Supplementary Information

ZIKV Inhibitors Based on Pyrazolo[3,4-*d*]pyridazine-7-one Core: Rational Design, *in vitro* Evaluation and Theoretical Studies

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Contents

Figure S1-S27: Spectral data ([1]H-NMR, HRMS spectrum, and HPLC/GC-FID) of synthesized compounds

7a

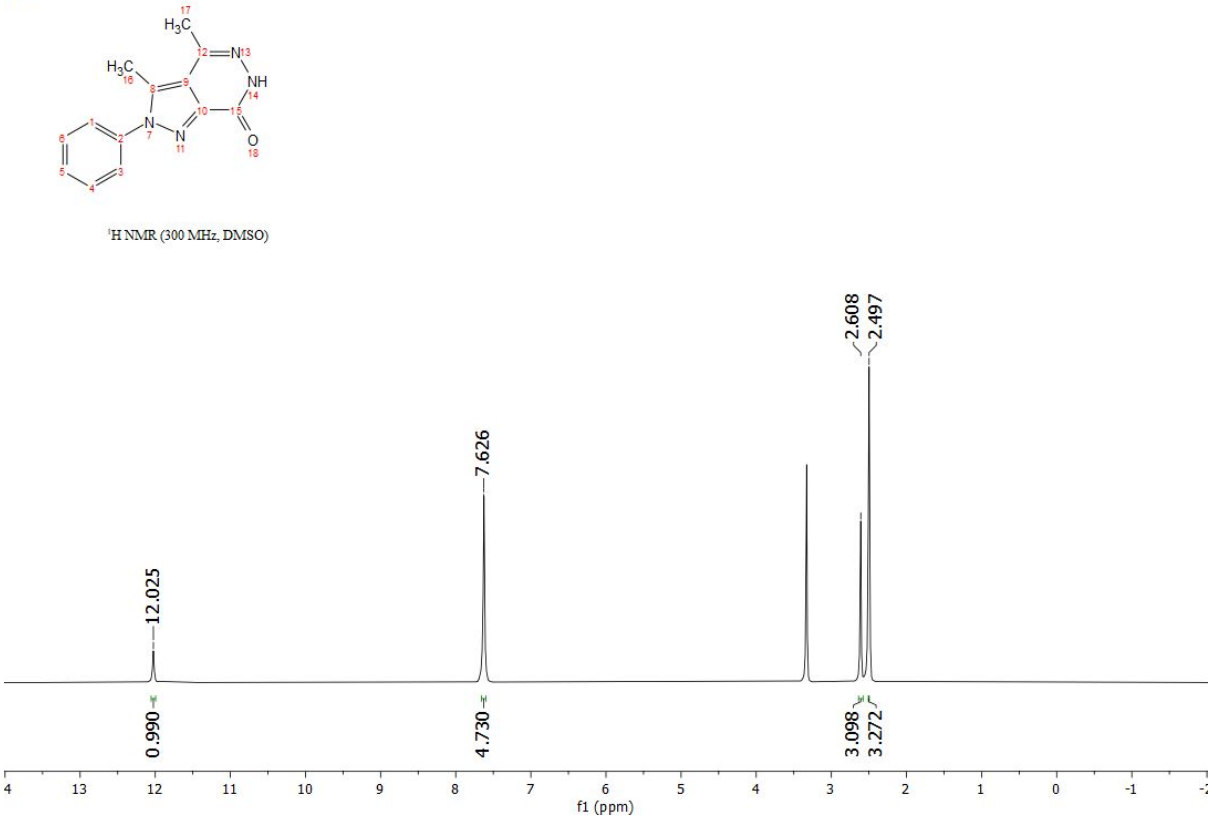


Figure S1. [1]H-NMR spectrum of **7a**

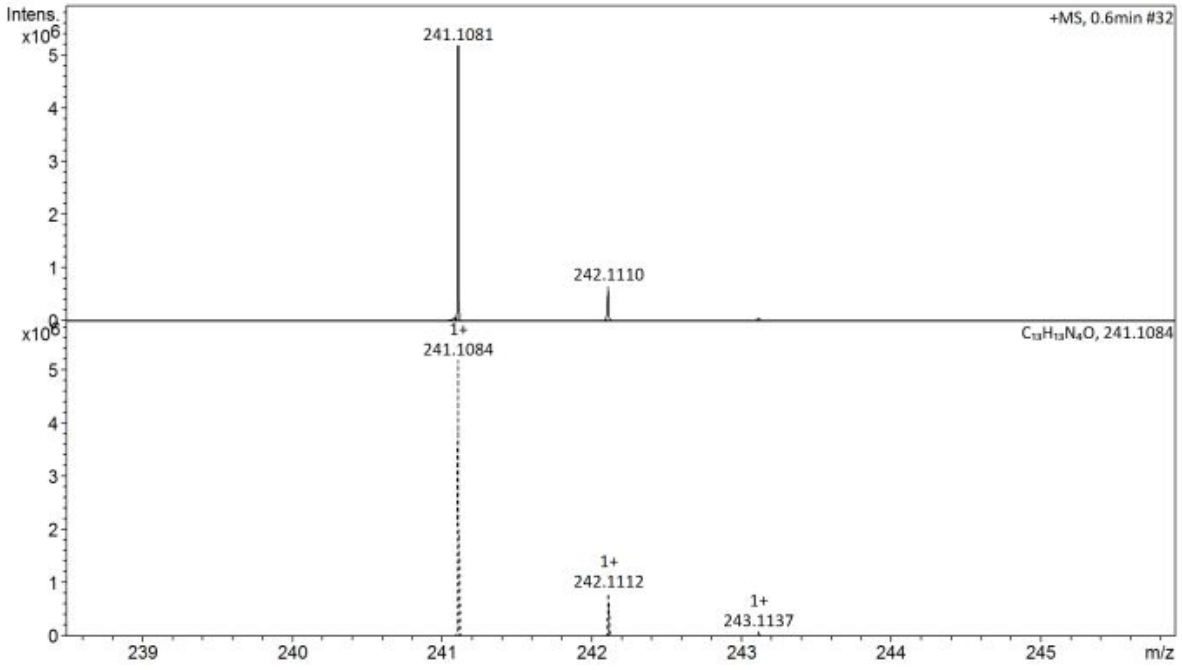


Figure S2. HRMS spectrum of **7a**

7b

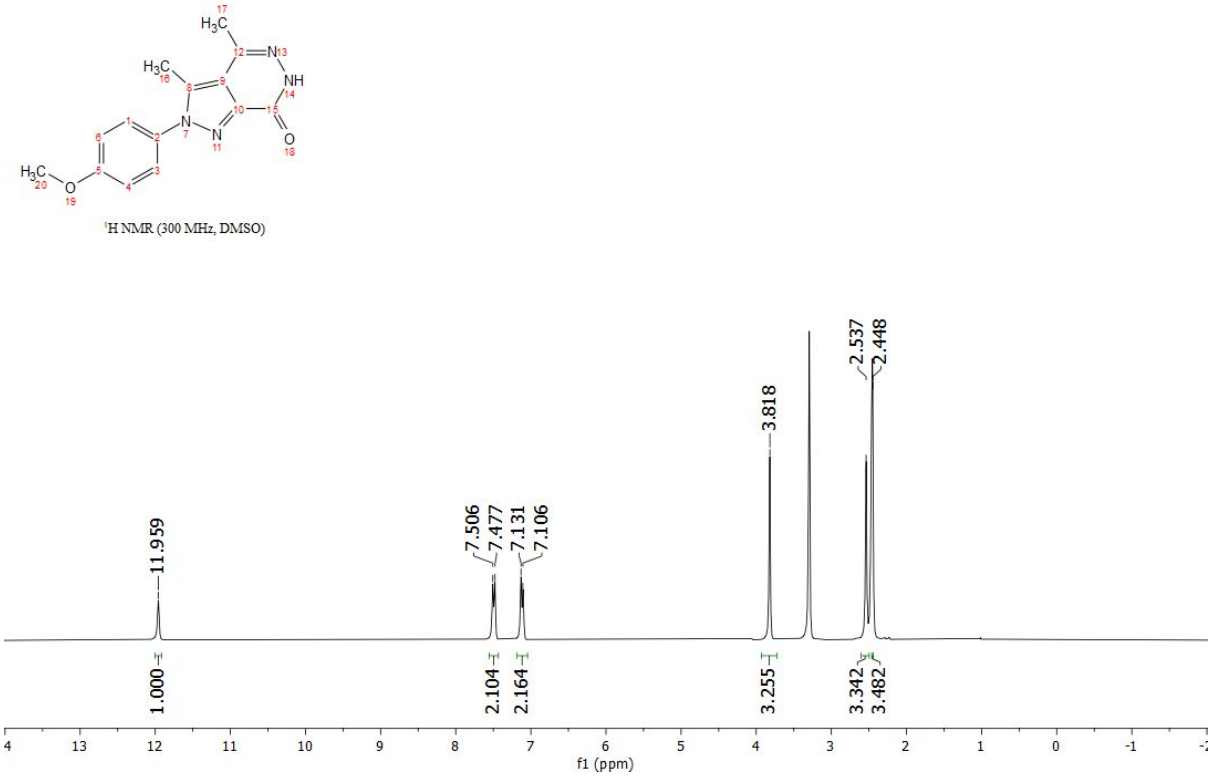


Figure S3. ^{1}H-NMR spectrum of **7b**

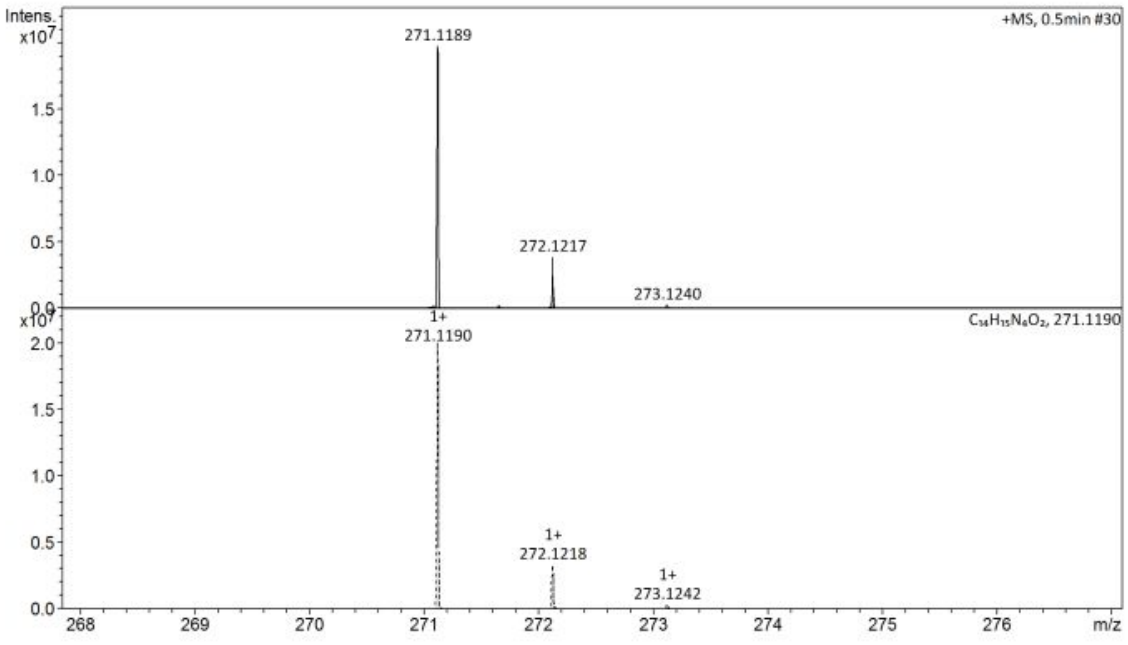


Figure S4. HRMS spectrum of **7b**

9a

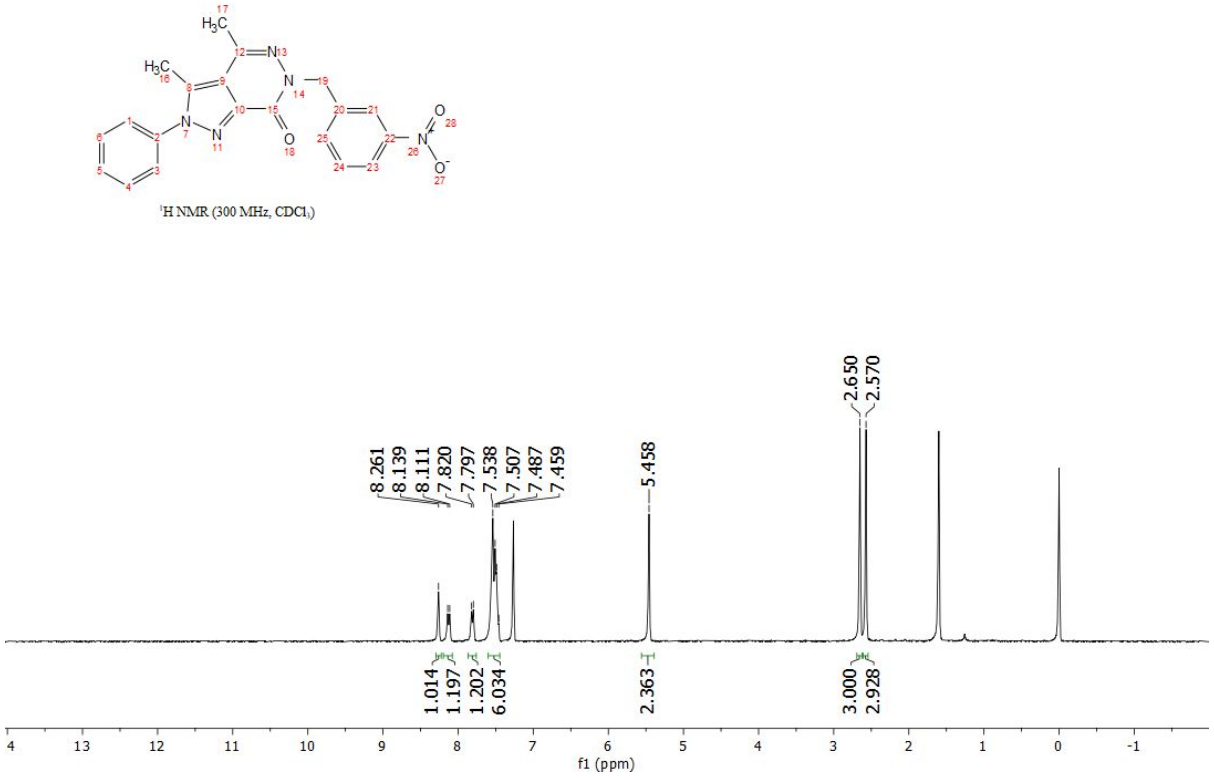


Figure S5. ^{1}H-NMR spectrum of **9a**

9b

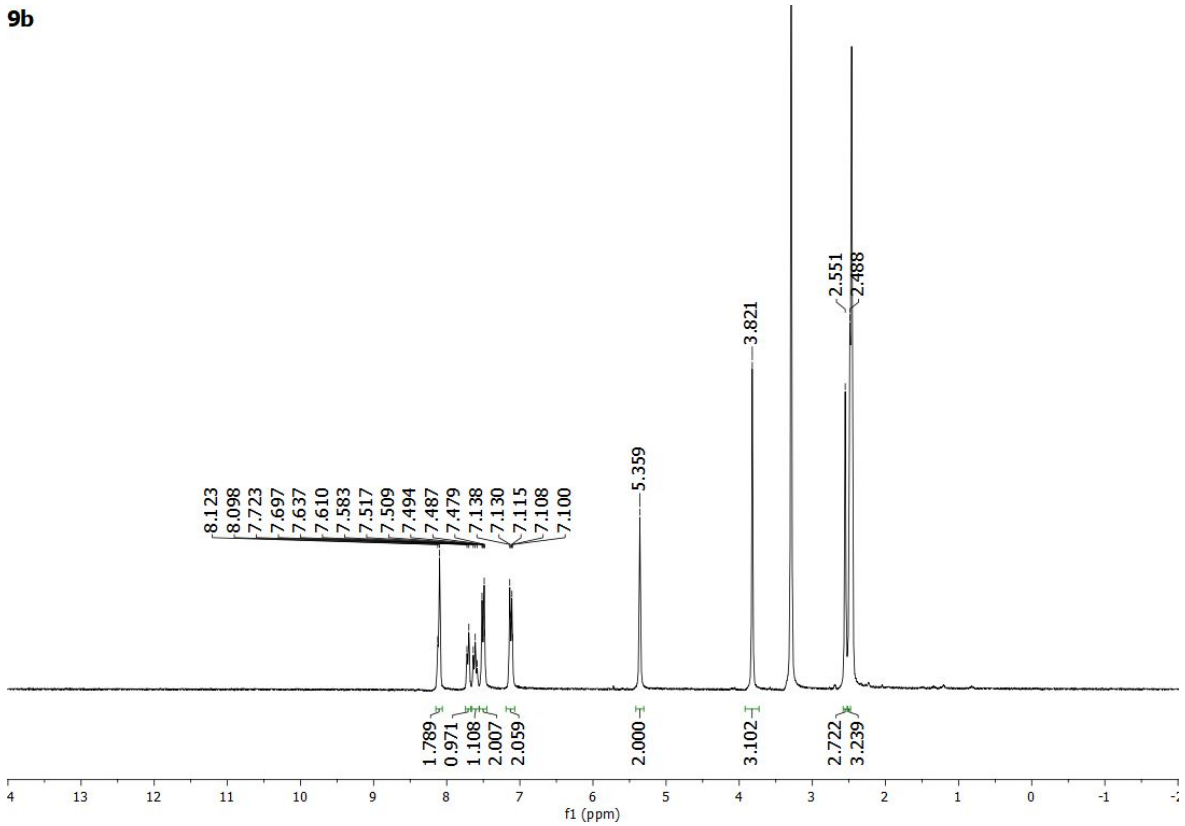


Figure S6. ^{1}H-NMR spectrum of **9b**

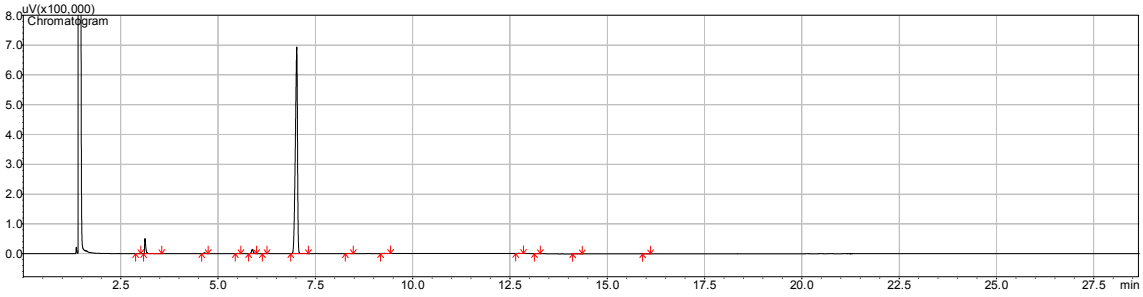


Figure S7. GC chromatogram of **9b**. GC-FID 2010 model (Shimadzu) in the following conditions: Programmed Temperature Vaporizer (PTV) in direct inject mode at 250ºC using hydrogen as carrier gas and column flow in 2.04 mL/min at a constant pressure mode. The column oven temperature program started 150°C (hold 1 min), then programmed at 10°C/min to 320 °C (hold 4 min). The total analytical time was 30 min. The detector temperature was 320°C.

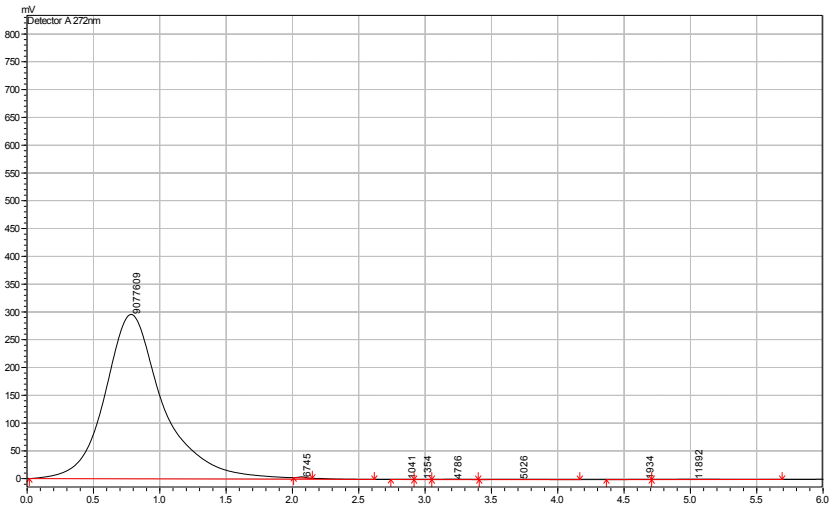
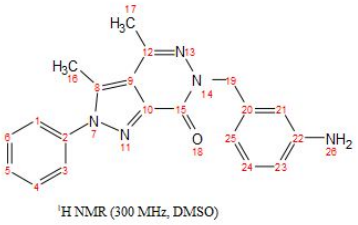


Figure S8. HPLC chromatogram of **9b** under the following analytical condition: system: Agilent 1260 HPLC system; column: Agilent Eclipse ZORBAX XDB-C18 (46×250 mm; 5 µm); flowrate: 1.0 mL/min; column temperature: 40°C; UV wavelength: 272 nm; solvent system: 0-10 min: 100% MeOH.

10a



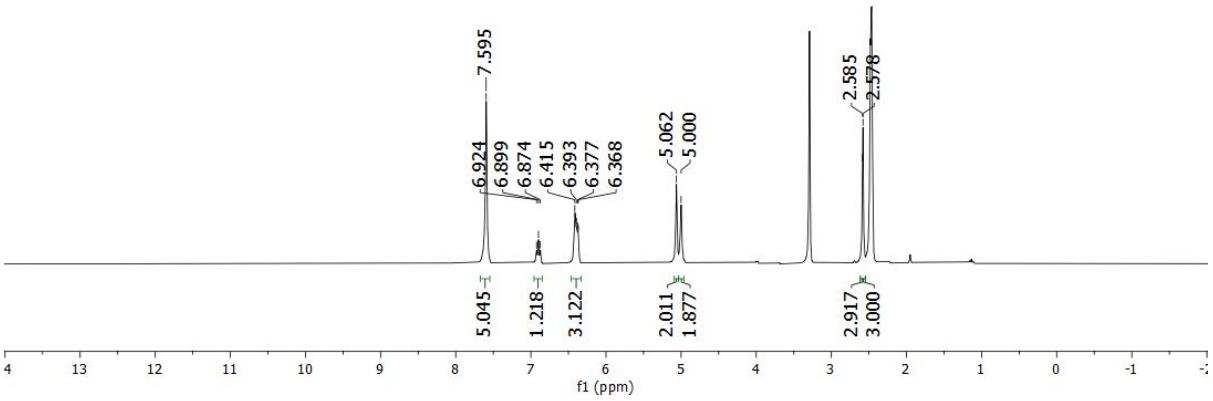


Figure S9. ^{1}H-NMR spectrum of **10a**

10b

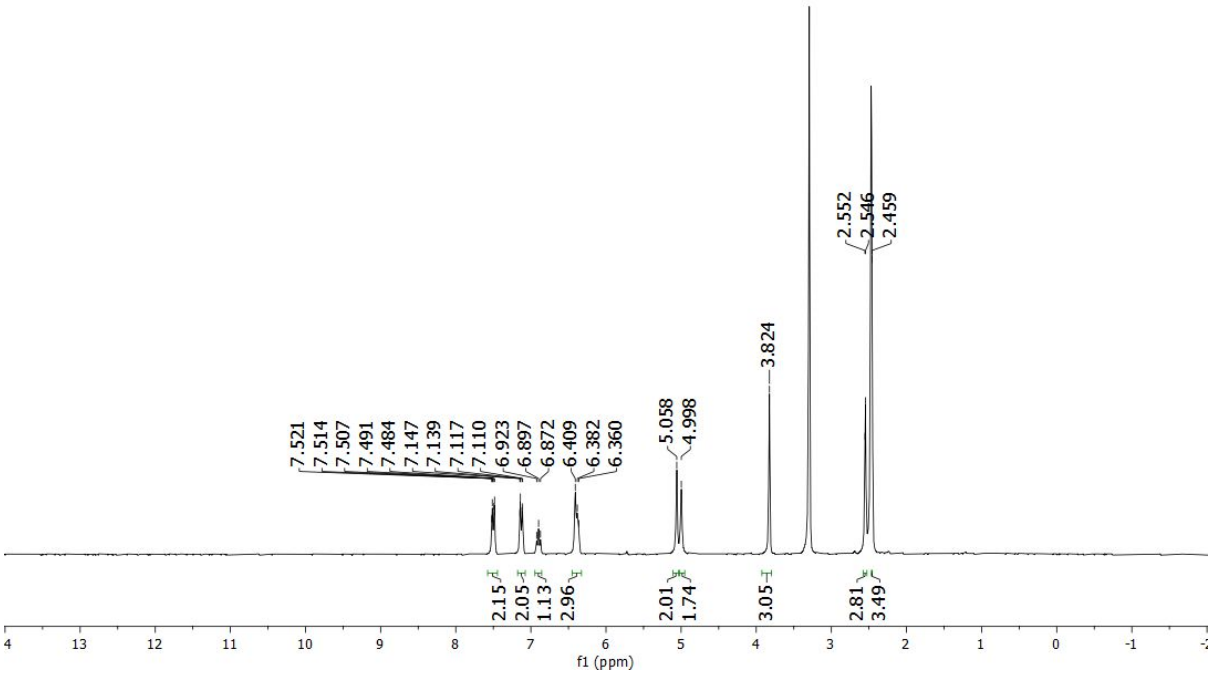


Figure S10. ^{1}H-NMR spectrum of **10b**

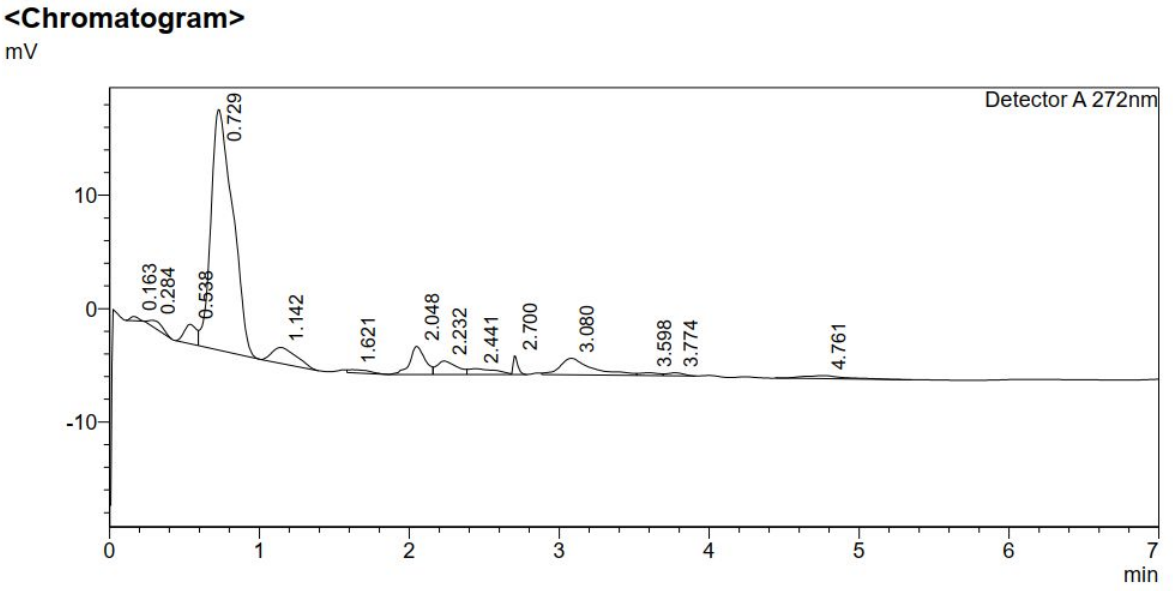


Figure S11. HPLC chromatogram of **10b** under the following analytical condition: system: Agilent 1260 HPLC system; column: Agilent Eclipse ZORBAX XDB-C18 (46×250 mm; 5 μm); flowrate: 1.0 mL/min; column temperature: 40°C; UV wavelength: 272 nm; solvent system: 0-10 min: 100% MeOH.

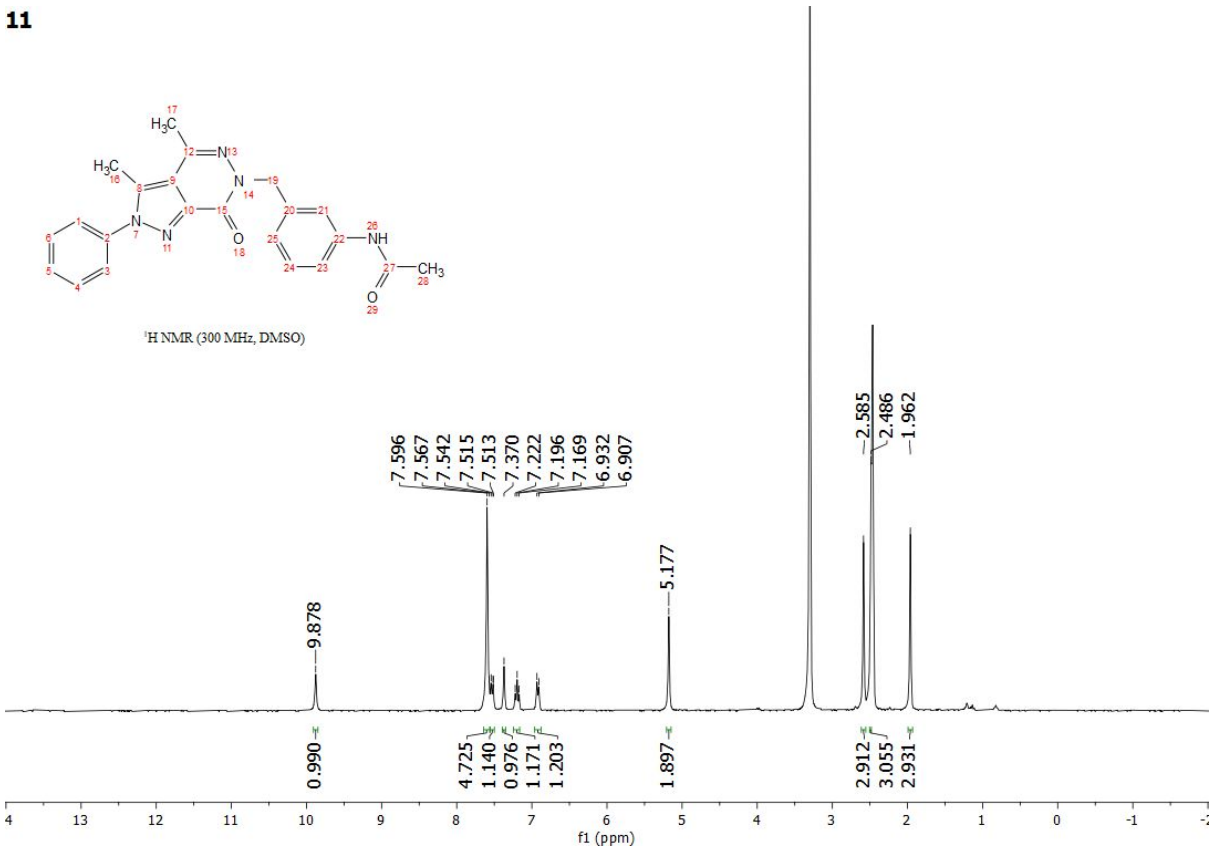


Figure S12. ^{1}H-NMR spectrum of **11**

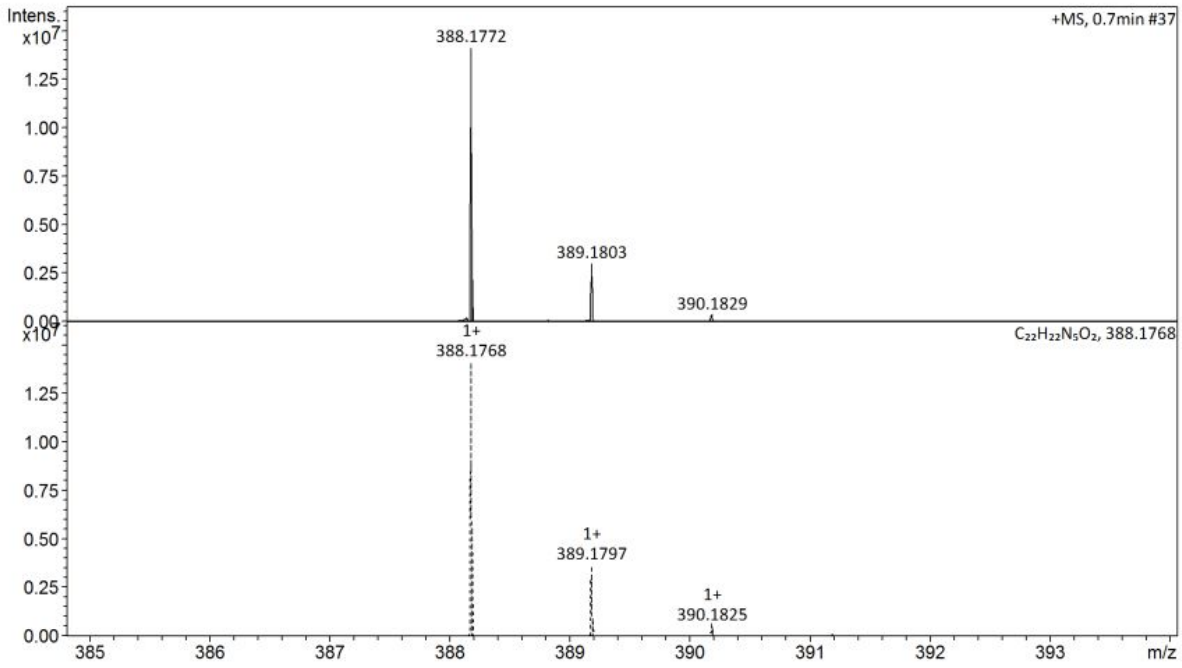


Figure S13. HRMS spectrum of **11**

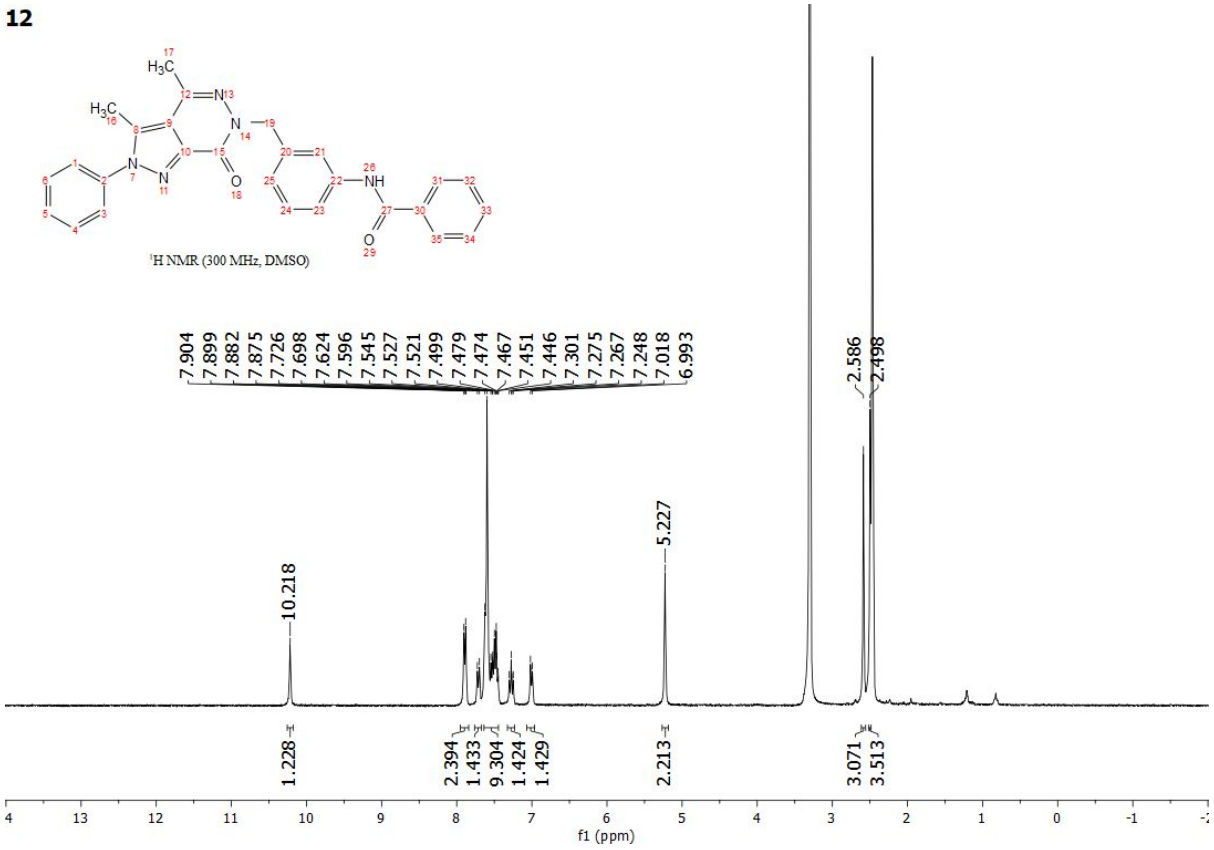


Figure S14. ^{1}H-NMR spectrum of **12**

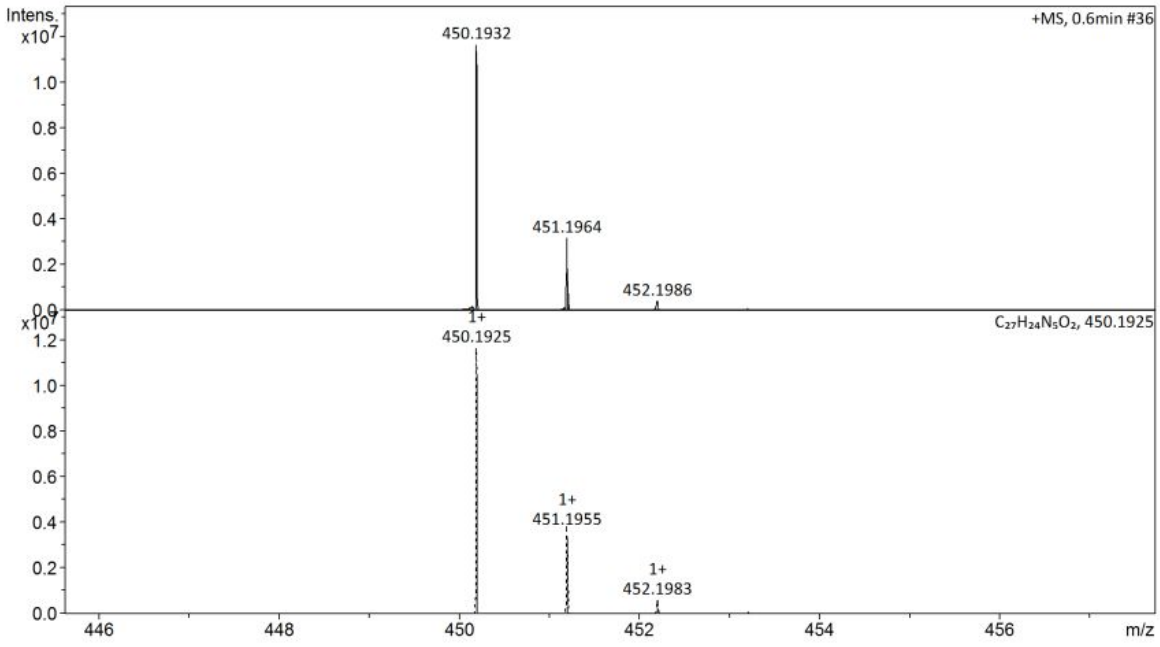


Figure S15. HRMS spectrum of **12**

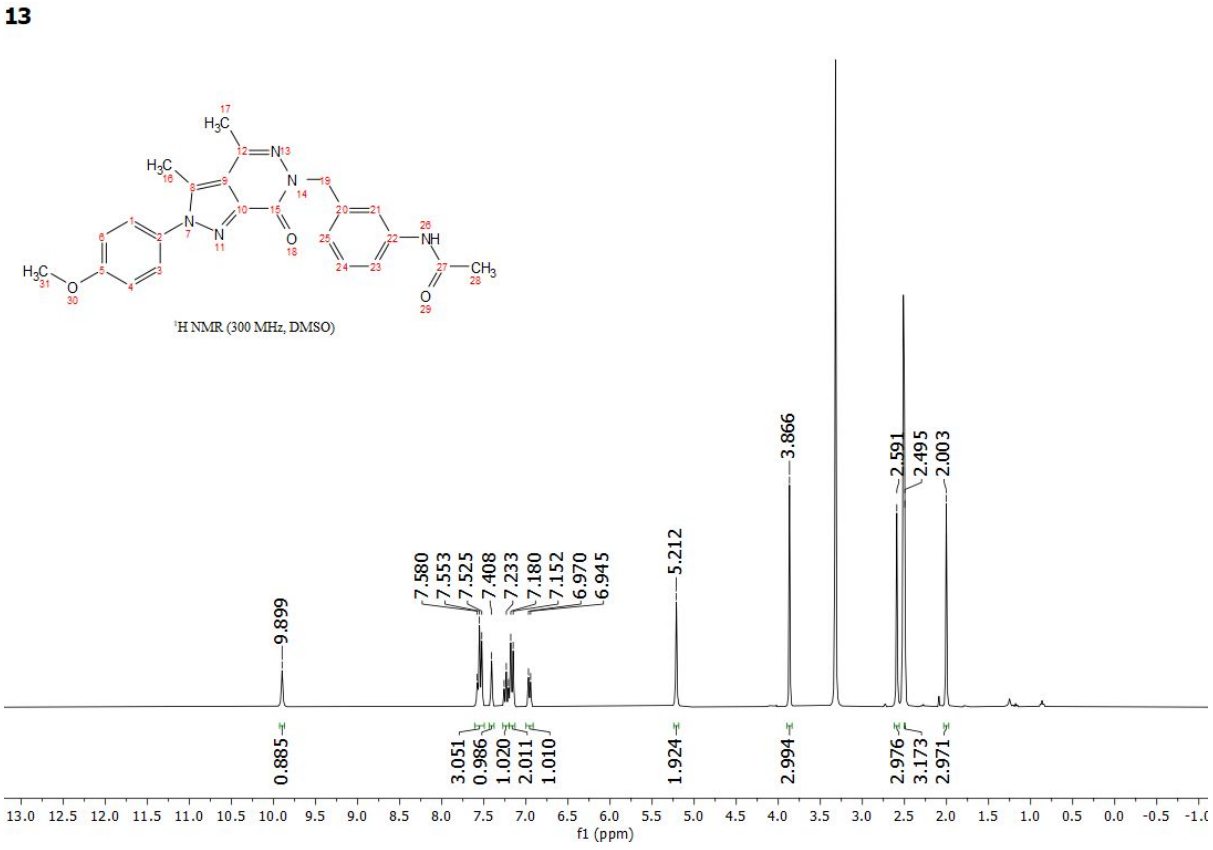


Figure S16. ^{1}H-NMR spectrum of **13**

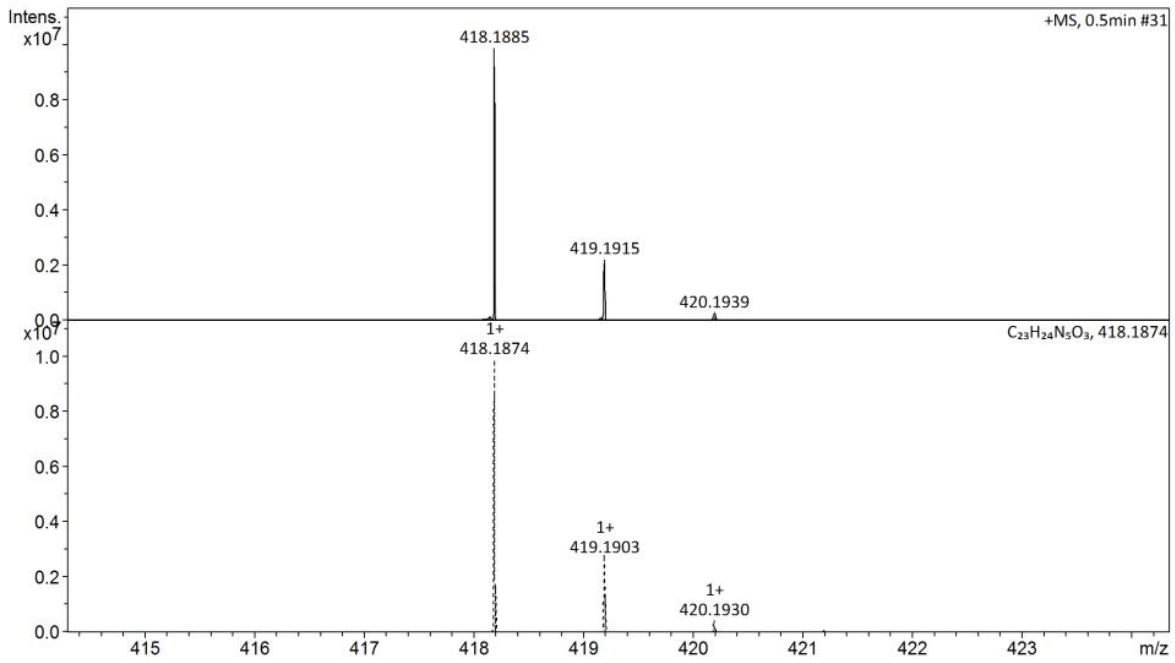


Figure S17. HRMS spectrum of **13**

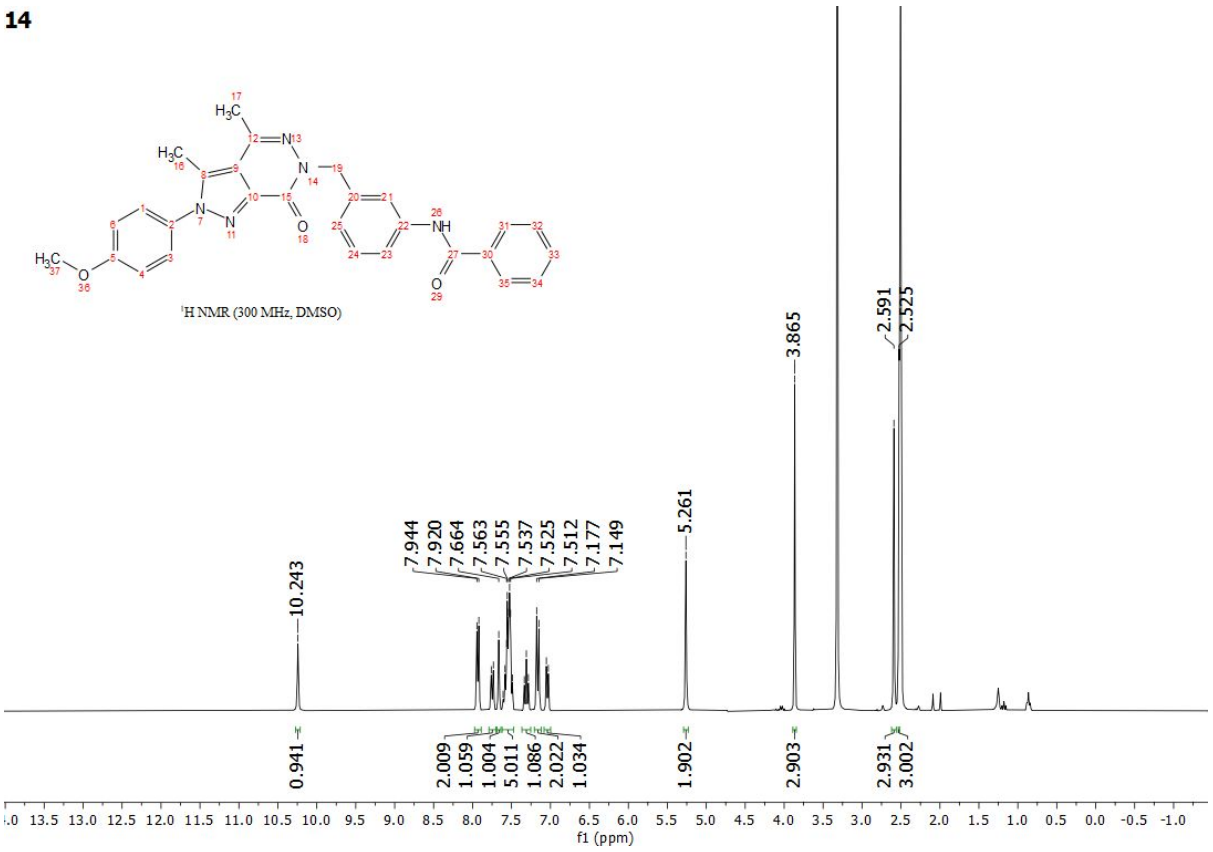


Figure S18. ^{1}H-NMR spectrum of **14**

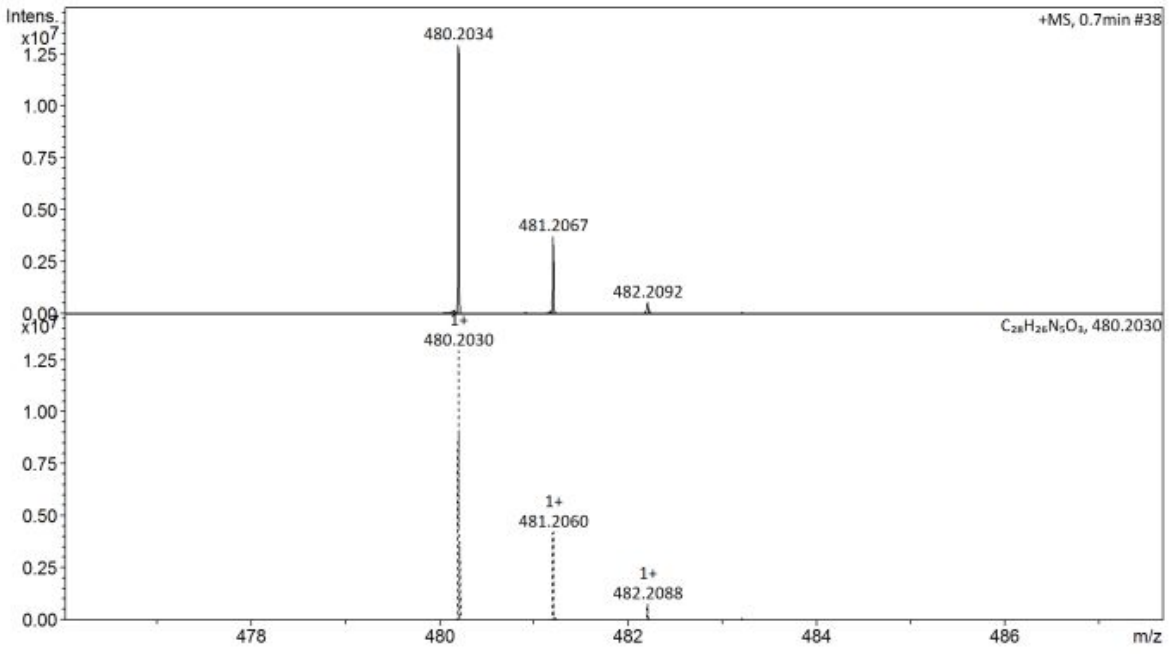


Figure S19. HRMS spectrum of **14**

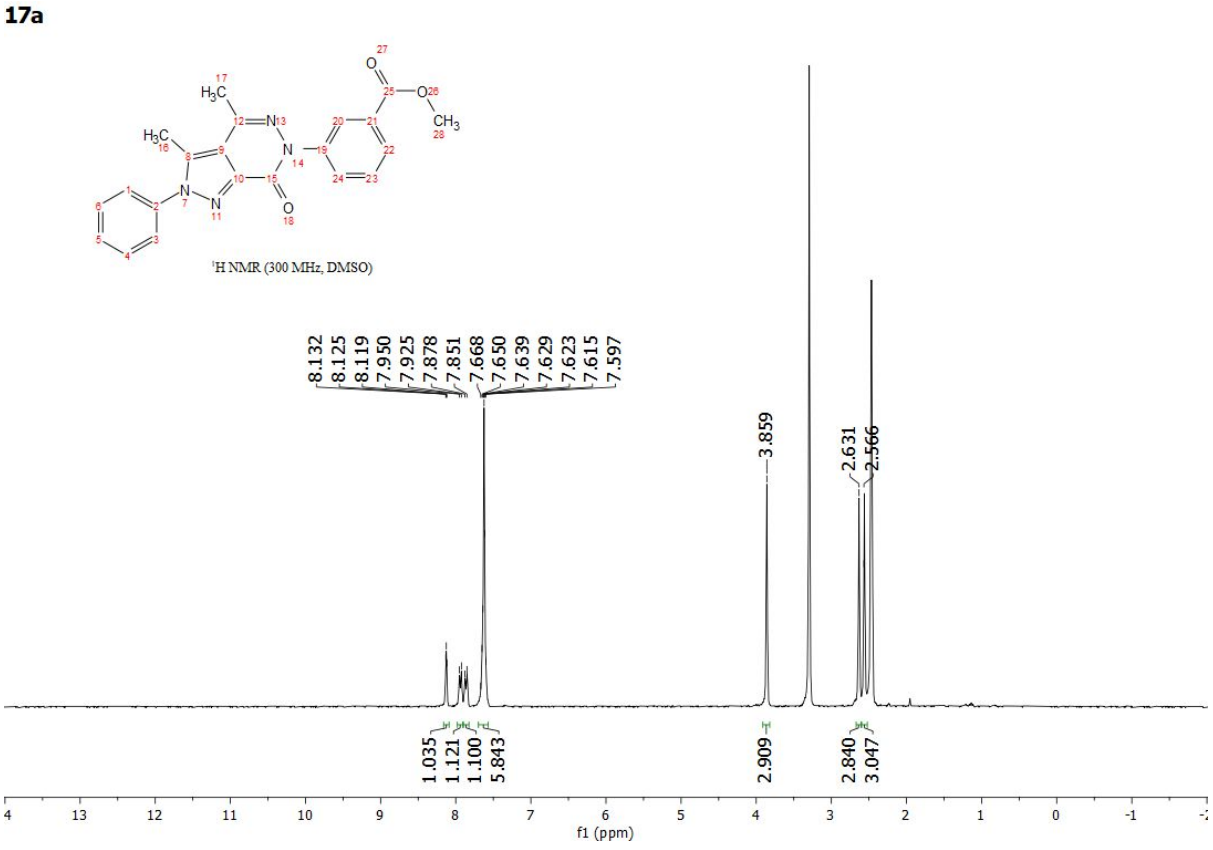


Figure S20. ^{1}H-NMR spectrum of **17a**

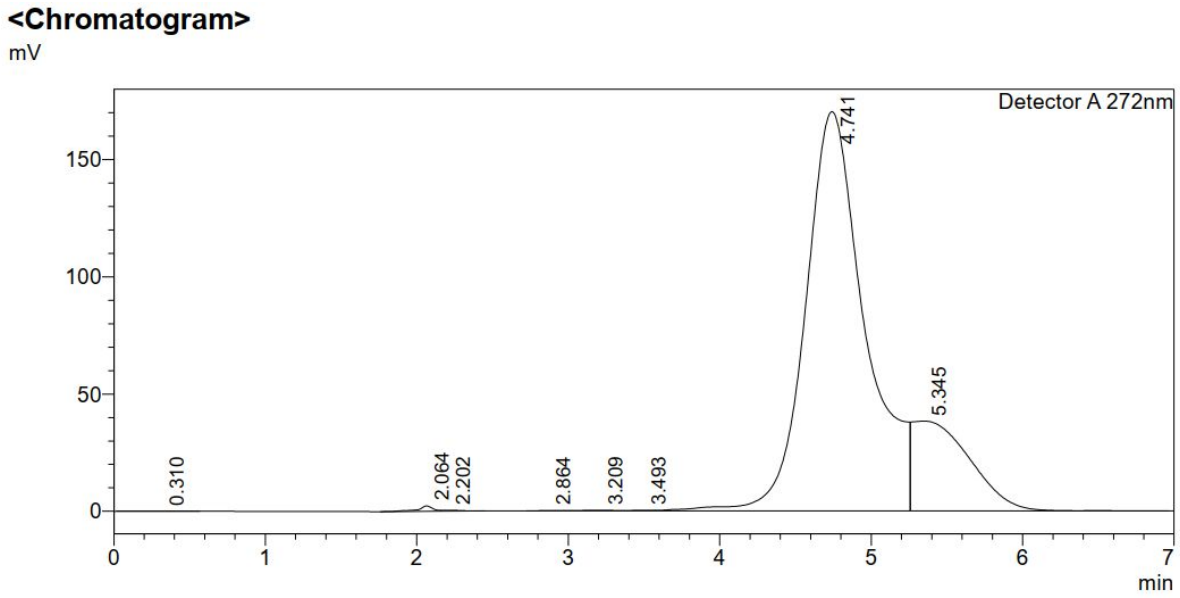


Figure S21. HPLC chromatogram of **17a** under the following analytical condition: system: Agilent 1260 HPLC system; column: Agilent Eclipse ZORBAX XDB-C18 (46×250 mm; 5 μm); flowrate: 1.0 mL/min; column temperature: 40°C; UV wavelength: 272 nm; solvent system: 0-10 min: 100% MeOH.

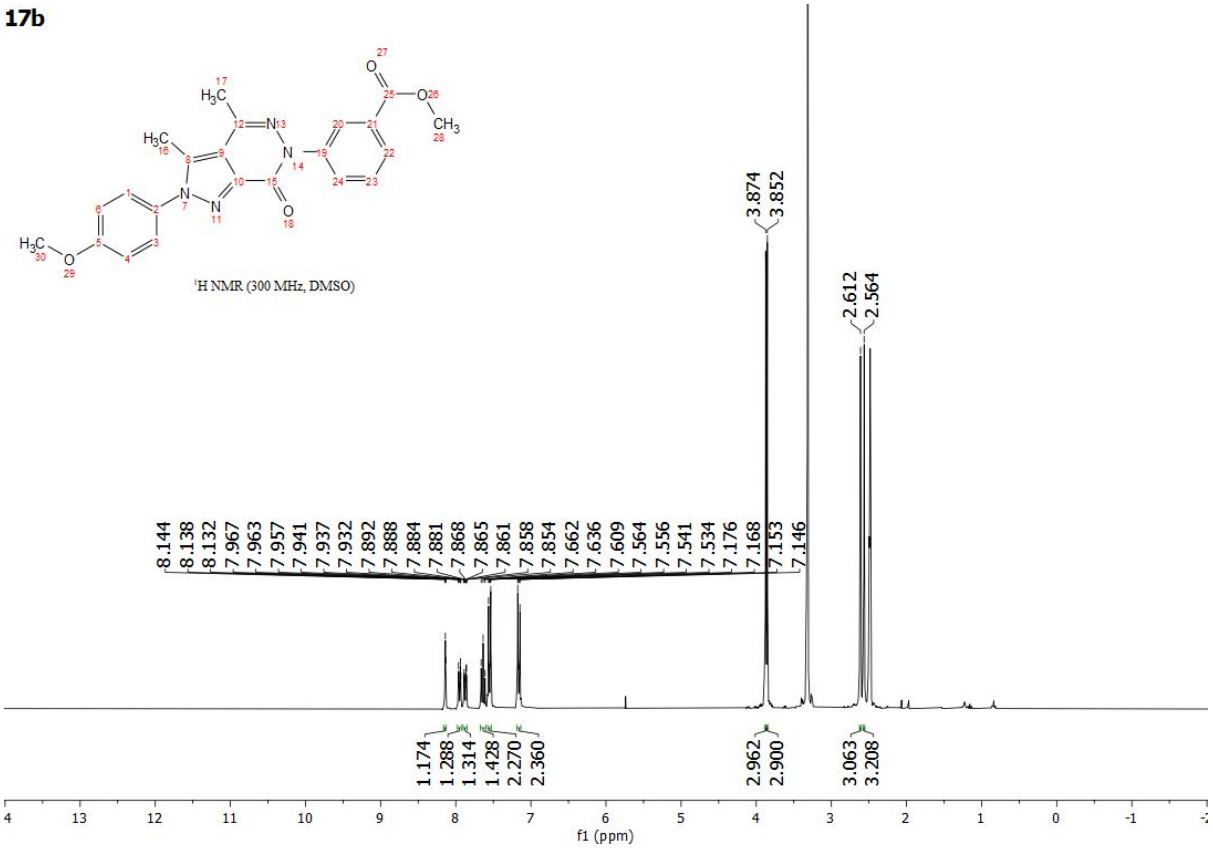


Figure S22. ^{1}H-NMR spectrum of **17b**

18a

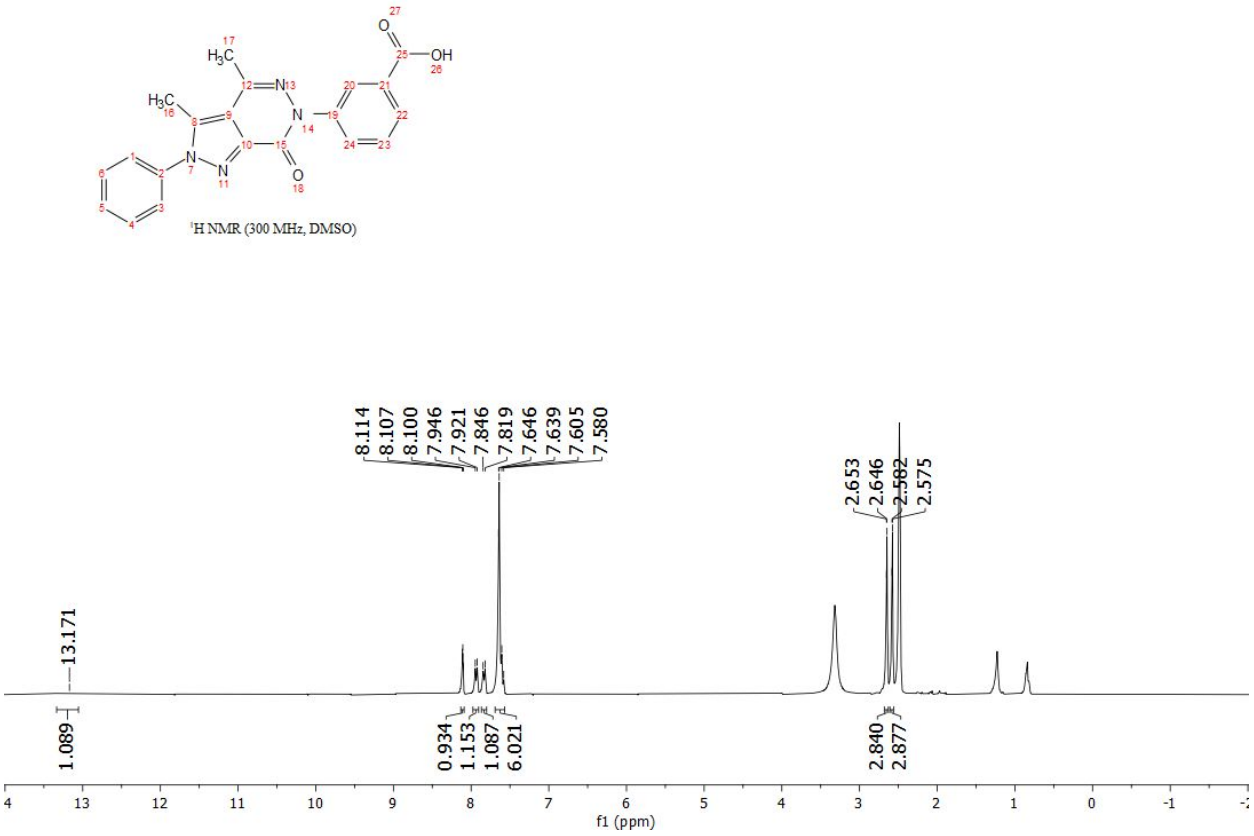


Figure S23. [1]H-NMR spectrum of **18a**

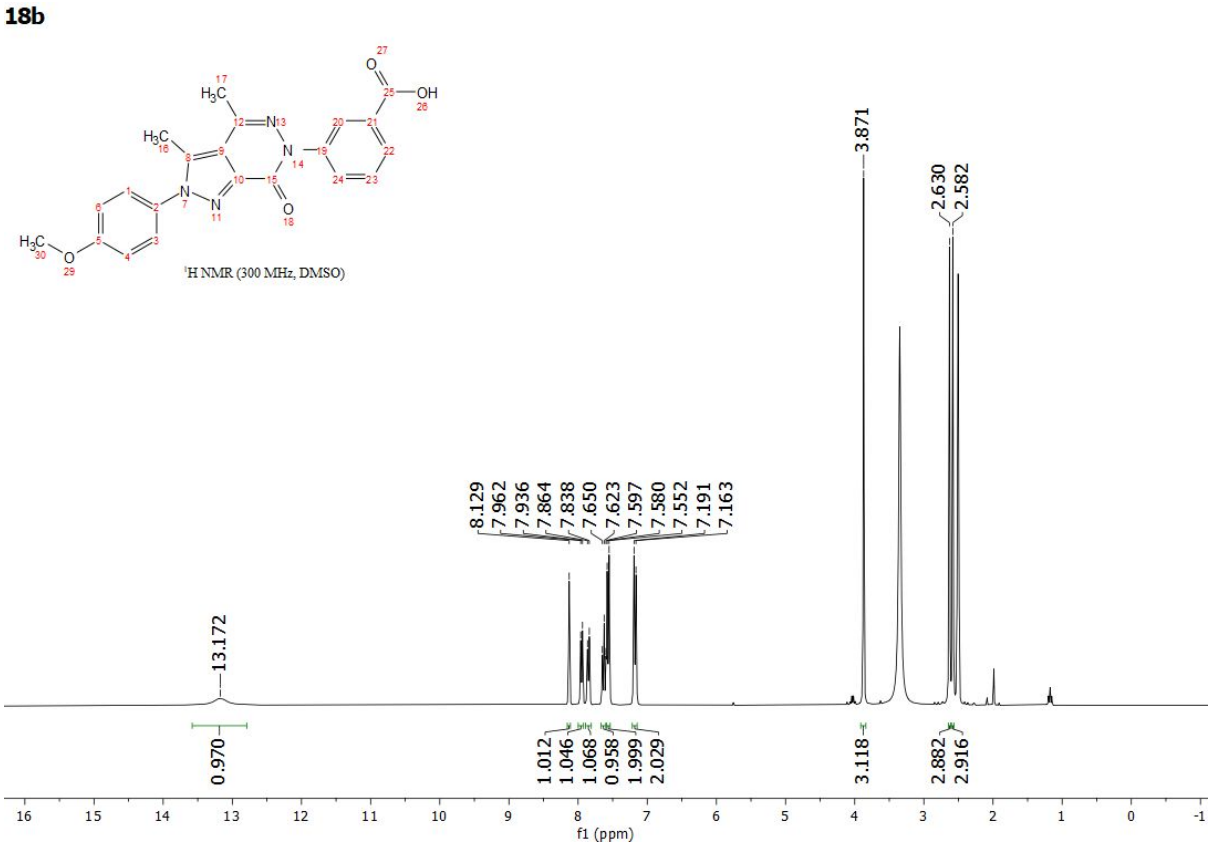


Figure S24. [1]H-NMR spectrum of **18b**

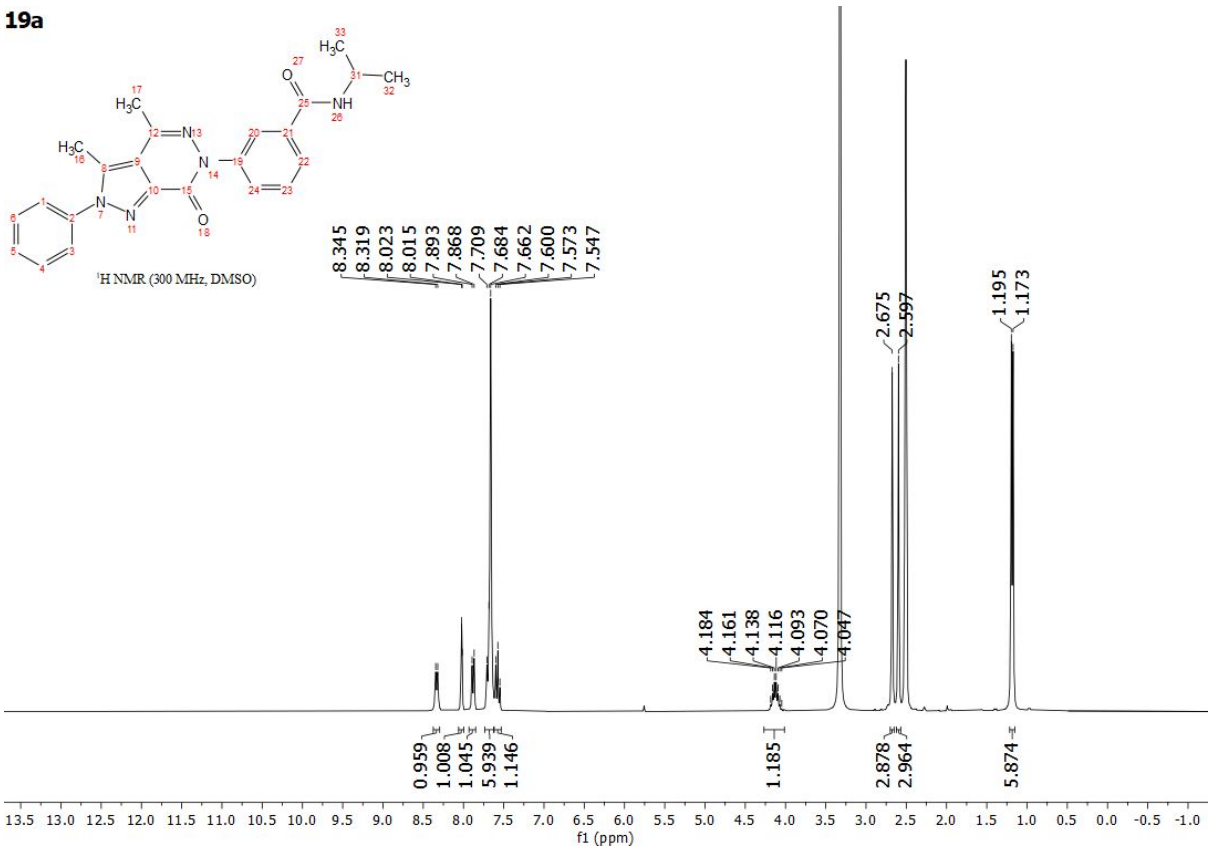


Figure S25. ^{1}H-NMR spectrum of **19a**

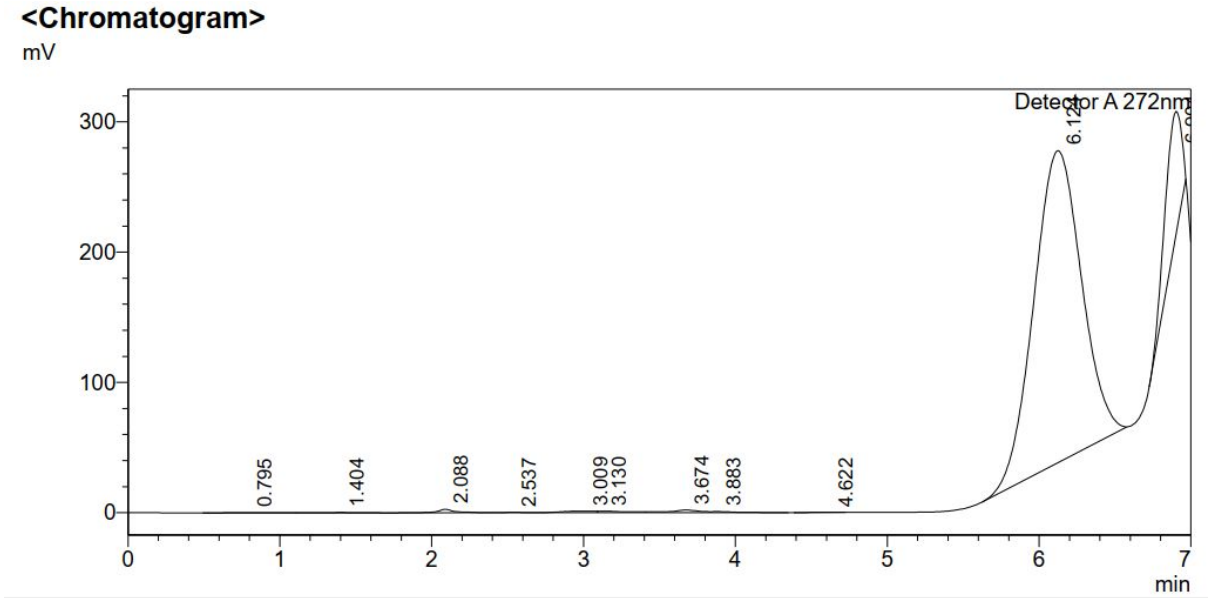


Figure S26. HPLC chromatogram of **19a** under the following analytical condition: system: Agilent 1260 HPLC system; column: Agilent Eclipse ZORBAX XDB-C18 (46×250 mm; 5 μm); flowrate: 1.0 mL/min; column temperature: 40°C; UV wavelength: 272 nm; solvent system: 0-10 min: 100% MeOH.

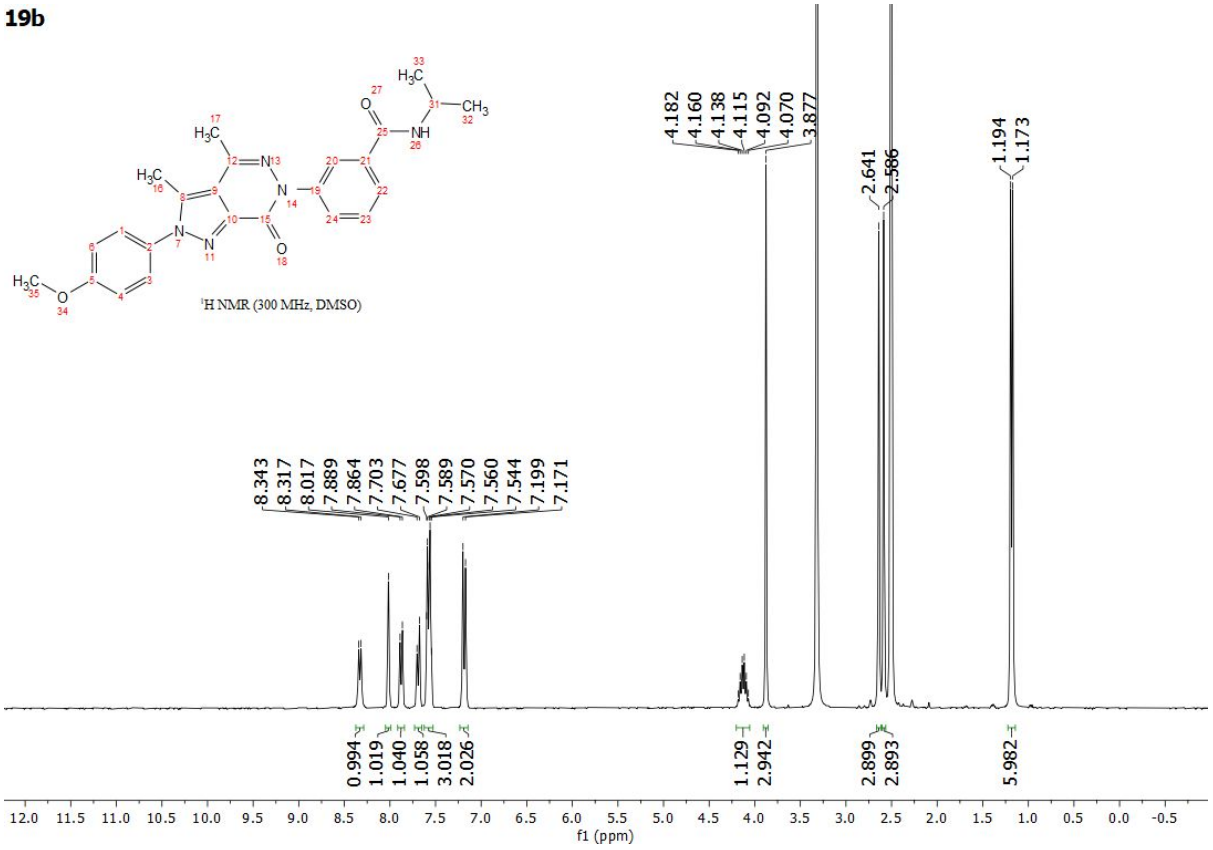
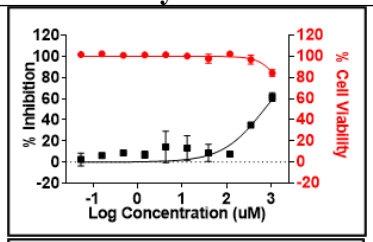
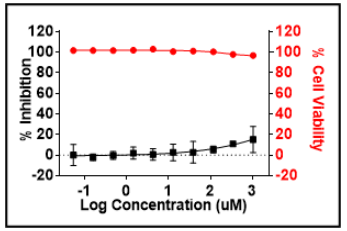
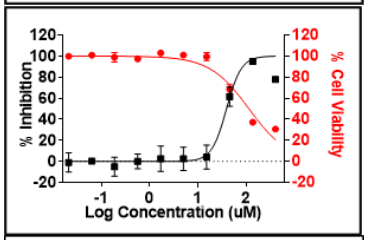
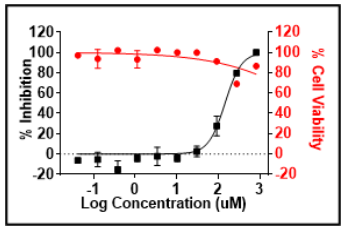
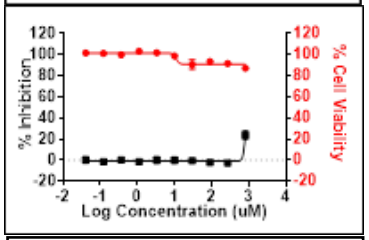
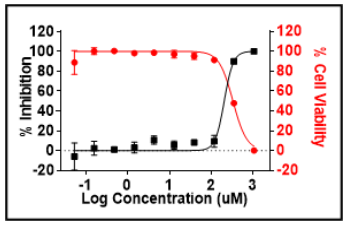
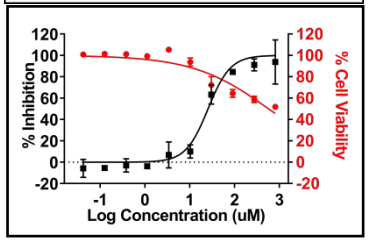
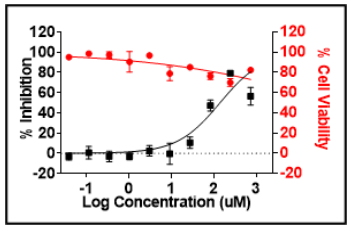


Figure S27. ^{1}H-NMR spectrum of **19b**

Biological assay for all compounds (Table S1 and Figure S28)

Table S1. Dose-response curves of compounds against the ZIKV

Compound	Curve analysis	Compound	Curve analysis
7a		10a	
7b		10b	
9a		11	
9b		12	

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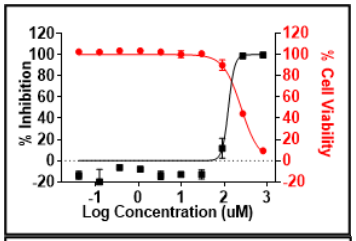

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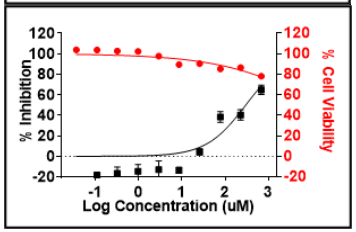
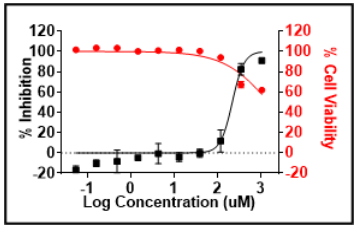
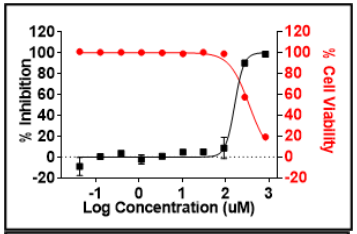


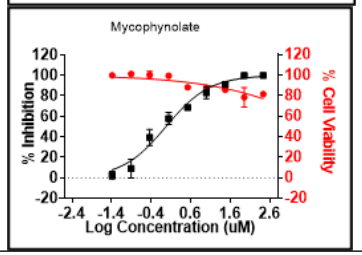
17a



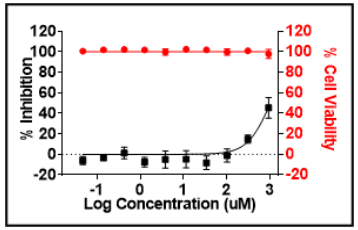
17b



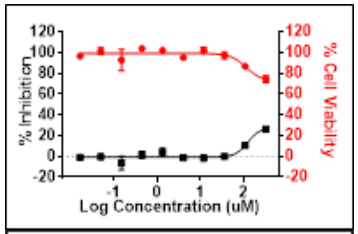
MPA



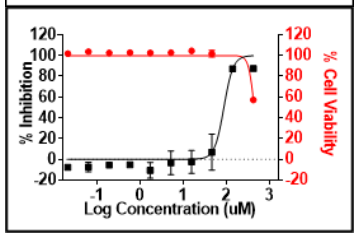
18a



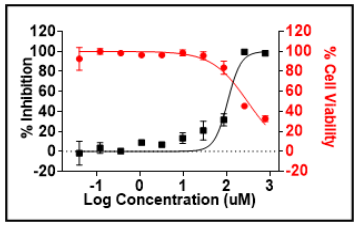
18b



19a



19b



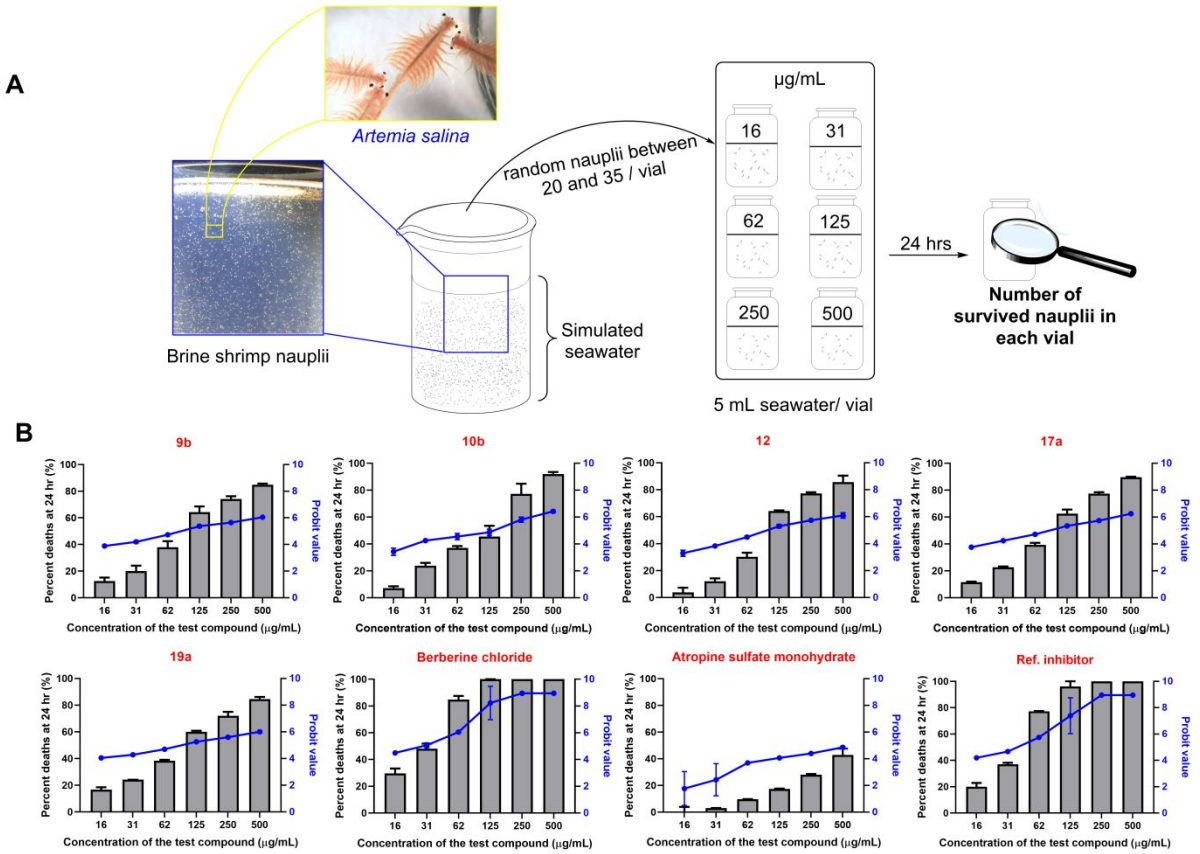


Figure S28. A: Brine shrimps (*Artemia salina*) were used for the investigation of cytotoxic activity. The naupliis are considered dead when there is no movement for 30 seconds. All the tests were replicated three times. (Copyright of super macro close up of *A. salina* was purchased from Depositphotos Inc. United States, https://depositphotos.com/377593032/stock-photo-super-macro-close-artemia-salina.html). B: Cytotoxic potential of compounds and standard drugs against *Artemia salina*. The percentage of deaths of shrimps is shown in the gray column. Probit values are shown in the blue line. The ZIKV inhibitor (berberine derivative-**4d**) was described by us previously published and it was used as standard (DOI:10.1155/2021/5567111)

Molecular docking and pharmacophore model (Figure S29-S30 Table S2)

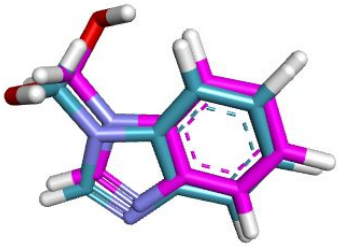
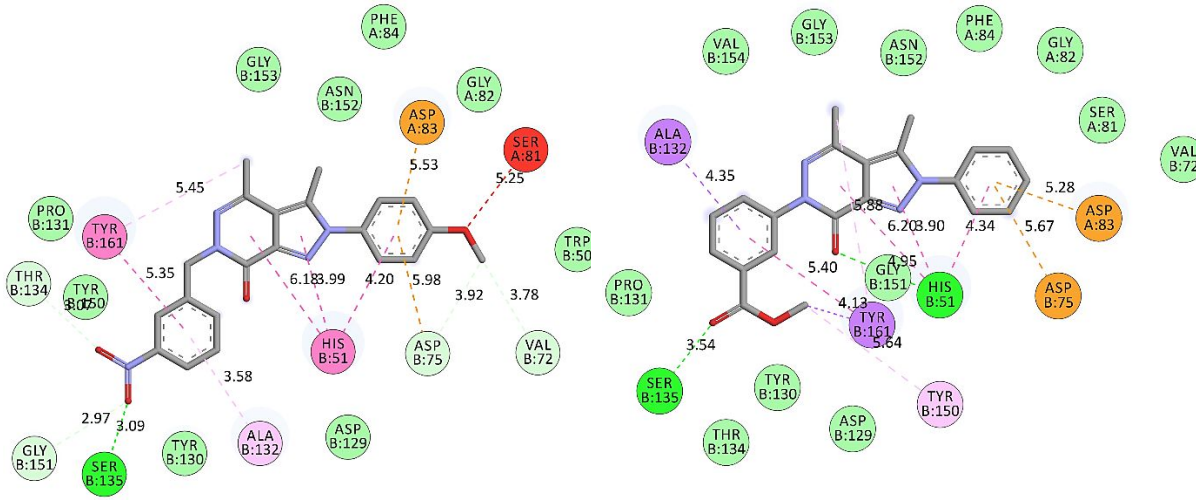


Figure S29. Redocking (blue) and co-crystallized benzimidazole-1-ylmethanol (violet) in the NS2B-NS3 binding site suggested. RMSD value of 0.5936 for backbone chain using Maestro



9b **17b**

Interaction

Conventional Hydrogen Bond

Carbon Hydrogen Bond

Unfavorable Acceptor-Acceptor

Pi-Anion

Pi-Pi Stacked

Pi-Pi T-shaped

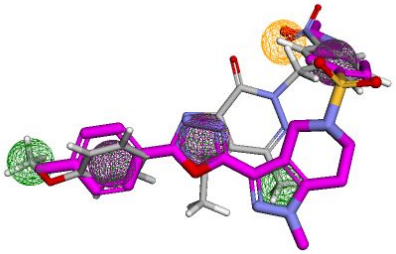
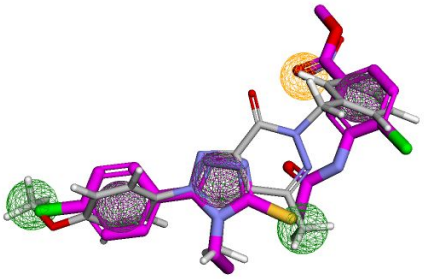
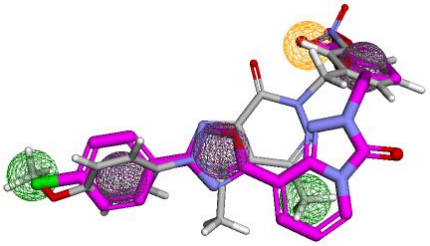
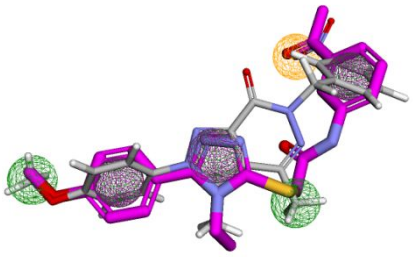
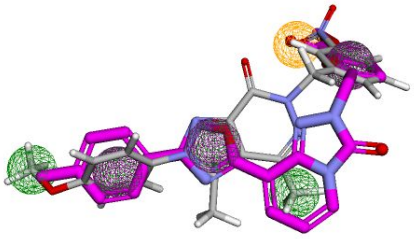
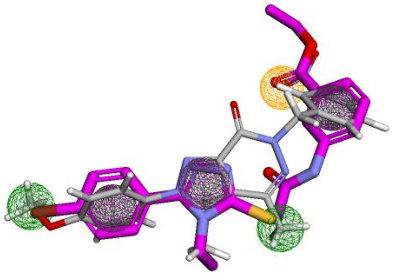
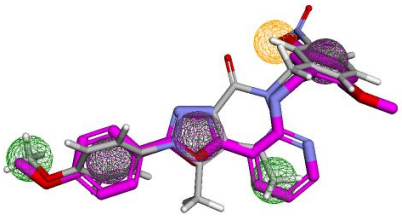
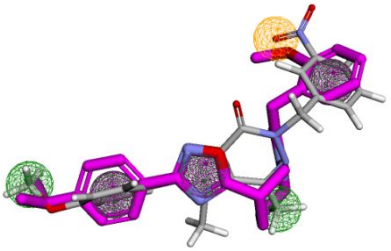
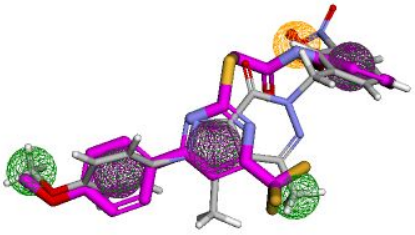
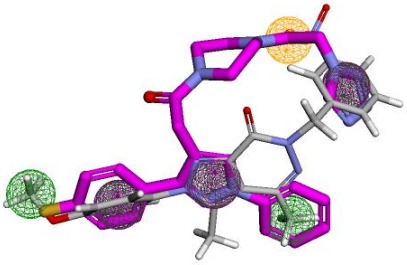
Van der Waals

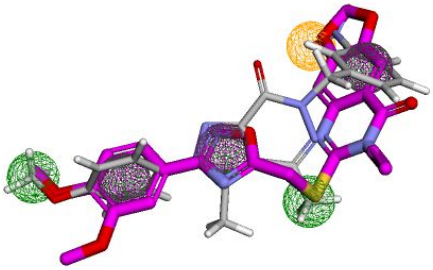
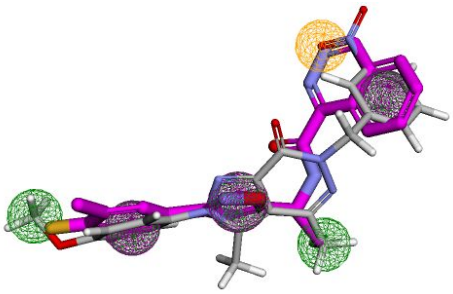
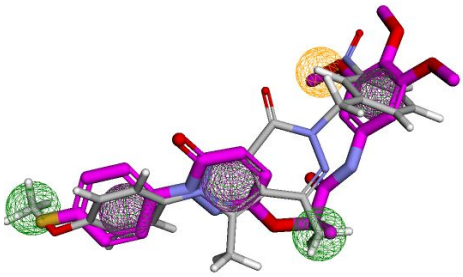
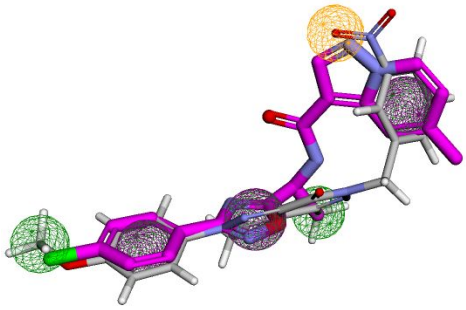
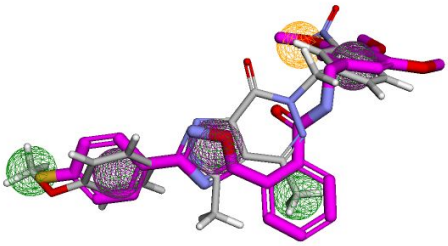
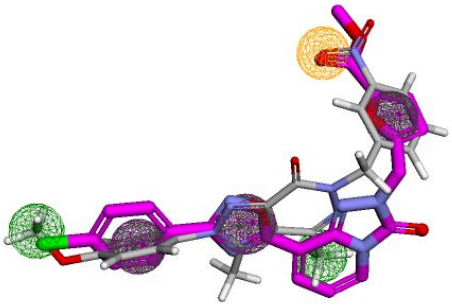
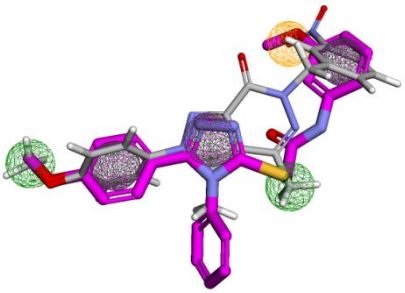
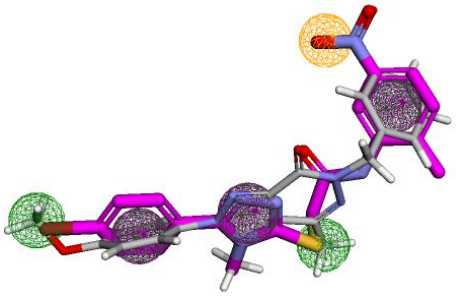
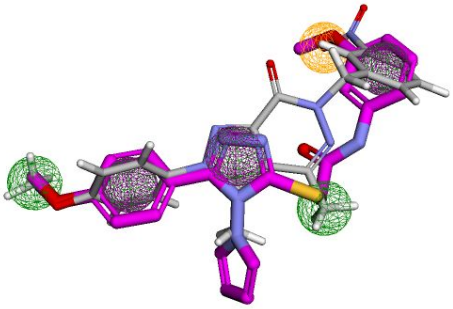
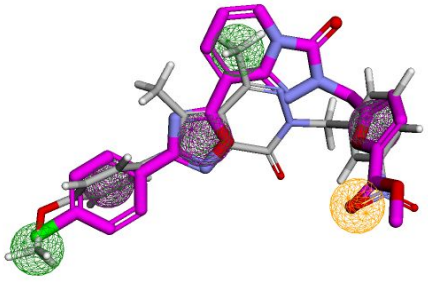
Pi-Alkyl

Pi-sigma

Figure S30. Interaction and distance of compounds **9 b** and **17b** with amino acids in the NS2B-NS3 binding site.

Table S2. Top ten molecules from virtual screening protocol

Top	ChemDiv database		ZINC database	
	Molecules	RMSD	Molecules	RMSD
1	 L856-0019	0.438	 ZINC1064562	0.430
2	 M177-0899	0.449	 ZINC874077	0.434
3	 M177-0896	0.468	 ZINC33412328	0.436
4	 M034-2432	0.494	 ZINC1360047	0.440
5	 6466-0245	0.505	 ZINC520429136	0.441

6	 L541-0235	0.505	 ZINC1266162581	0.443
7	 L324-0607	0.507	 ZINC766850835	0.444
8	 F177-1440	0.512	 ZINC49006630	0.446
9	 D419-0114	0.512	 ZINC976479	0.447
10	 G856-7804	0.514	 ZINC49006630	0.449