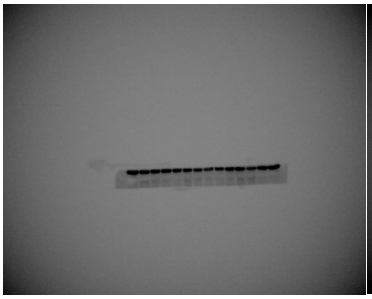


Fig.4. GAPDH (lane 1–7)

Fig.4. iNOS (lane 1–7)

Fig.4. NF-κB (lane 1–7)

Fig.4. p-NF-κB (lane 1–7)

Fig.5. COX-2 (lane 8–14)

Fig.5. GAPDH (lane 8–14)

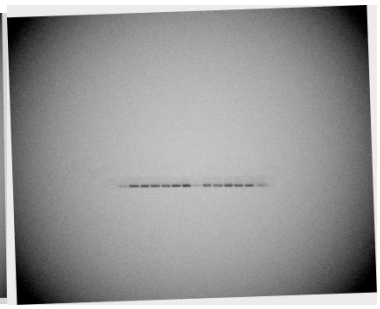


Fig.5. iNOS (lane 8–14)

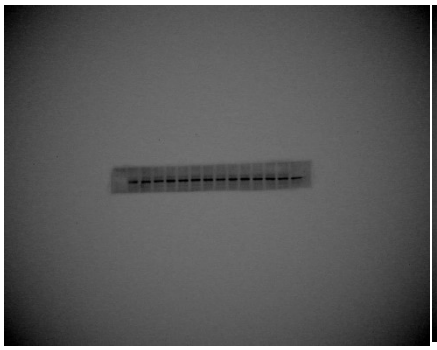


Fig.5. NF-κB (lane 8–14)

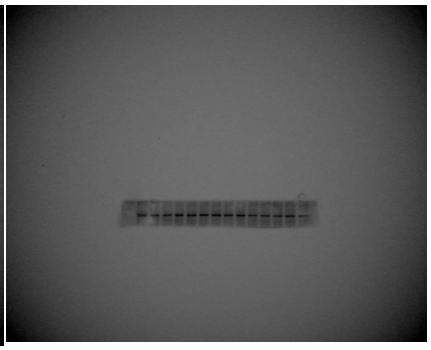


Fig.5. p-NF-κB (lane 8–14)

Figure S29. Effect of compounds (−)-**1** and (+)-**2** on production of nitrite oxide induced by LPS.

Cell were treated with LPS with or without compounds for 24 hours, the culture supernatants were collected and centrifuged. The production of nitrite oxide was measured using the Griess Kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. In short, 50 µl of the cell supernatants were mixed with 50 µl Griess regent I and II, then the absorbance at 560 nm wavelength was measured using a microplate reader (BioTek, USA). Data represent mean ± SEM values of three experiments. ***P < 0.0001 compared with LPS alone. Dexamethansone (DEX) was used as a positive control.

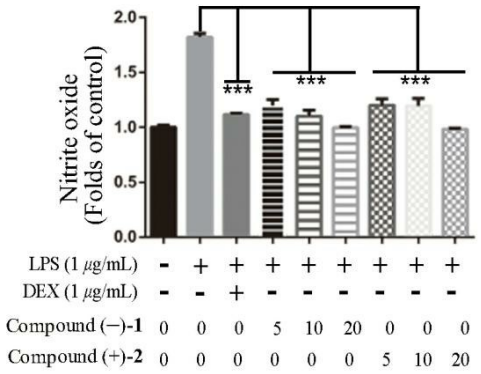


Figure S30. The results of western blot experiments were repeated for 5 times.