Azonazine, A Novel Dipeptide from a Hawaiian Marine Sediment-Derived Fungus, *Aspergillus insulicola*

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[Supporting Information]

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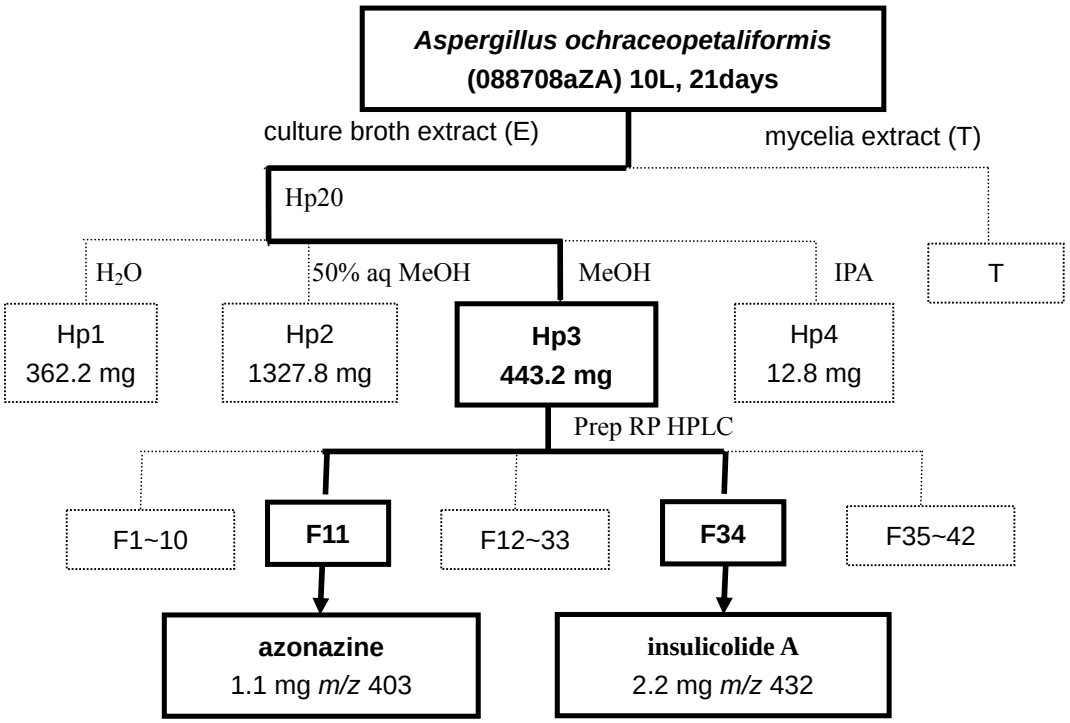
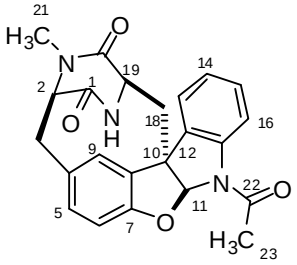
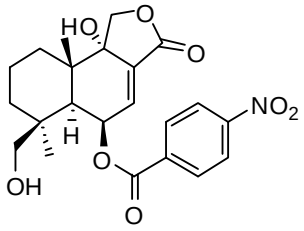
Chart S1. Extraction scheme of azonazine and insulicolide A





azonazine



insulicolide A

Table S1. NMR data in CD_3CN of azonazine

No.	δ_H (J)[a]	δ_C (DEPT)[b,c]	gCOSY	gHMBC[d]	1D-NOE
1		169.49 (s)			
2	4.09 d (7.2)	66.26 (d)	3	1,3,4,20,21	3a,3b,21
3	a 3.08 dd (14.4,7.2)	39.21 (t)	2	1,2,4,5,9	2,3b,5,21
	b 3.49 d (14.4)				2,3a,9
4		133.20 (s)			
5	6.95 dd (7.8,1.2)	131.51 (d)	6,9	3,6,7,8,9	
6	6.67 d (8.4)	110.83 (d)	5	4,7,8,9	
7		158.99 (s)			
8		131.98 (s)			
9	7.50 s	126.42 (d)		3,5,6,7,10	
10		59.06 (s)			
11	6.58 s	106.95 (d)		7,8,12,17,18,22	18b,23
12		135.34 (s)			
13	7.59 d (7.2)	124.13 (d)	14	10,15,16,17	
14	7.19 t (7.8)	125.72 (d)	13,15	10,12,13,16,17	13,15
15	7.29 t (7.8)	129.61 (d)	14,16	12,13,16,17	
16	8.13 brs ($w_{1/2}\approx7.8$)	117.29 (d)	15		
17		142.48 (s)			
18	a 2.49 dd (16.8,1.8)	43.35 (t)	19	8,10,11,12,19,20	9,13,18b,NH
	b 2.84 dd (16.8,5.4)				11,18a,19
19	4.26 d (5.4)	54.80 (d)	18,NH	1,10,18,20	18a,18b,NH
20		165.51 (s)			
21	2.40 s	32.83 (q)		2,20	2,3a, 5
22		171.34 (s)			
23	2.42 s	24.16 (q)		22	
NH	7.04 brs		19	2,20	

[a] Recorded at 600 MHz. [b] Recorded at 150 MHz. [c] Multiplicities inferred from by DEPT and HMQC experiments. [d] Protons showing long-range correlation with indicated carbon.

Table S2. Disk diffusion assay data for small scale crude (EtOAc) extract of *Aspergillus ochraceopetaliformis*

Extract conc. (mg/ml)	L1210	Colon38	CFU-GM	HCT-116	H-125	LNCaP	OVC-5	U251N	MDA	PANC-1	CEM
66		**>1000**		600	600	**700**	550	600	550	500	**400**
66						**800**					**650**
16	**750**	**>1000**				**400**					**400**
4	**400**	**650**	600								

*(200 zone units = 6 mm).

L1210 (murine lymphocytic), Colon 38 (murine colon adenocarcinoma), CFU-GM (granulocyte macrophage colony forming units; murine), HCT-116 (human colon adenocarcinoma), H-125 (human lung adenocarcinoma), LNCaP (human prostate adenocarcinoma), OVC-5 (human ovarian carcinoma), U251N (human glioma cells), MDA (human melanoma cells), PANC-1 (human pancreatic carcinoma), CEM (human leukemic lymphoid)

Significant selectivity is defined by a difference of 250 zone units.

Table S3. Disk diffusion assay data for azonazine and insulicolide A (c 1.0 mg/mL)

Compound Name	L1210	Colon 38	HCT-116	H-125
Azonazine	0	0	0	0
Insulicolide A	**300**	**150**	**200**	**250**

*(200 zone units = 6 mm).

L1210 (murine lymphocytic), Colon 38 (murine colon adenocarcinoma), HCT-116 (human colon adenocarcinoma), H-125 (human lung adenocarcinoma)

Significant selectivity is defined by a difference of 250 zone units.

Table S4. Summary of possible configurations for azonazine supporting final structure as type **I**

Type	Configurations*	ECD match with CD	Insights from MMX Models	Dignostic 1D-NOE*
I	2R, 10R, 11S, 19R	Yes	Different anisotropic environment with DKP C=O groups for aryl protons H9 (δ7.50) & H5 (δ6.95) as shown by structures in Figures S15 and S15a	Match with observed data (Figure S15): (1) *N*Me-21 to H-3a & to H-5; (2) H-3b to H-9; (3) H-18a to *N*H, to H-9, & to H-13
II	2R, 10S, 11R, 19R	–	Same as above for **I**, opposite absolute configuration at 10/11 vs. diazonamide with 10*R*,11*S*	Same as above for **I**
III	2S, 10S, 11R, 19S	No	–	–
IV	2S, 10R, 11S, 19S	–	Similar anisotropic environment with DKP C=O groups for aryl protons H9 (δ7.50) & H5 (δ6.95) as shown by structures in Figures S15 and S15a	Same as above for **I**
V	2S, 10R, 11S, 19R	Yes	Similar anisotropic environment with DKP C=O groups for aryl protons H9 (δ7.50) & H5 (δ6.95) as shown by structures in Figures S15 and S15a	Same as above for **I**
VI	2R, 10S, 11R, 19S	No	–	–

*1D-NOE spectrum shows the configuration of C10/C11 should be 10S,11R or 10R,11S.

Table S5. Molecular Taxonomy Sequencing Data for Collection Number 088708A with final identification as *Aspergillus insulicola*

UTHSC ID#	rRNA ITS	Beta-tubulin
R-4478	*Aspergillus* sp. OY10607 > 555/555 (100%) *Aspergillus ochraceopetaliformis* isolate NRRL 35668 > 555/555 (100%) *Aspergillus ochraceopetaliformis* isolate NRRL 35055 > 555/555 (100%) *Aspergillus ochraceopetaliformis* isolate NRRL 4752 > 555/555 (100%) *Aspergillus insulicola* isolate NRRL 6138 553/565 (97%)	*Aspergillus ochraceopetaliformis* isolate NRRL 35668 beta-tubulin gene >543/543 (100%) *Aspergillus ochraceopetaliformis* isolate NRRL 4752 beta-tubulin gene >542/543 (99%) *Aspergillus ochraceopetaliformis* isolate NRRL 5224 beta-tubulin gene >542/543 (99%) *Aspergillus insulicola* isolate NRRL 6138 beta-tubulin gene, 529/544 (97%)
R-4586 glycerol stock	*Aspergillus ochraceoceous* 18S 584/587 (99%) *Aspergillus insulicola* isolate NRRL 6138 580/585 (99%) *Aspergillus ochraceopetaliformis* strain RKI08-134 586/592 (98%)	*Aspergillus insulicola* isolate NRRL 6138 beta-tubulin gene, 543/544 (99%) *Aspergillus insulicola* strain CBS 38275 beta-tubulin-like 580/585 (99%) *Aspergillus pseudoelegans* isolate NRRL 35671 beta-tubulin 530/544 (97%) *Aspergillus ochraceopetaliformis* isolate NRRL 4752 529/544 (97%) *Aspergillus ochraceous* strain CBS 10808 396/456 (86%)

Figure S1. [1]HNMR Spectrum of azonazine (CD_3CN, 600MHz)

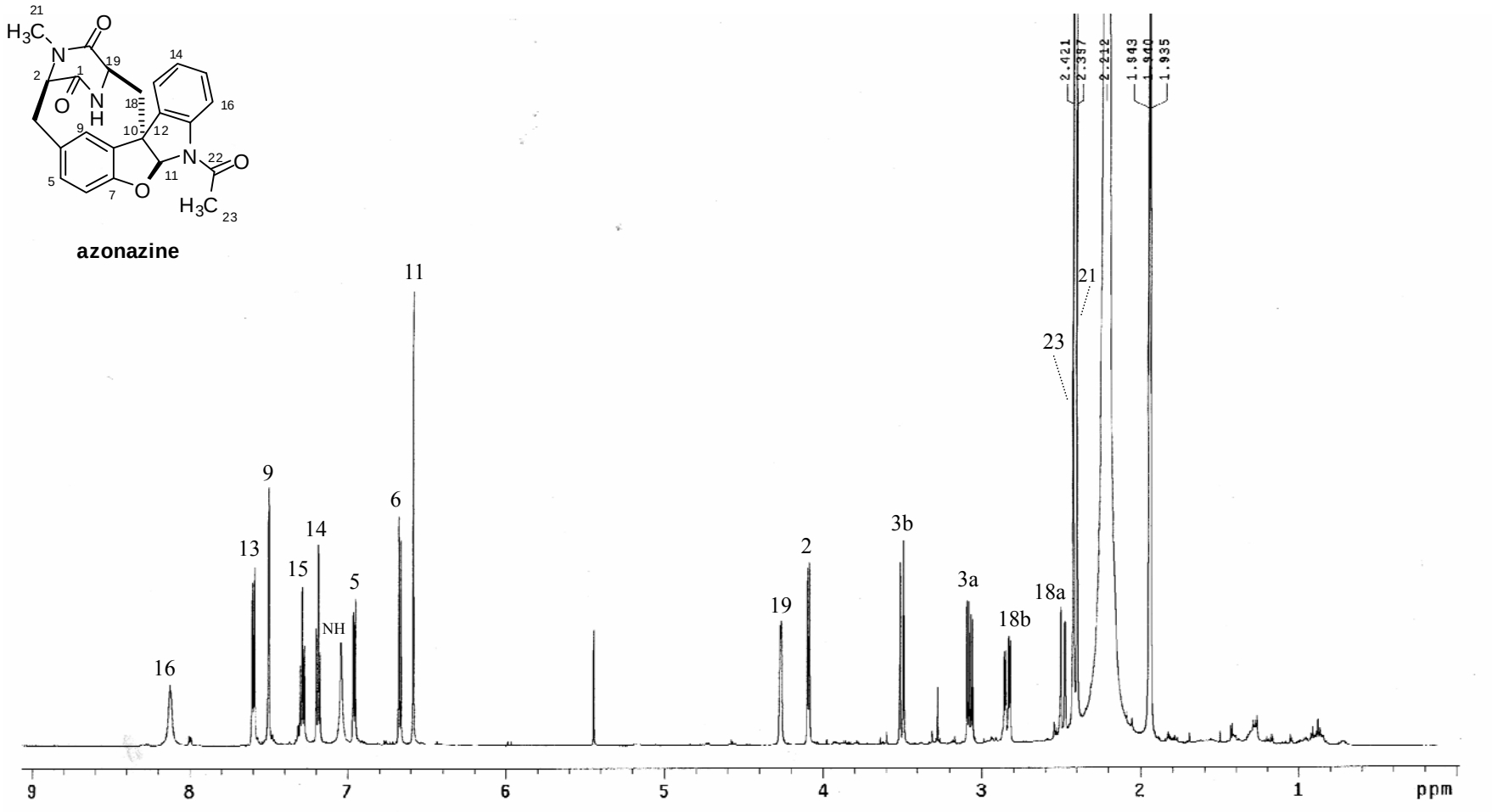


Figure S2. ^{13}CNMR Spectrum of azonazine (CD_3CN, 150MHz)

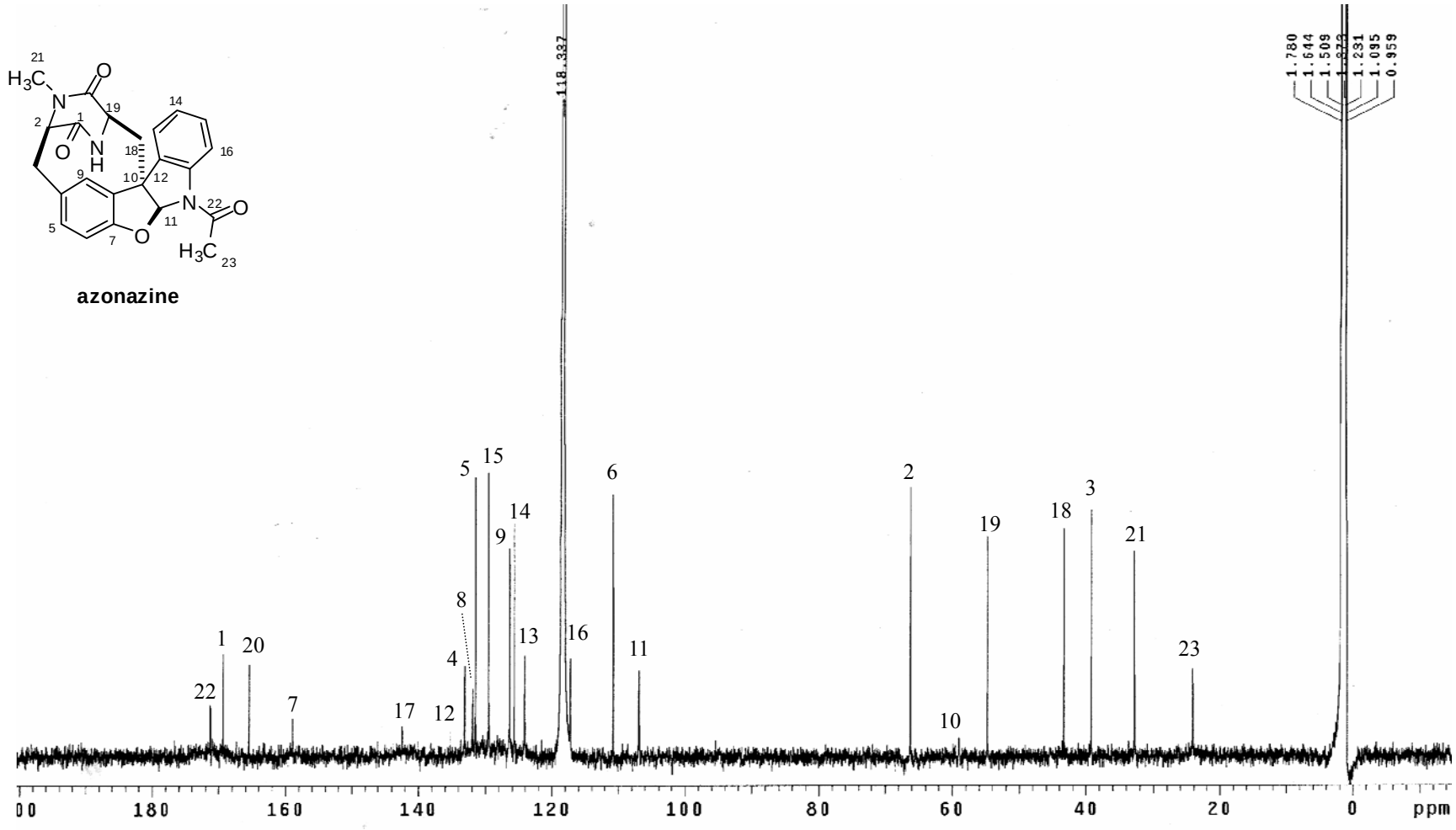
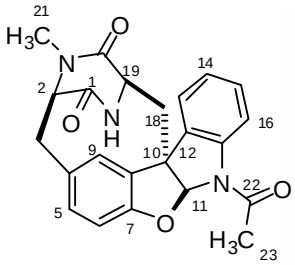


Figure S3. DEPT Spectrum of azonazine (CD_3CN, 150MHz)



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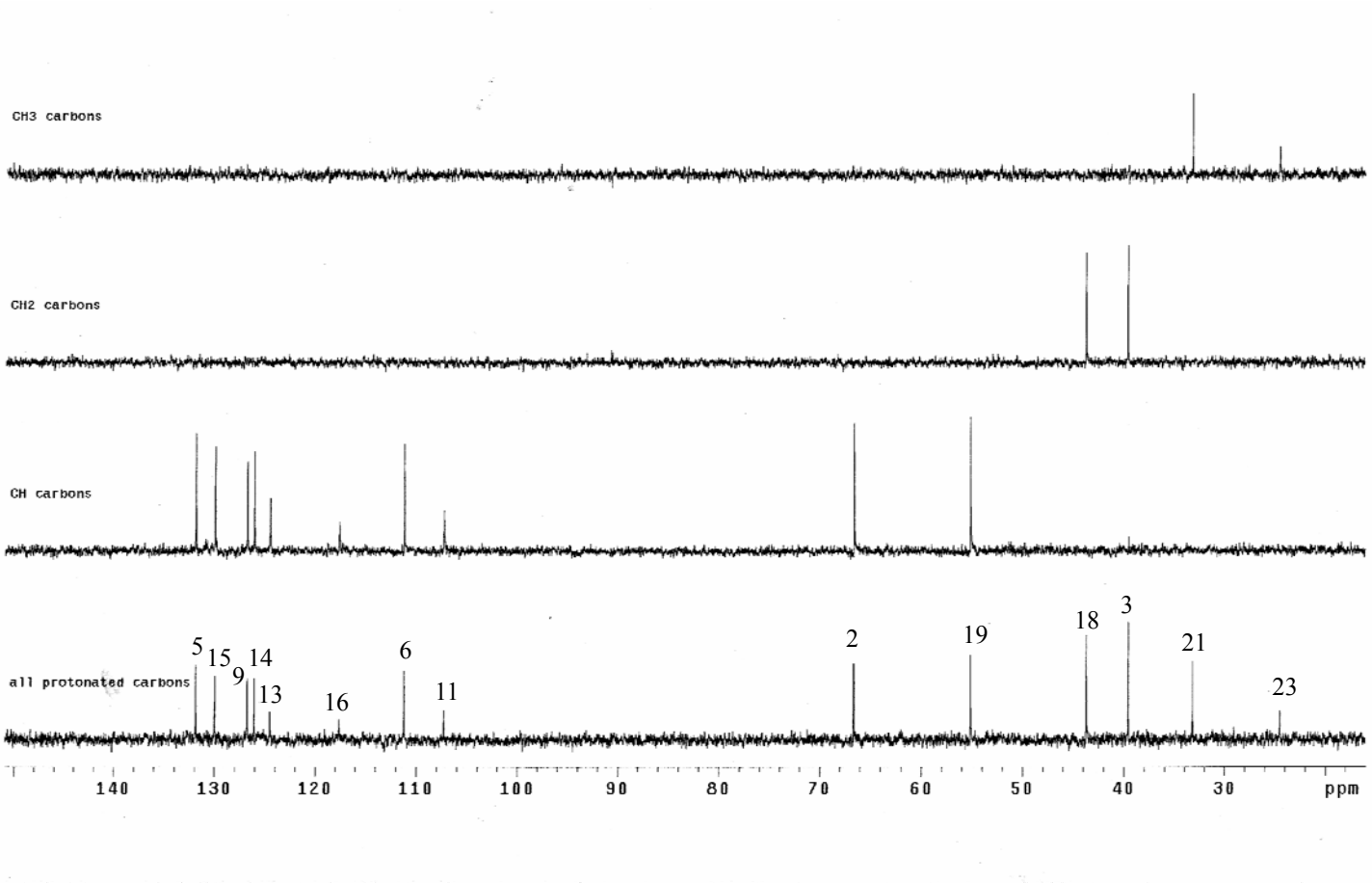
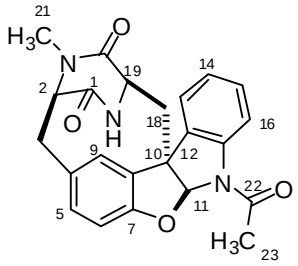


Figure S4. gCOSY Spectrum of azonazine (CD_3CN, 600MHz)



azonazine

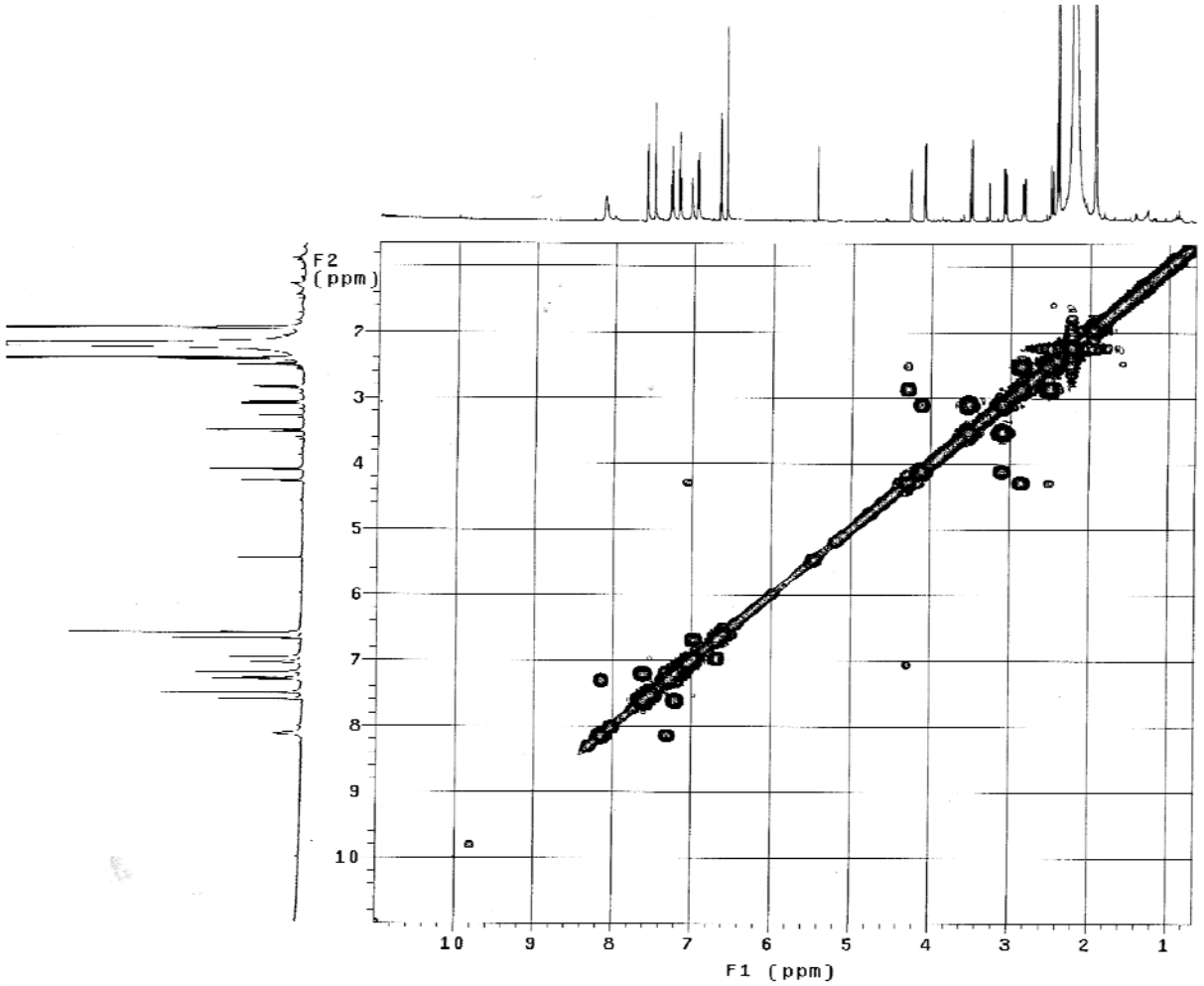
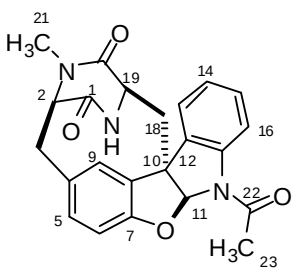


Figure S5. HMQC Spectrum of azonazine (CD_3CN, 600MHz)



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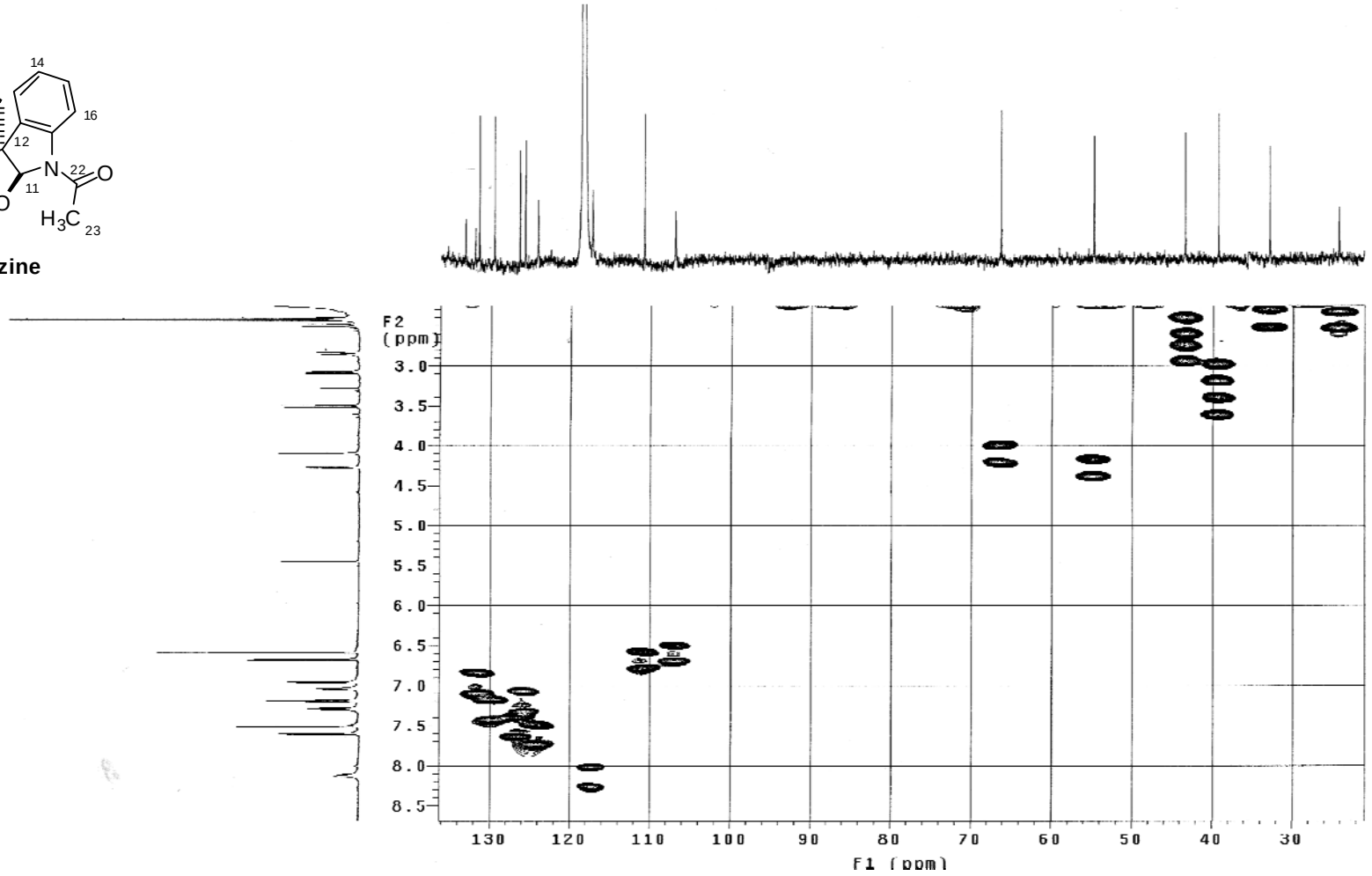
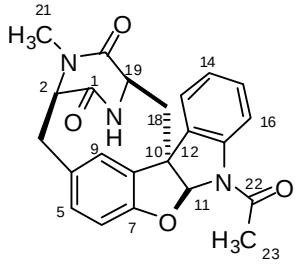


Figure S6. HMBC Spectrum of azonazine (CD_3CN, 600MHz)



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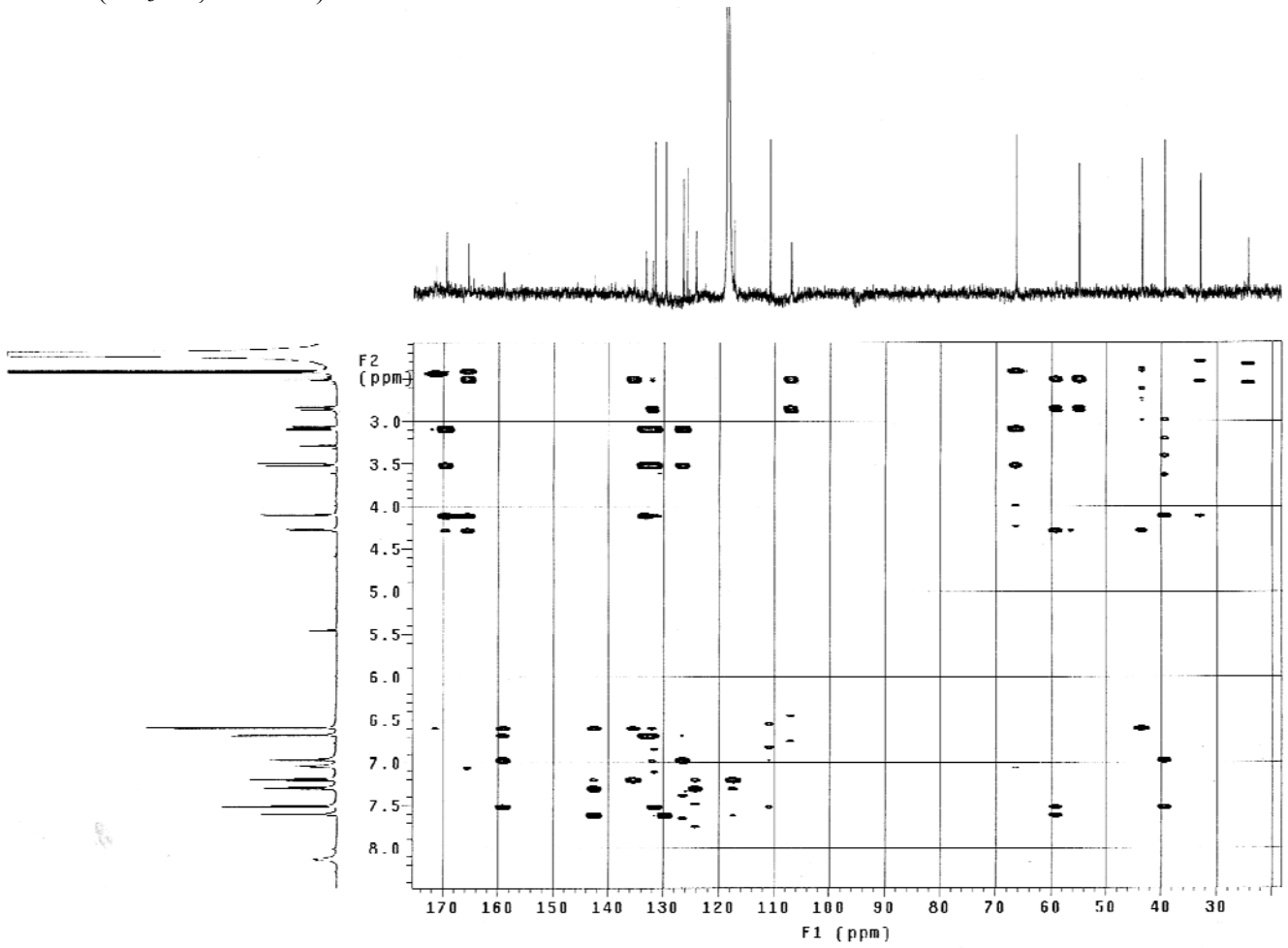


Figure S7. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

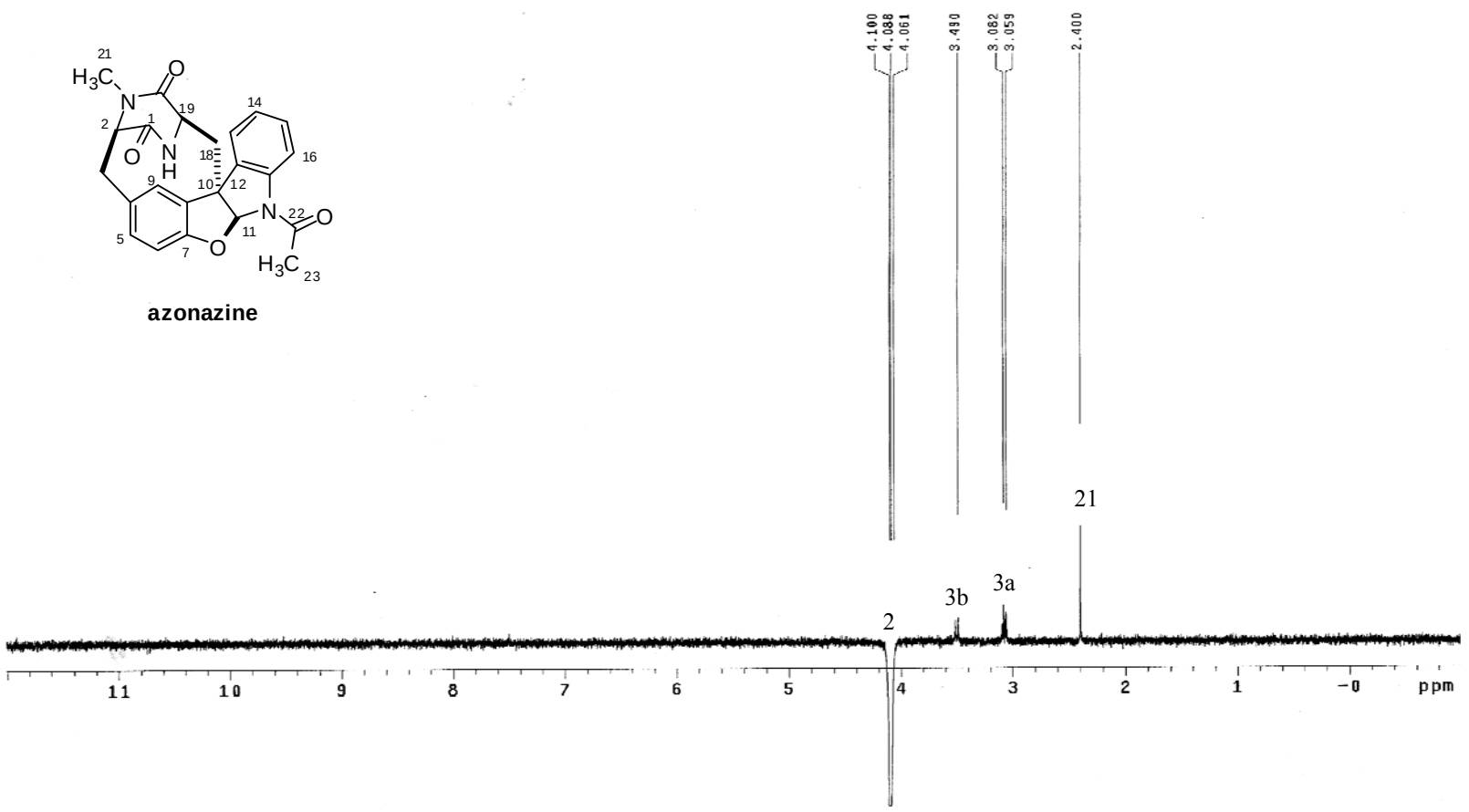


Figure S8. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

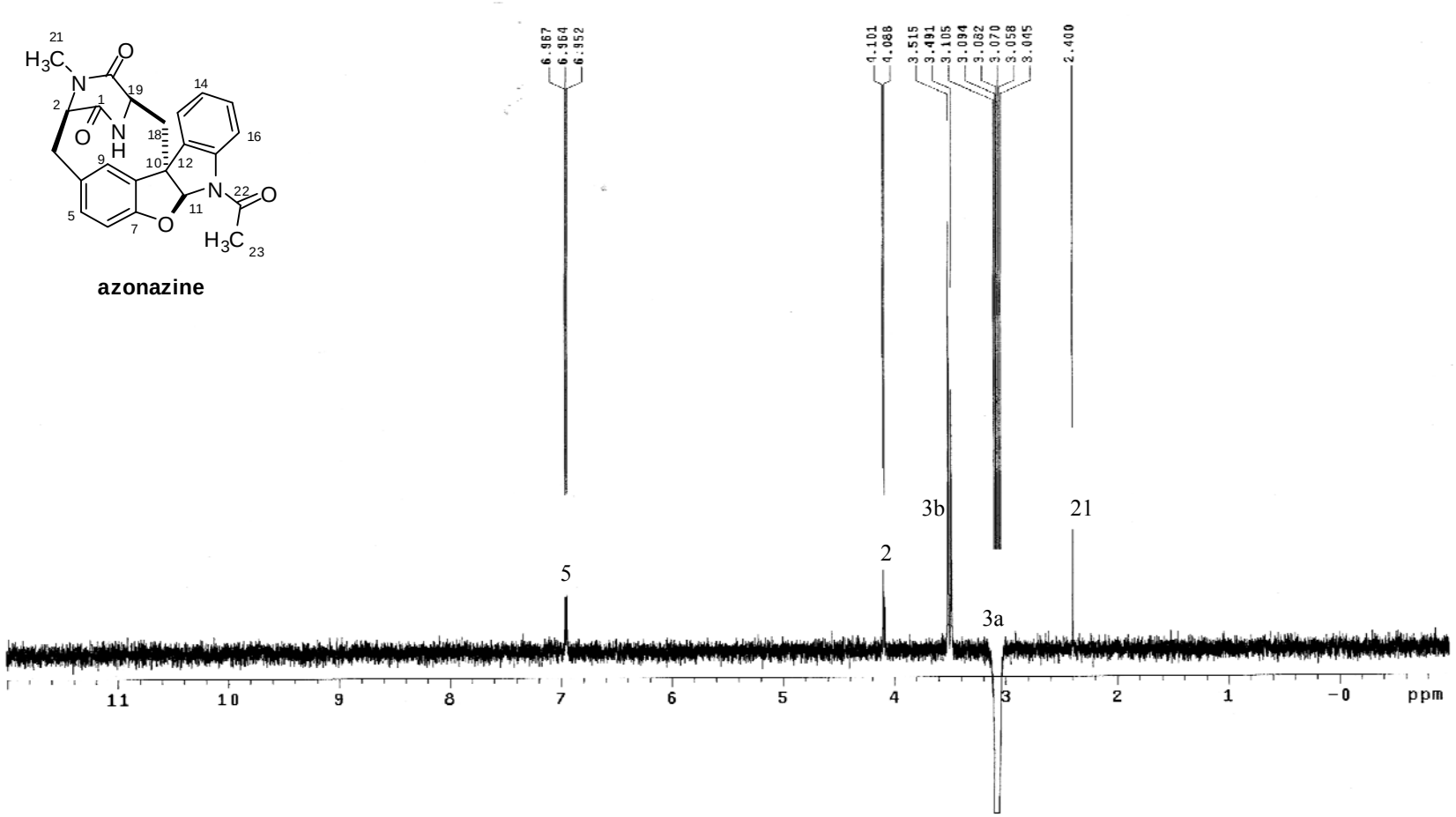


Figure S9. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

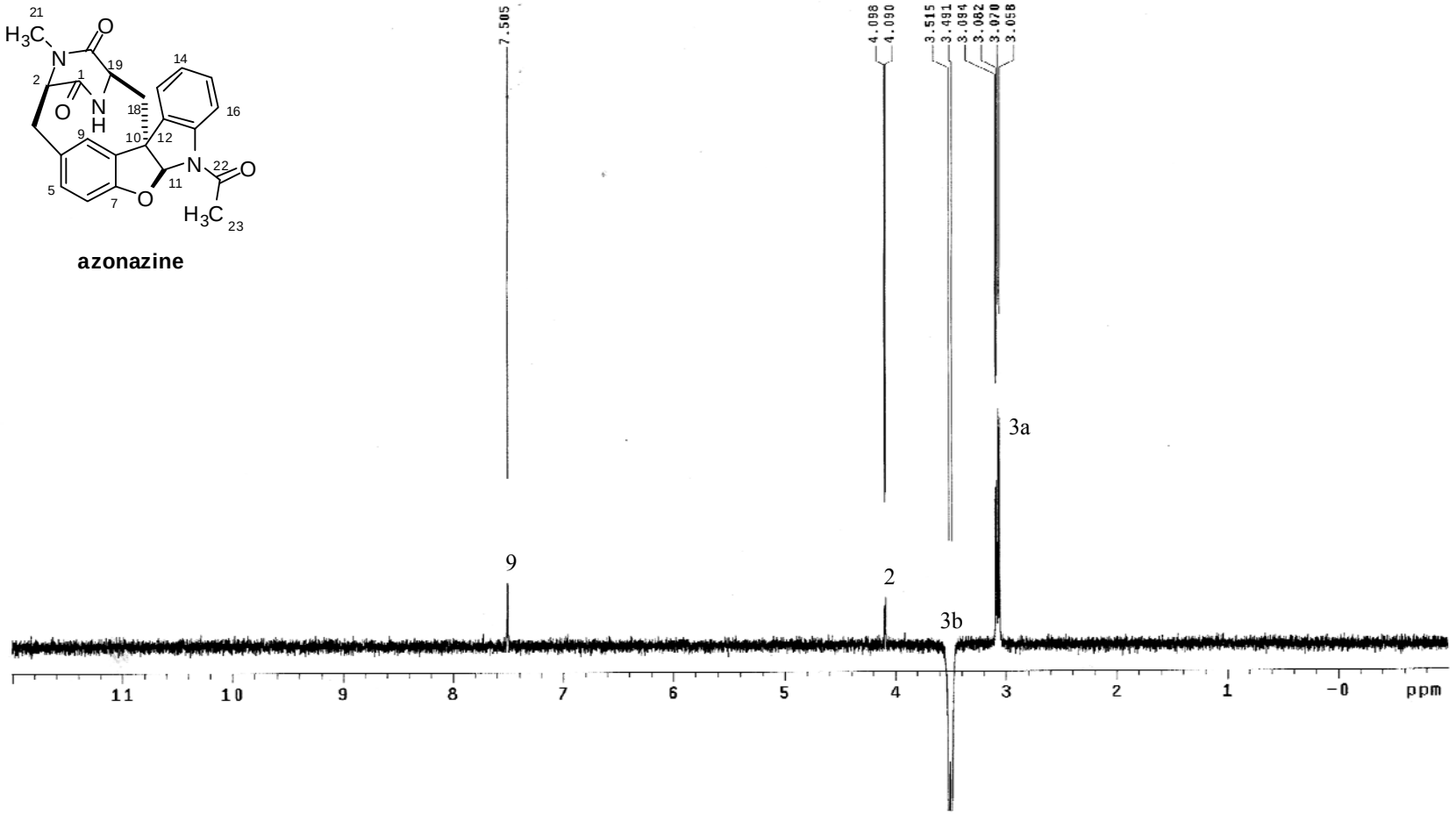


Figure S10. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

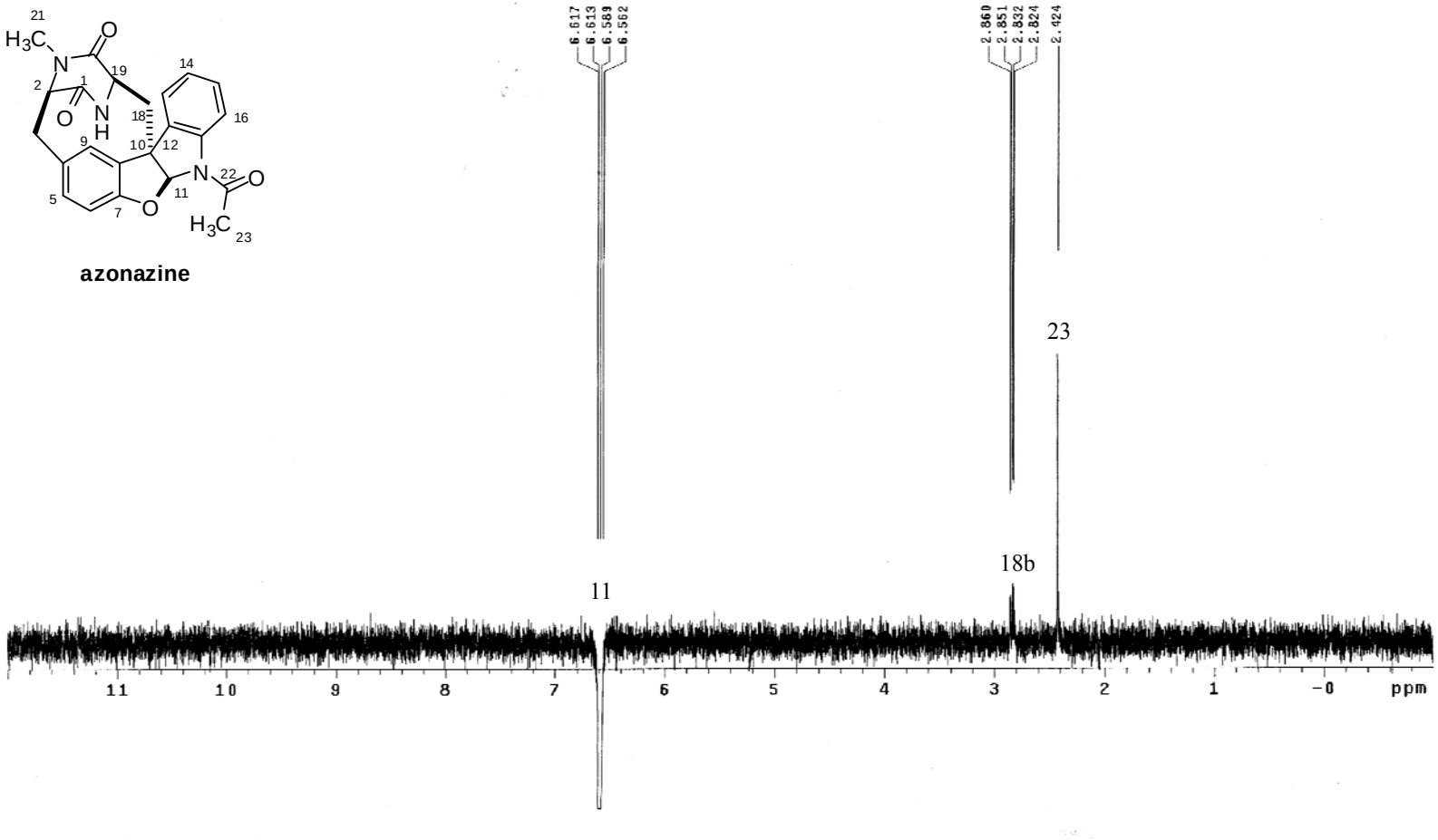


Figure S11. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

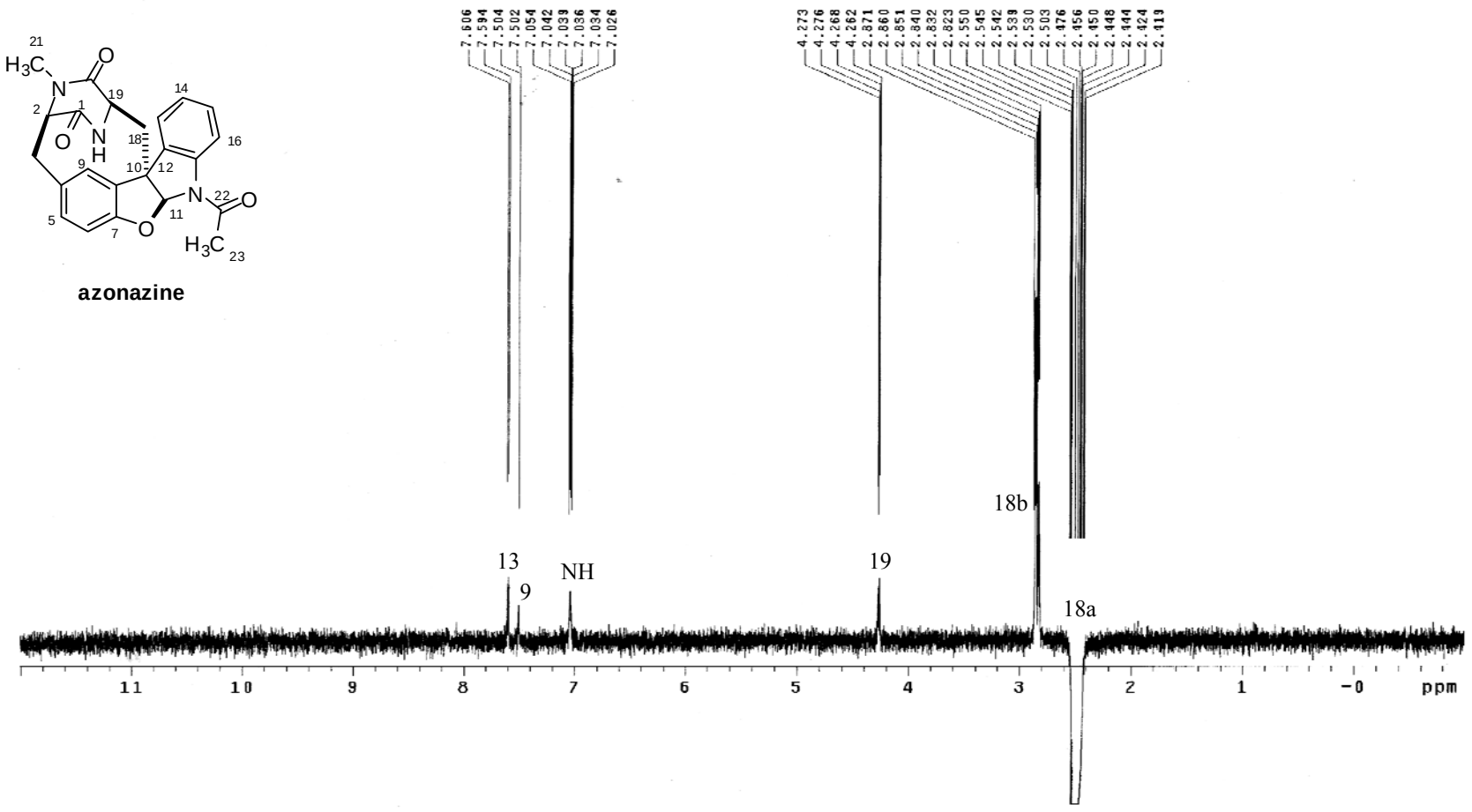


Figure S12. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

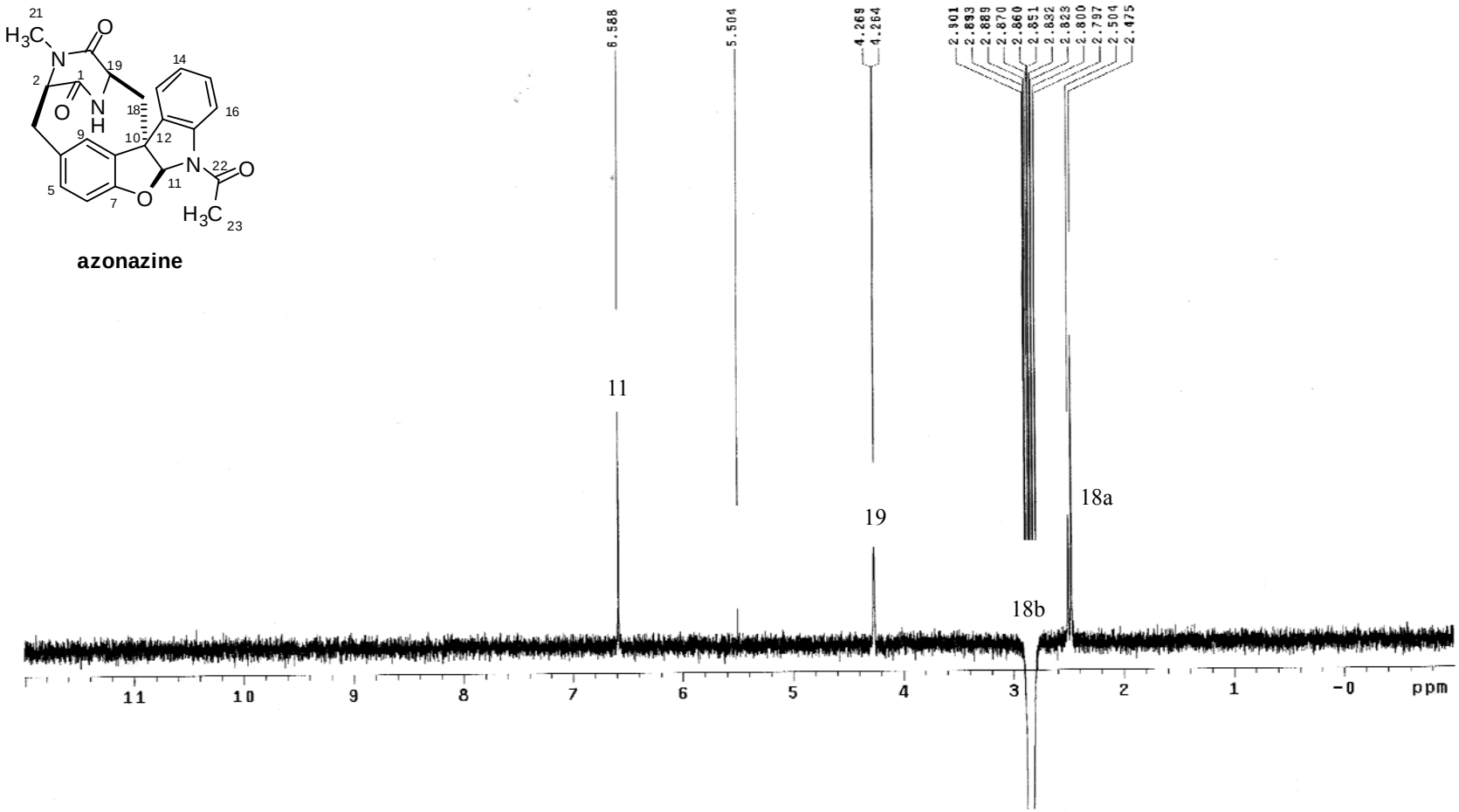


Figure S13. 1D-NOE Spectrum of azonazine (CD_3CN, 600MHz)

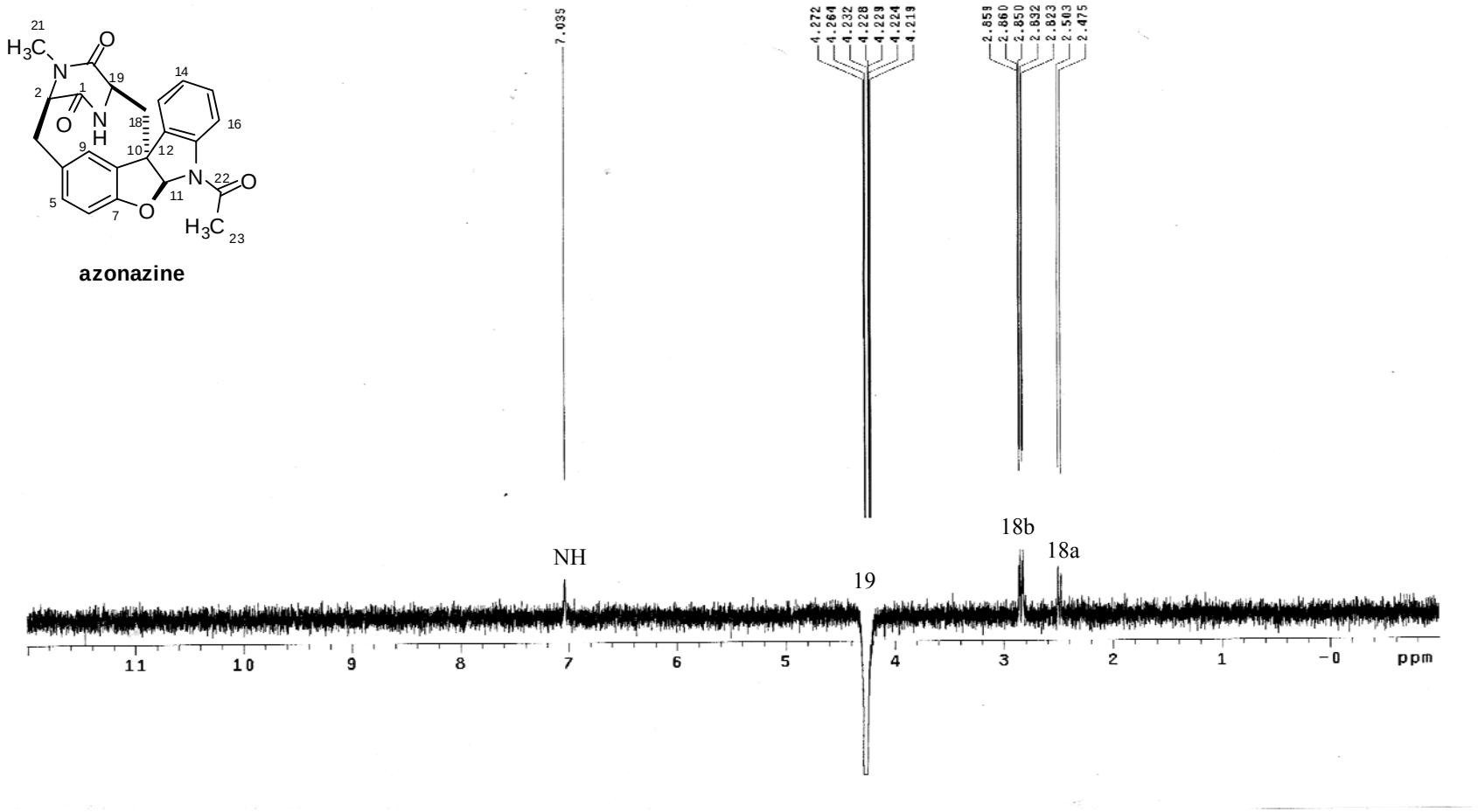
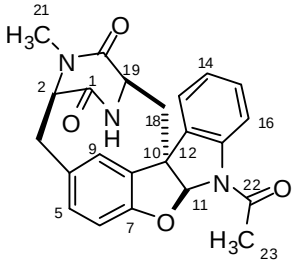


Figure S14. UV spectrum of azonazine (MeOH)



azonazine

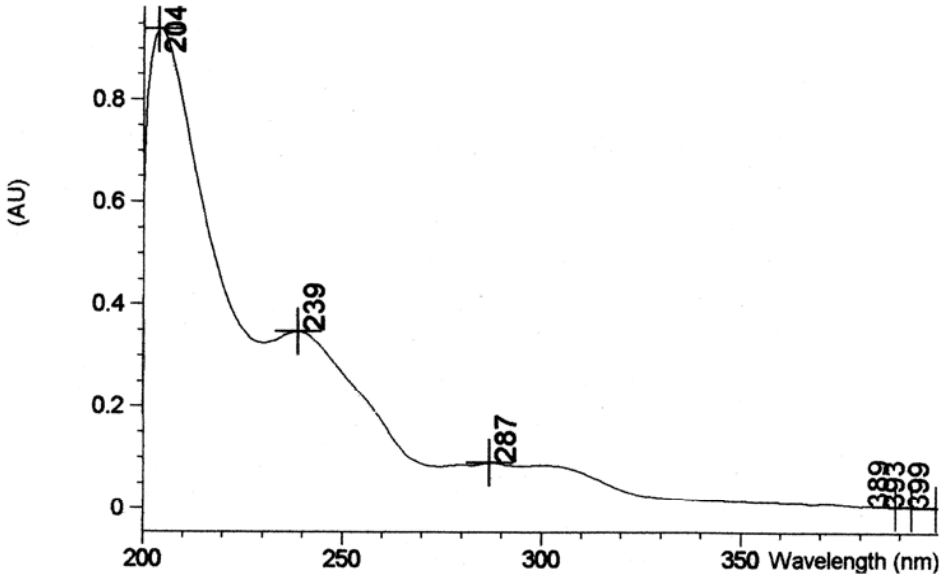


Figure S15. Energy minimized models of **I** – **VI** with selected NOEs

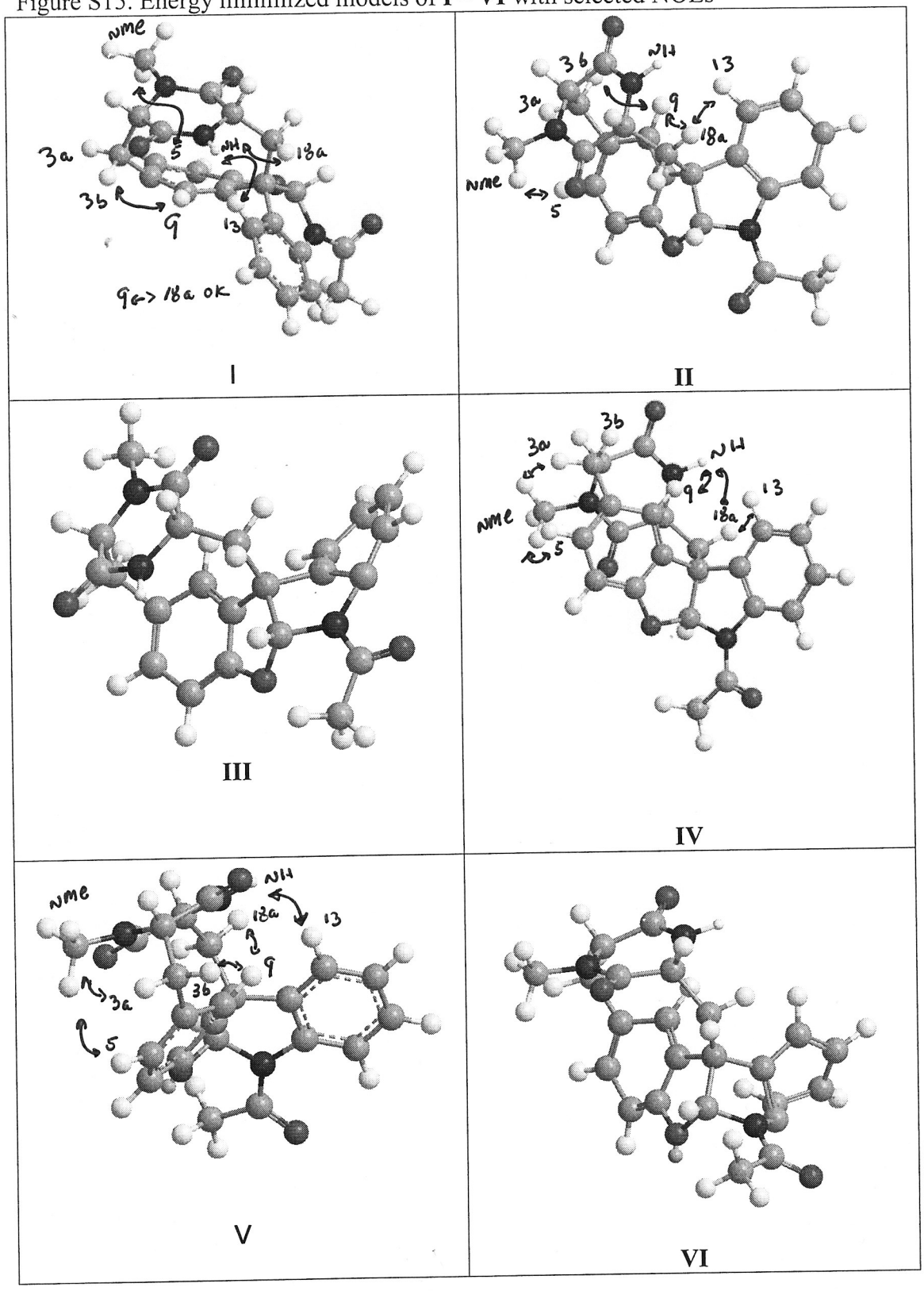


Figure S15a. Additional views of selected models.

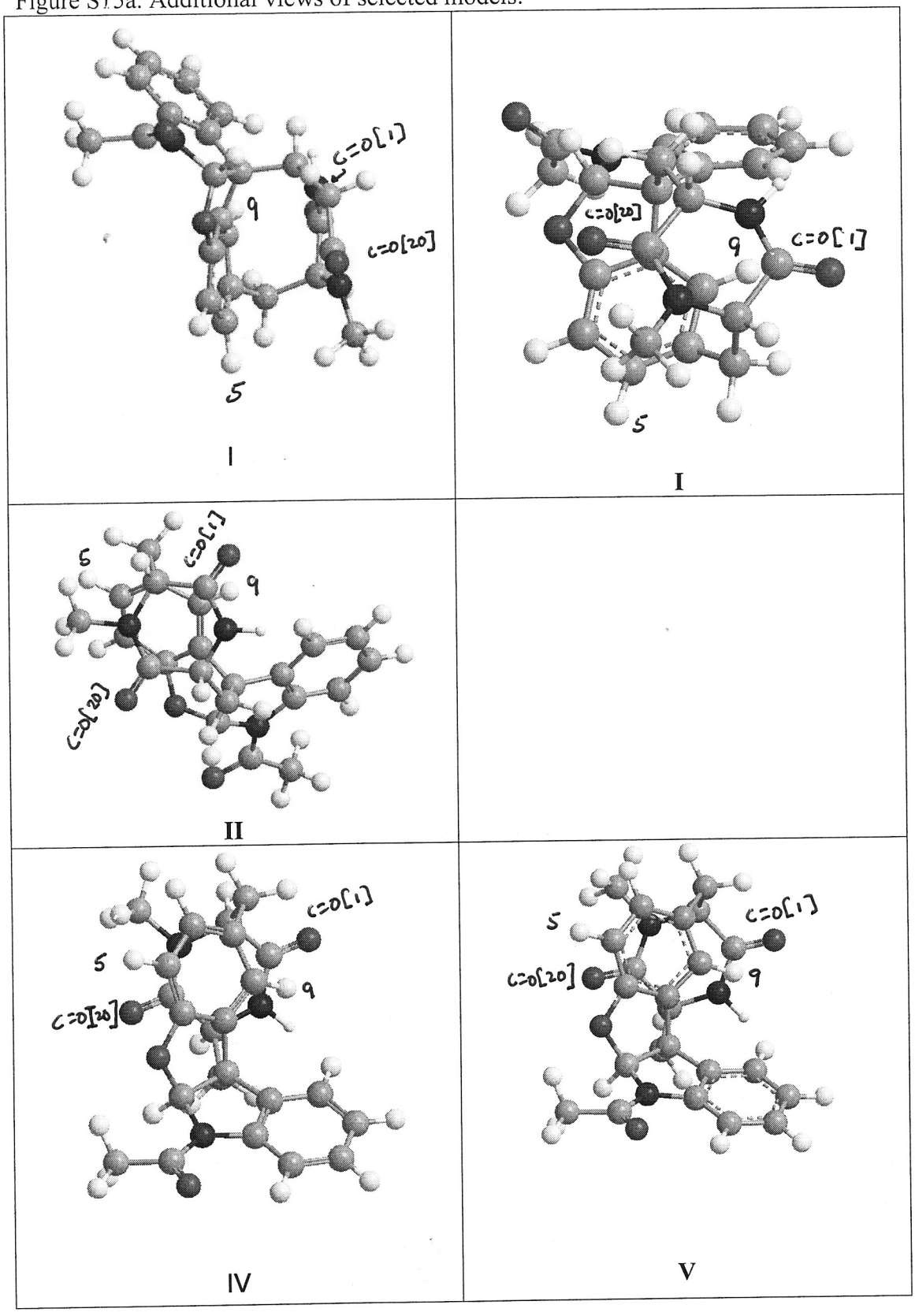


Figure S16. Plausible biosynthetic assembly of (+)-2*R*, 10*R*, 11*S*, 19*R*-azonazine

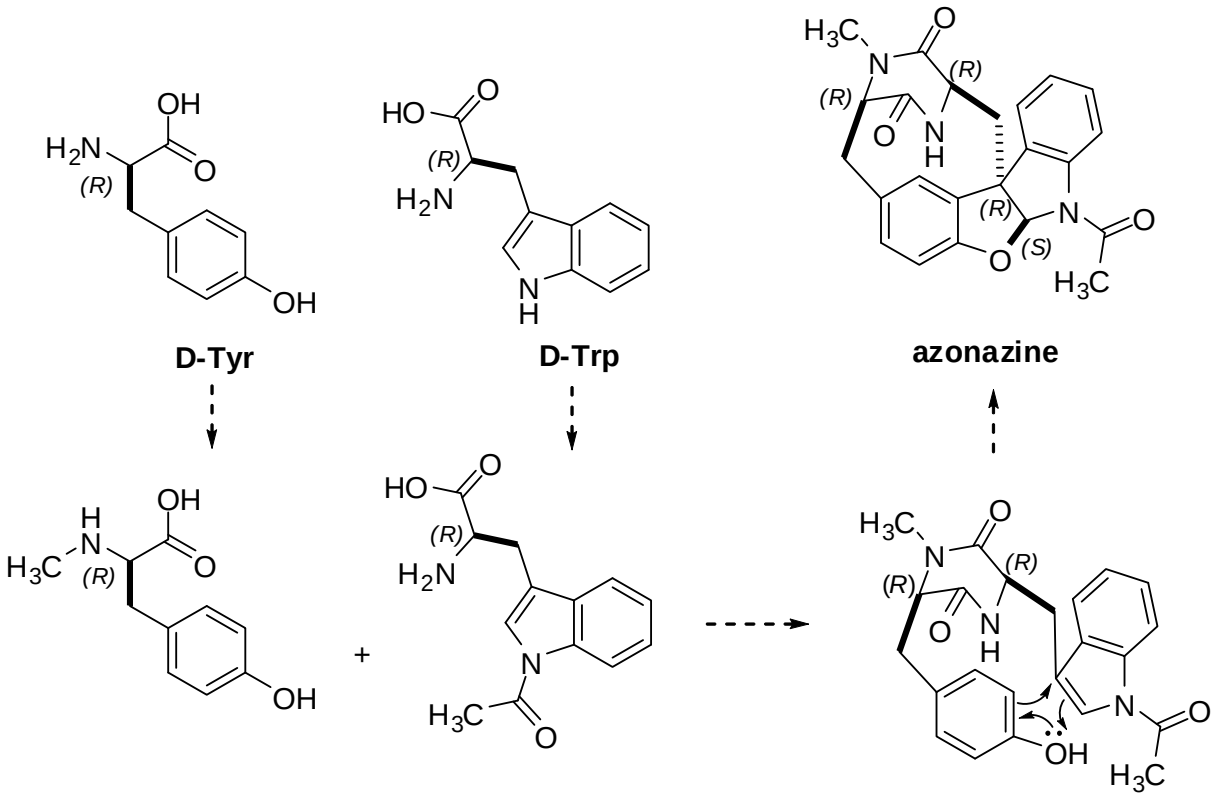
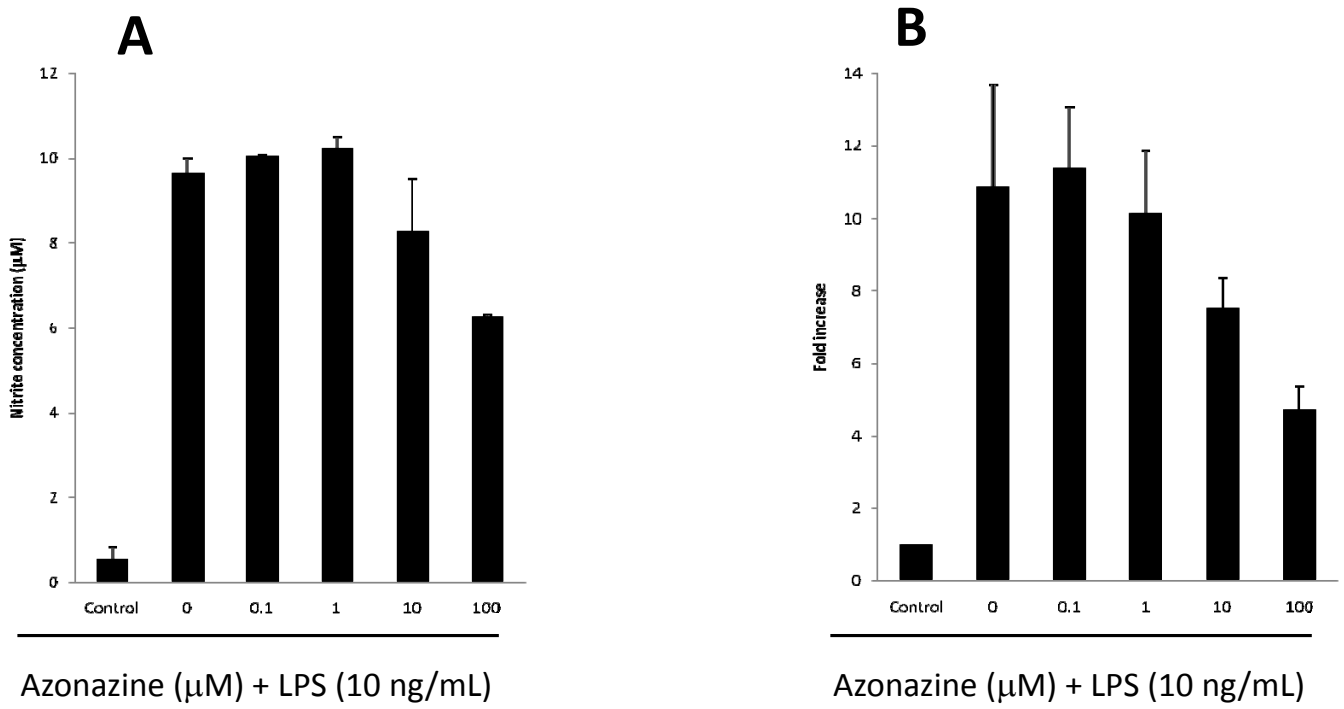


Figure S17. Inhibition of nitrite production and NFκB signaling by (+)-azonazine in RAW 264.7 cells



* A, Effect of azonazine on nitrite production. Cells in 96-well plate were treated with mixture of azonazine and LPS (10ng/mL) for 20h. B, Inhibition of LPS-induced NFκB signaling.