

Supplementary Information

Synergistic Inhibition of Urease by Novel Chromene-Dihidropirimidinone Hybrids: A Combined Synthetic, Biological, and Molecular Dynamics Study

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This publication is part of the special issue “Organic Synthesis - BMOS”

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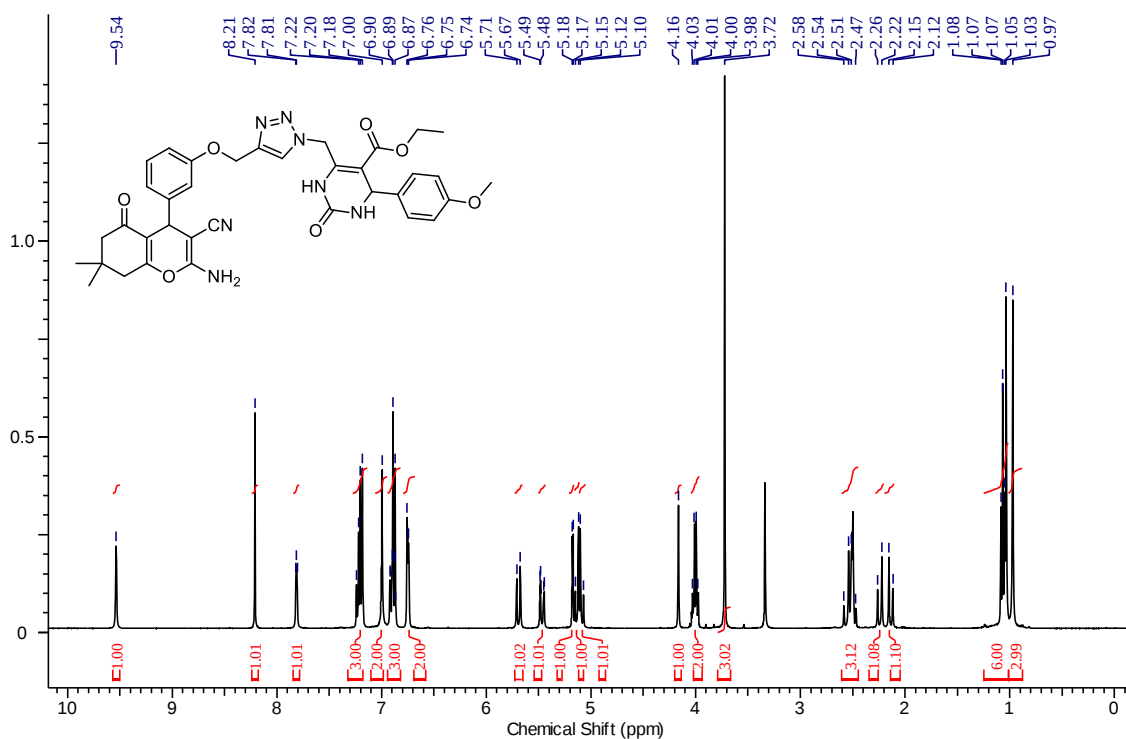


Figure S3. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of hybrid compound **4b**.

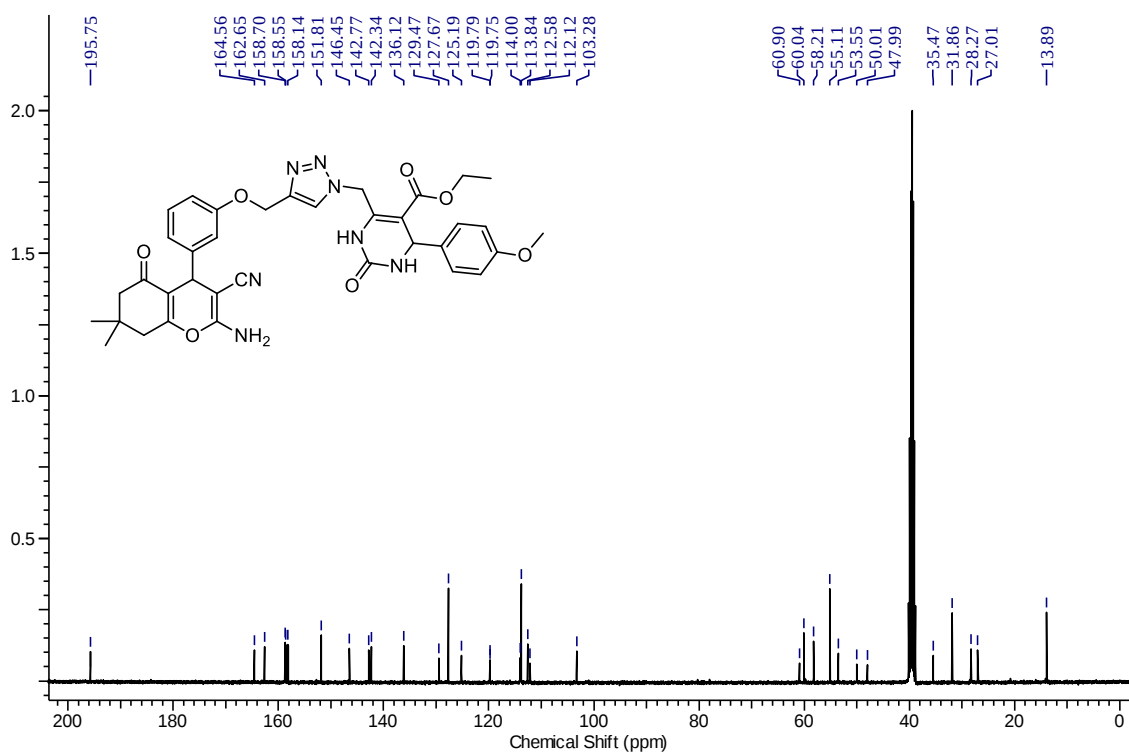


Figure S4. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆) of hybrid compound **4b**.

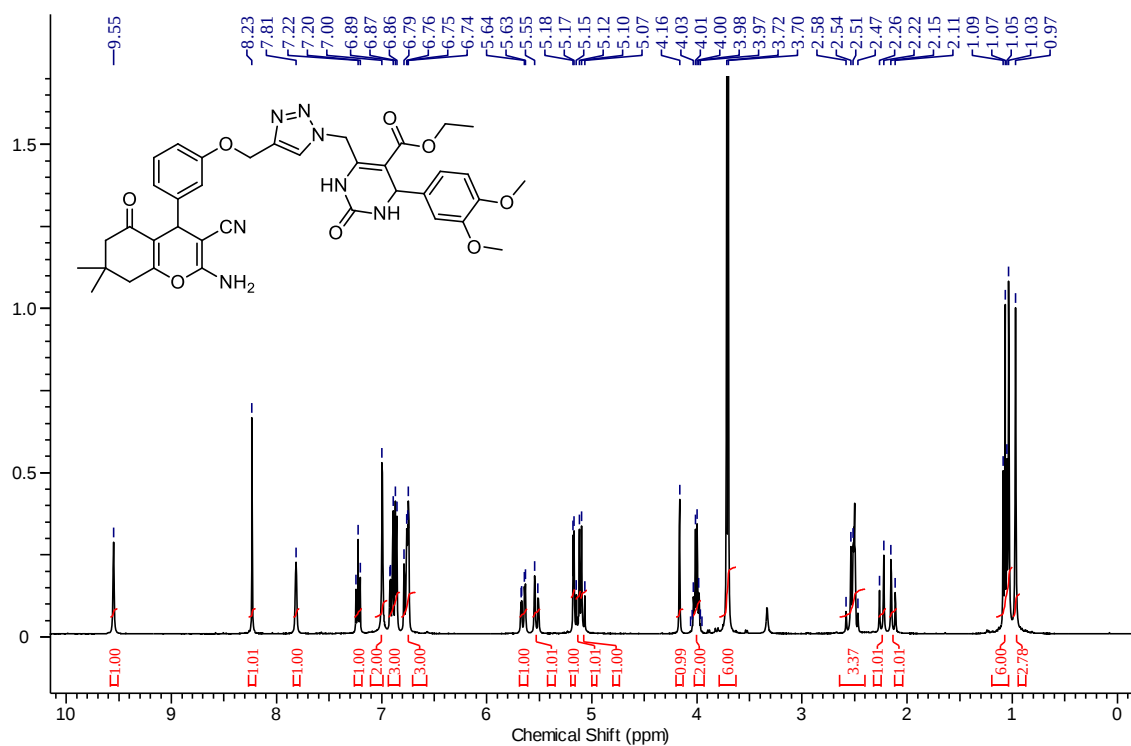


Figure S5. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of hybrid compound 4c.

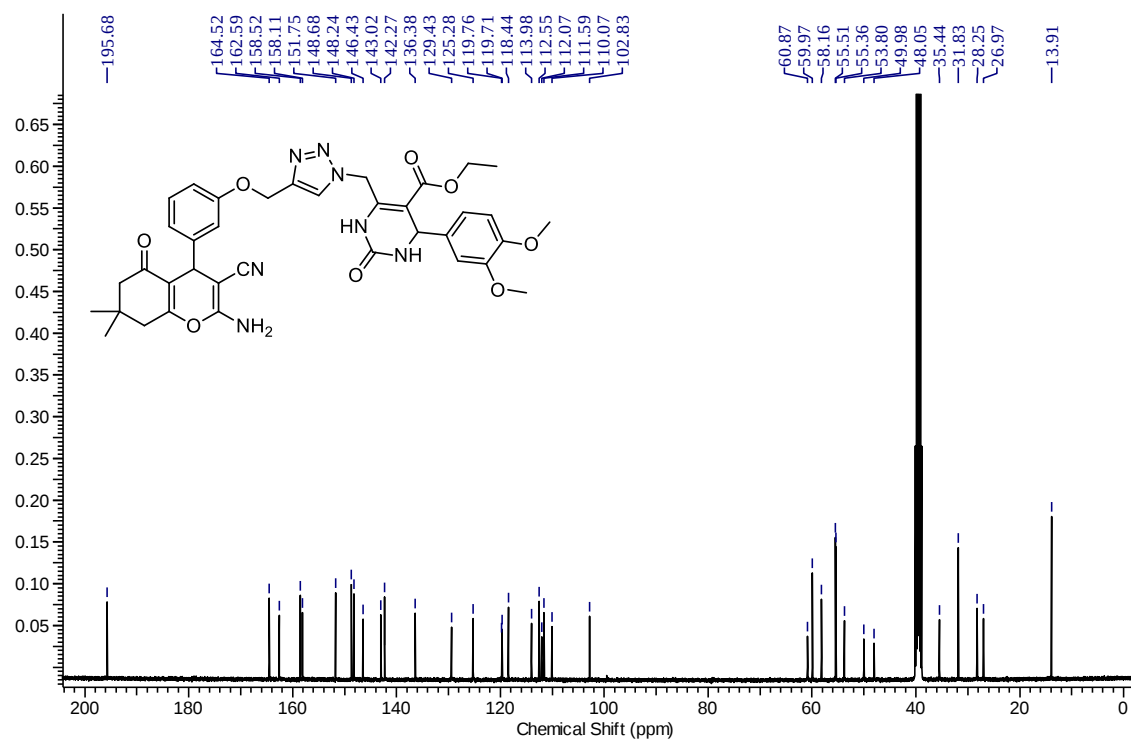


Figure S6. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆) of hybrid compound 4c.

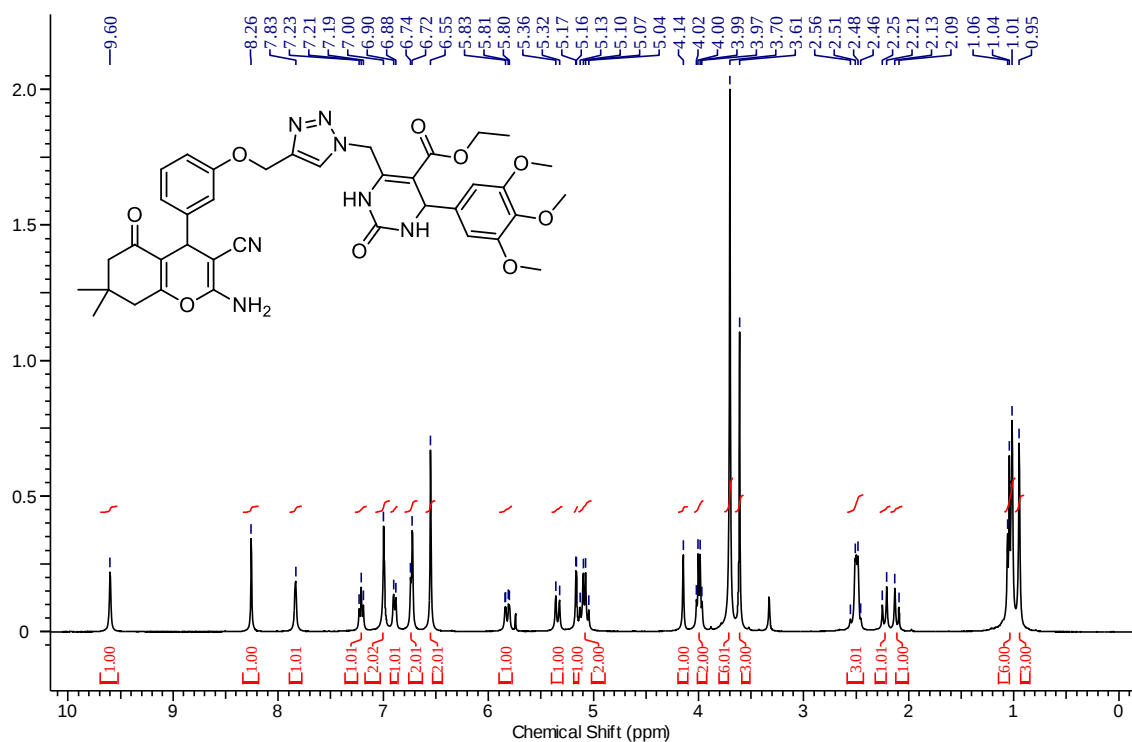


Figure S7. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of hybrid compound **4d**.

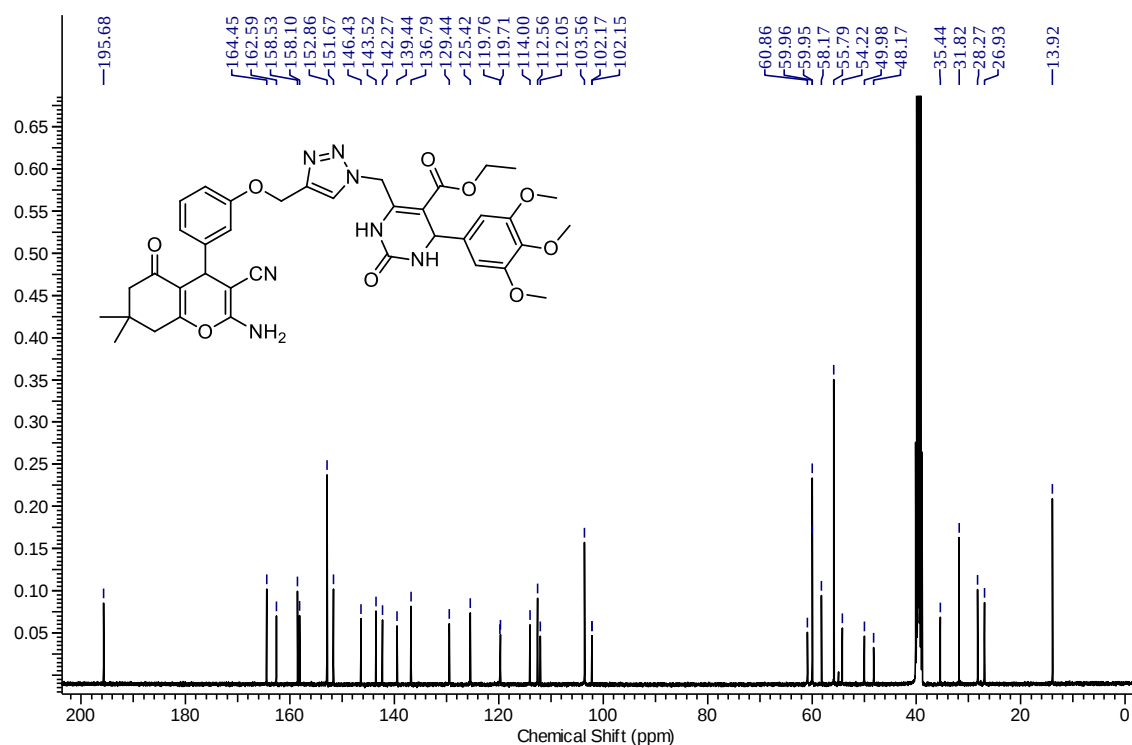


Figure S8. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of hybrid compound **4d**.

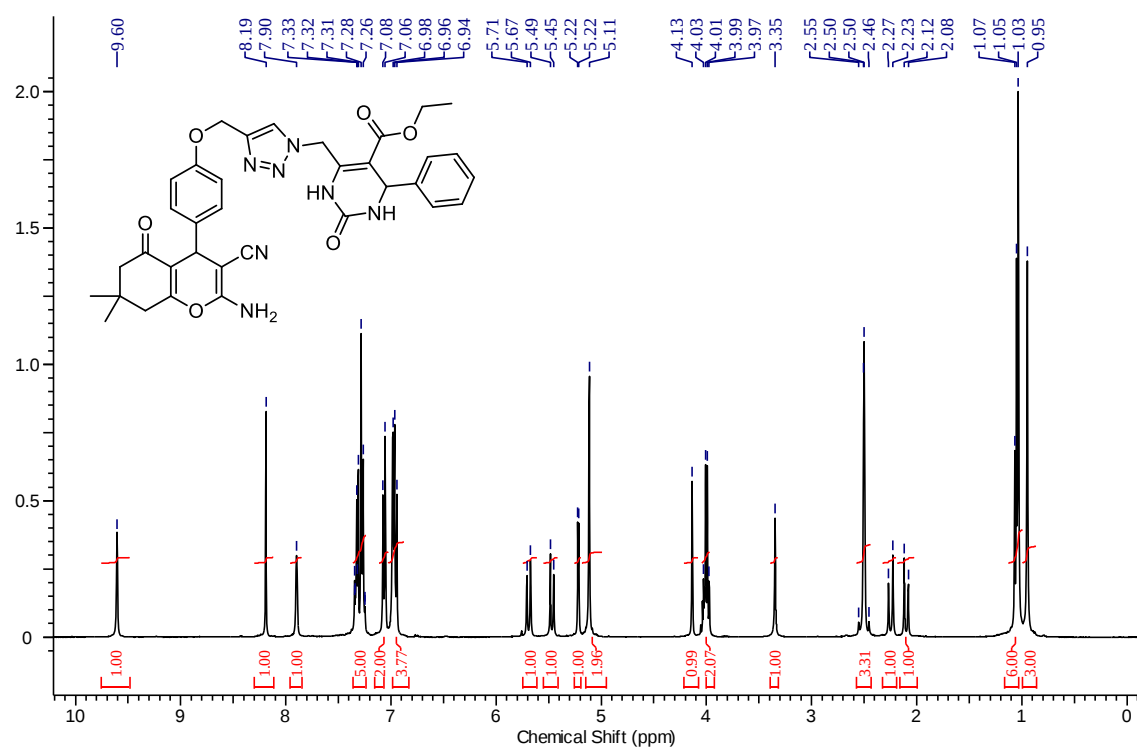


Figure S9. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of hybrid compound **4e**.

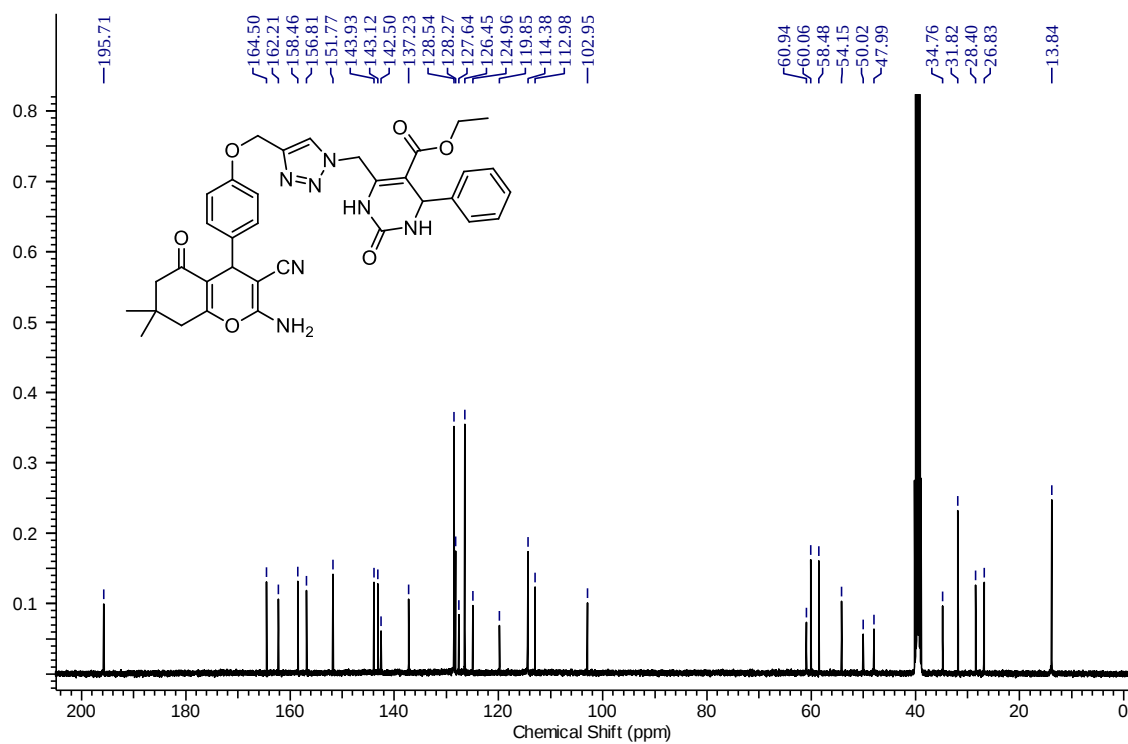


Figure S10. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆) of hybrid compound **4e**.

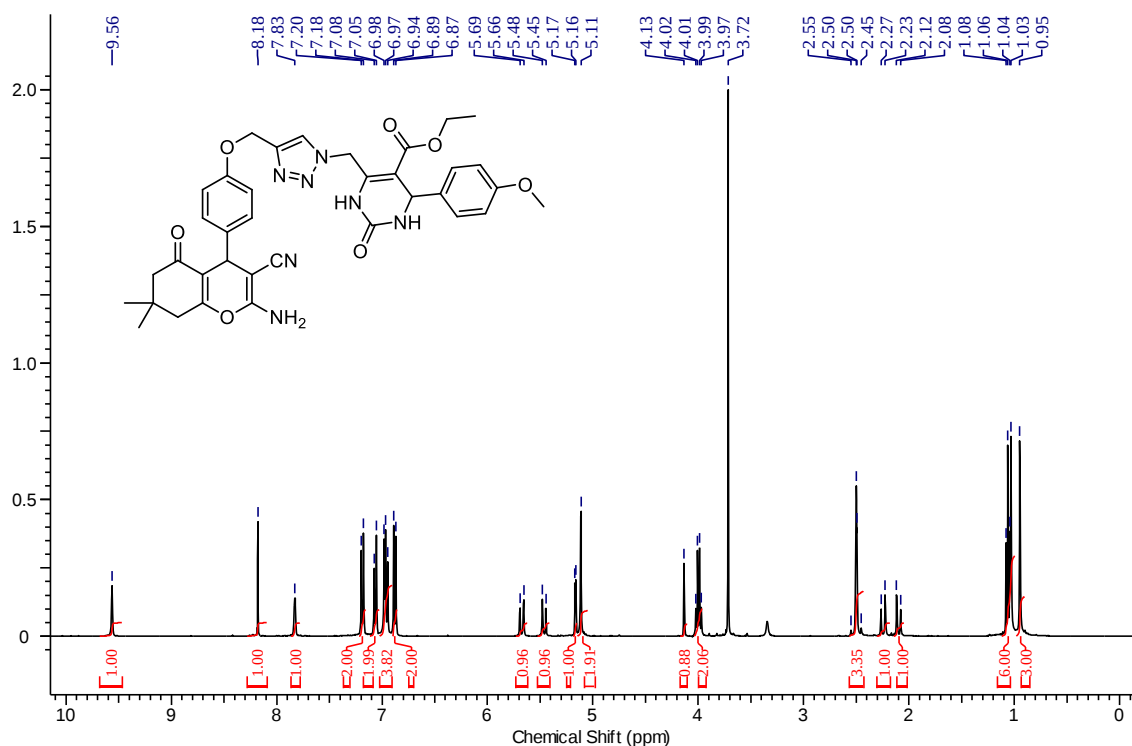


Figure S11. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of hybrid compound **4f**.

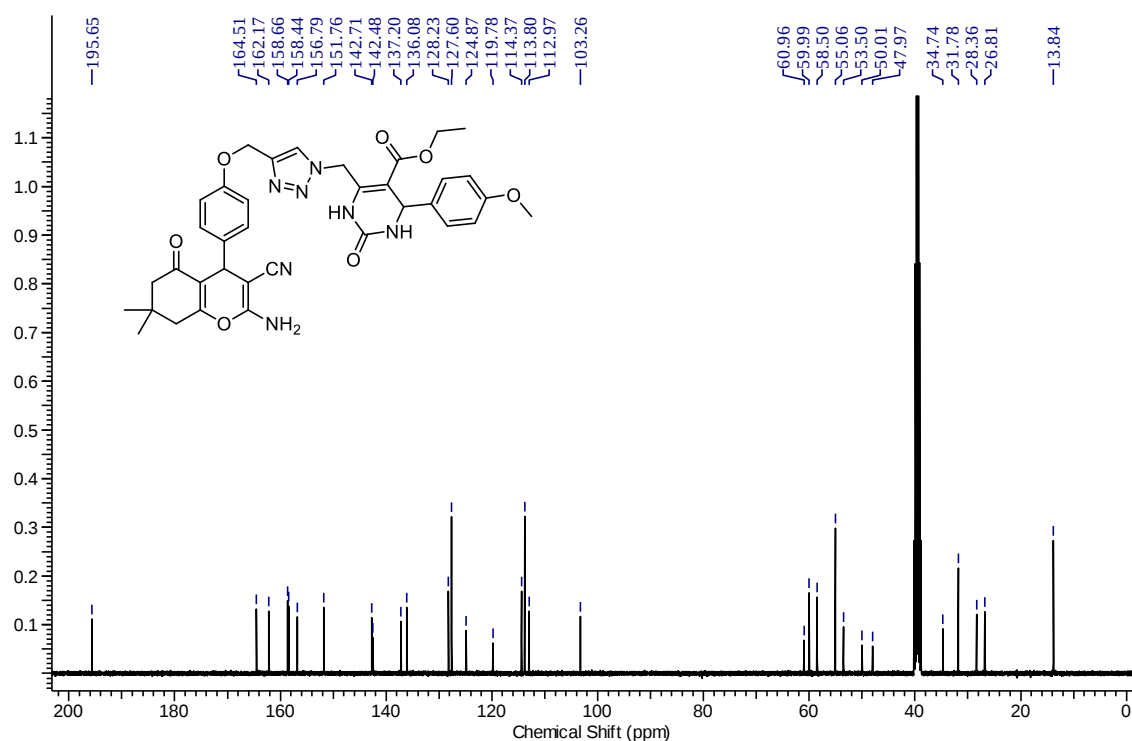


Figure S12. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of hybrid compound **4f**.

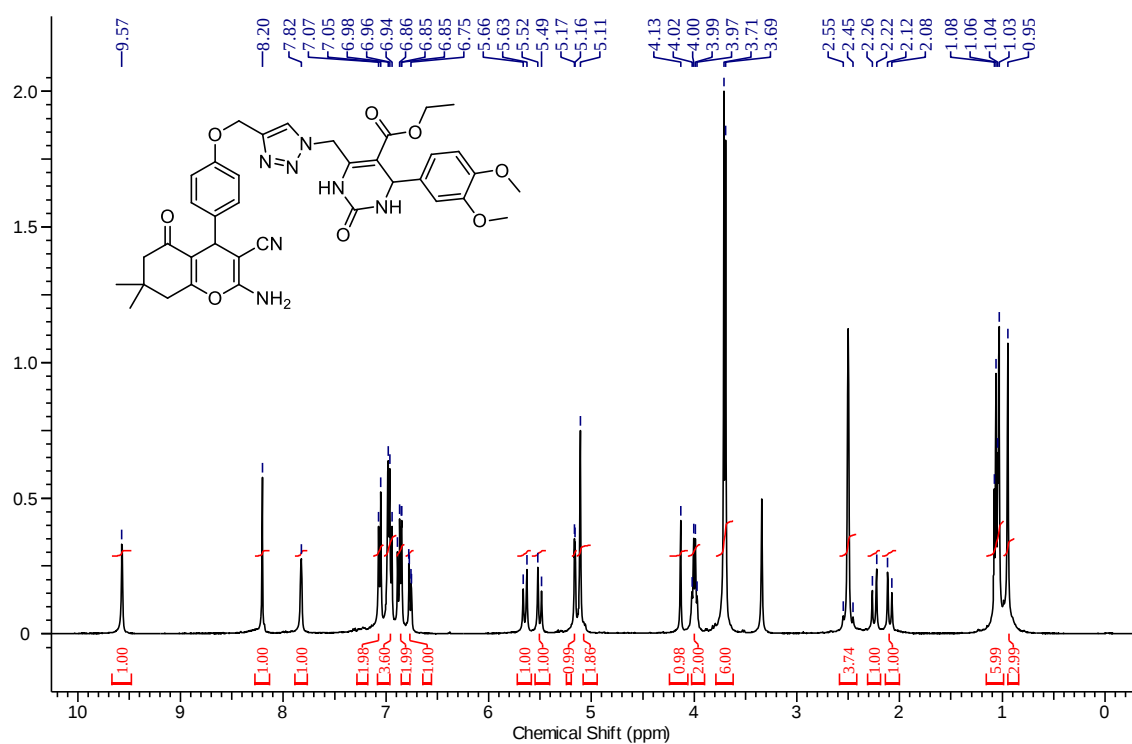


Figure S13. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of hybrid compound **4g**.

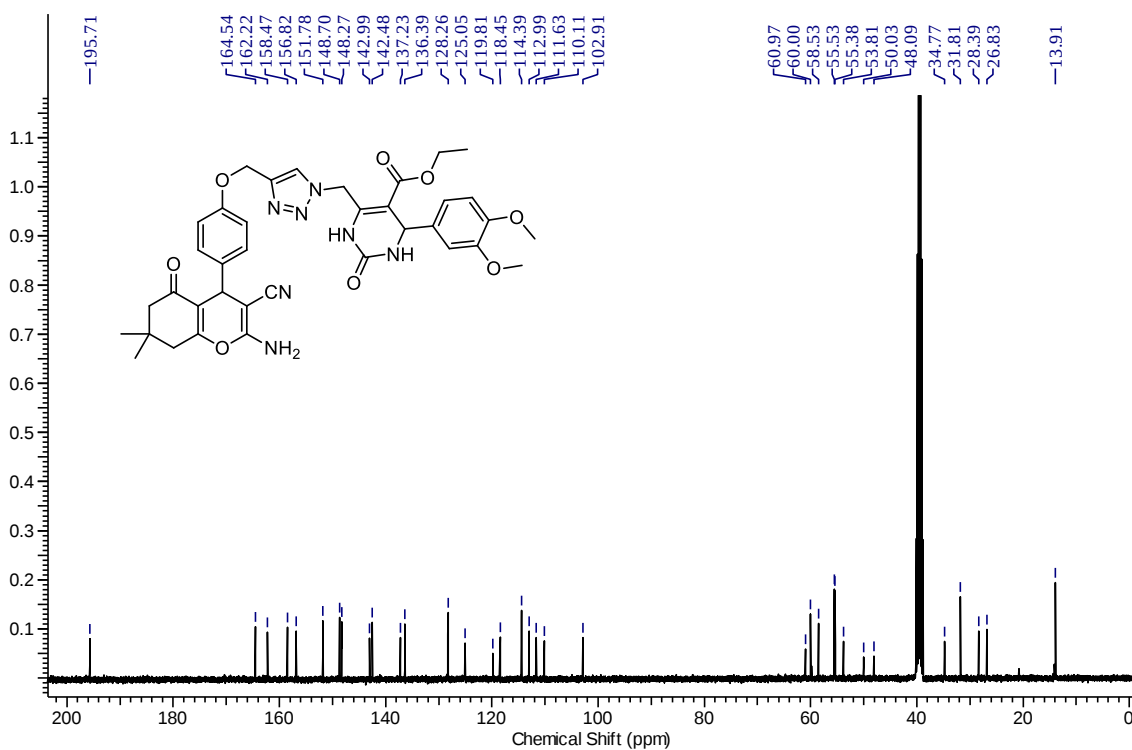


Figure S14. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of hybrid compound **4g**.

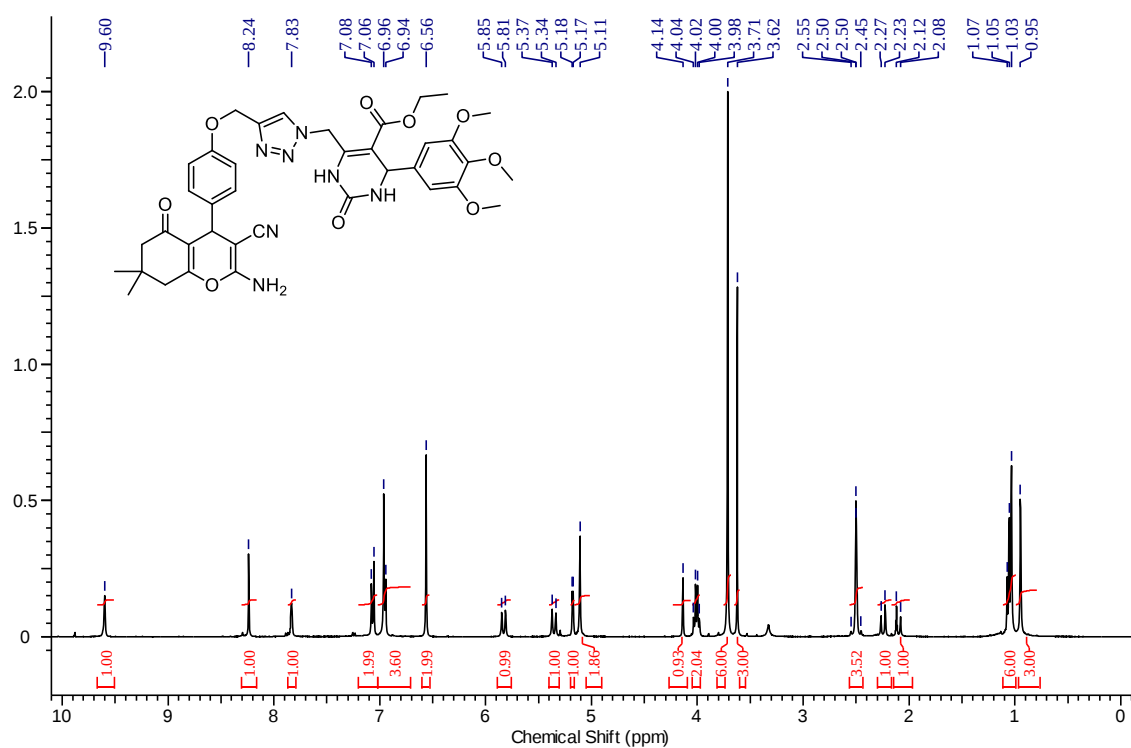


Figure S15. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of hybrid compound **4h**.

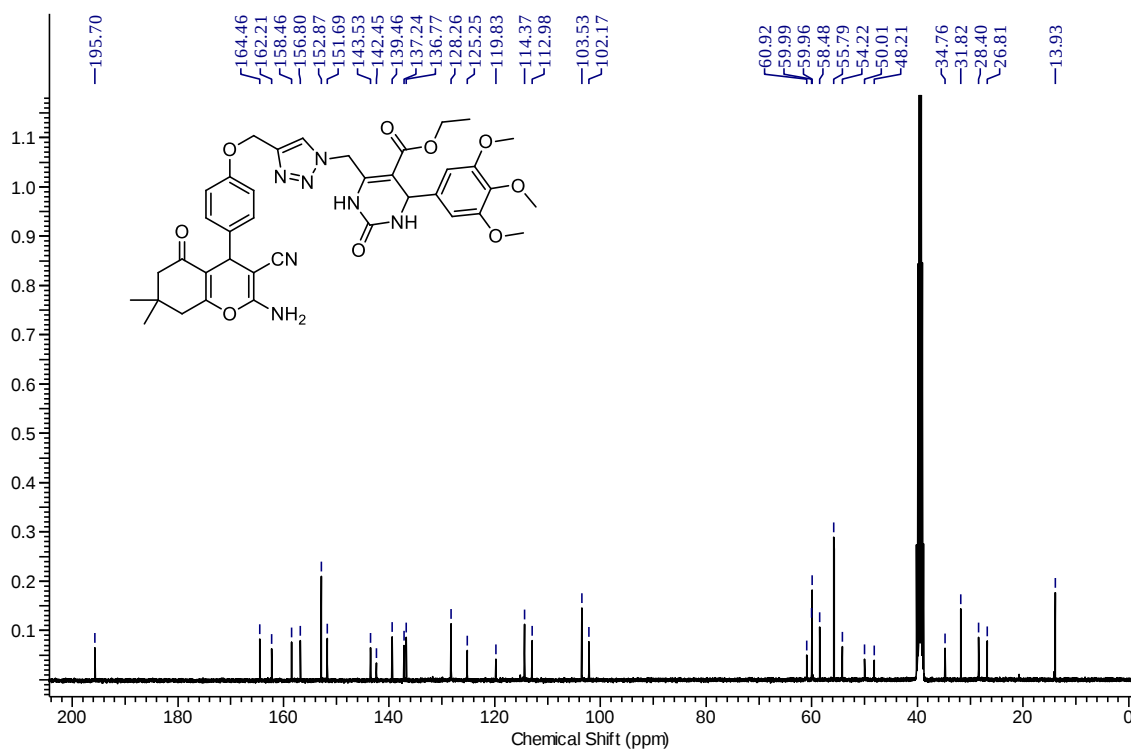


Figure S16. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of hybrid compound **4h**.

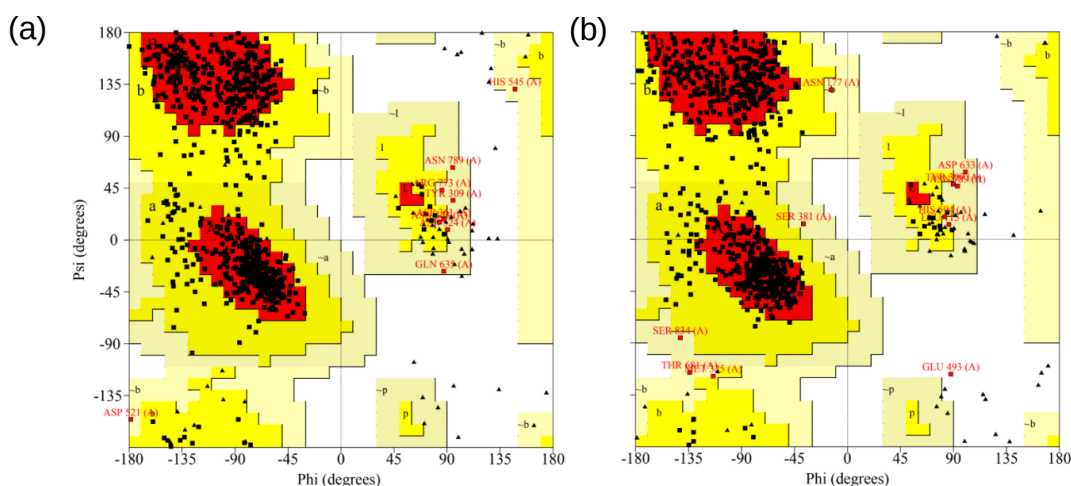


Figure S17. Ramachandran plot analysis of urease before and after molecular dynamics simulation. (a) Native enzyme structure, showing 81.5% of residues in the most favored regions, 17.0% in additionally allowed regions, and 1.4% in disallowed regions. (b) Urease complexed with compound 4h after molecular dynamics simulation, exhibiting 82.7% of residues in the most favored regions, 16.0% in additionally allowed regions, and 1.3% in disallowed regions. The comparable distribution of residues across favored and allowed regions indicates preservation of the overall stereochemical quality and structural integrity of the enzyme upon ligand binding.

Table S1. Effect of chromene-DHPM hybrids on the activity of type III urease from *Canavalia ensiformis*. Experiment conducted in technical quadruplicate. Reactions contained 12.5 mU urease, 20 mM urea, and each compound test at 100 μ M

Compound	Urease inhibition / %	
	Mean $\pm$ SD	
TIO	63.5 $\pm$ 3.5	
4a	34.7 $\pm$ 7.9	
4b	42.1 $\pm$ 13.6	
4c	35.4 $\pm$ 7.4	
4d	30.2 $\pm$ 9.7	
4e	24.4 $\pm$ 8.6	
4f	35.4 $\pm$ 5.7	
4g	29.9 $\pm$ 3.3	
4h	51.5 $\pm$ 6.2	
1a	30.3 $\pm$ 8.2	
3b	18.8 $\pm$ 4.9	
3d	0	

TIO: thiourea; SD: standard deviation.