

# Structure-Based Optimization of 1,2,4-Triazole-3-Thione Derivatives: Improving Inhibition of NDM-/VIM-Type Metallo- $\beta$ -Lactamases and Synergistic Activity on Resistant Bacteria

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**Table S1.** Structures of compounds and corresponding aldehydes used.

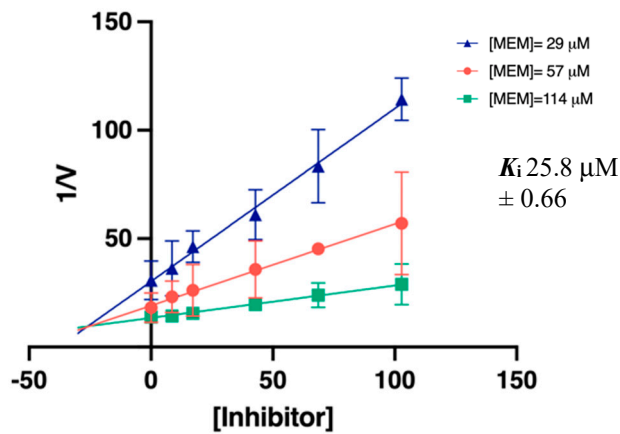
Cpd	Structure	Aldehyde	Cpd	Structure	Aldehyde
CP 17			CP 29		
CP 18			CP 30		
CP 19			CP 31		
CP 20			CP 32		
CP 21			CP 35		
CP 22			CP 44		
CP 23			CP 45		
CP 24			CP 46		
CP 25			CP 55		
CP 26			CP 56		
CP 27			CP 57		
CP 28			CP 58		

**Table S2.** The inhibitory activity of 4-amino-4H-1,2,4-triazole-3-thiol derivatives discussed here.

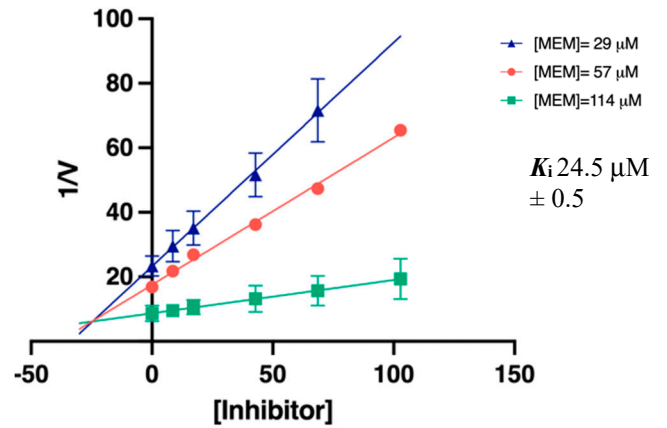
CODE	% Inib vs VIM <sup>a,b</sup> MBLs	NDM-1
CP 17	46 <sup>a</sup>	51
CP 18	33 <sup>a</sup>	>100
CP 19	15 <sup>a</sup>	24
CP 20	14 <sup>a</sup>	40
CP 21	32 <sup>a</sup>	38
CP 22	46 <sup>a</sup>	68
CP 23	NI	67
CP 24	28 <sup>a</sup>	64
CP 25	23 <sup>a</sup>	11
CP 26	NI	NI
CP 27	NI	36
CP 28	NI	NI
CP 29	17 <sup>a</sup>	NI
CP 30	18 <sup>a</sup>	NI
CP 31	NI	10
CP 32	17 <sup>a</sup>	20
CP 35	53 <sup>a, b</sup>	81
CP 44	42 <sup>a</sup> 47 <sup>b</sup>	24
CP 45	16 <sup>a</sup>	20
CP 46	42 <sup>b</sup>	15
CP 55	48 <sup>a</sup> 75 <sup>b</sup>	NI
CP 56	77 <sup>a</sup> 42 <sup>b</sup>	>100
CP 57	73 <sup>a</sup> >100 <sup>b</sup>	>100
CP 58	28 <sup>a</sup> 53 <sup>b</sup>	52

Tests were conducted at 25° C by following the absorbance variation observed upon reporter substrate (Meropenem 57 µM for NDM-1, 17 µM for VIM-2) or Nitrocefin (23 µM for VIM-1). The percentage of inhibition is reported except when ≤ 15% and in this case is indicated by NI “no inhibition”. Compounds concentration was <sup>a</sup>200 mM vs VIM-1, <sup>b</sup> 70 mM vs VIM-2 and 100 mM vs NDM-1. Assays were run after a 5-minutes incubation.

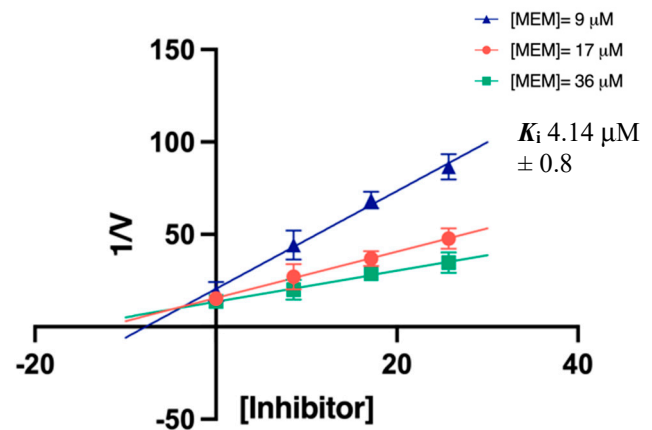
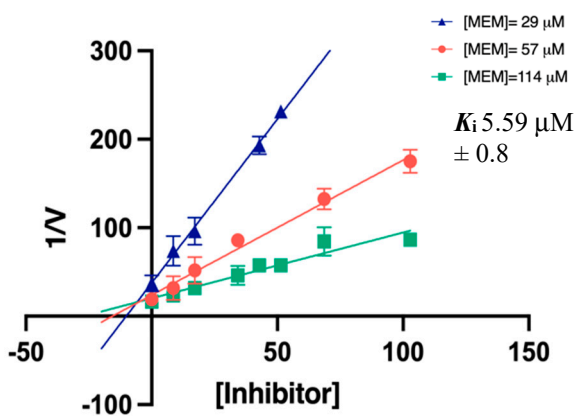
**Figure S1.** Dixon plots for compounds **CP 35**, **CP 56** against NDM-1(A,B) and **CP 57** against NDM-1 and VIM-2 (C,D). Compounds were tested after a 5-minutes incubation, using MEM as reporter substrate. The initial velocity of the reaction is measured as a function of inhibitor concentration at three fixed concentrations of MEM. The data were then plotted as  $1/V$  as a function of  $[I]$  for each substrate concentration, and the value of  $K_i$  determined from the  $x$ -axis value at which the lines intersect. Data points are mean  $\pm$  SD obtained from two or three independent experiments.



A



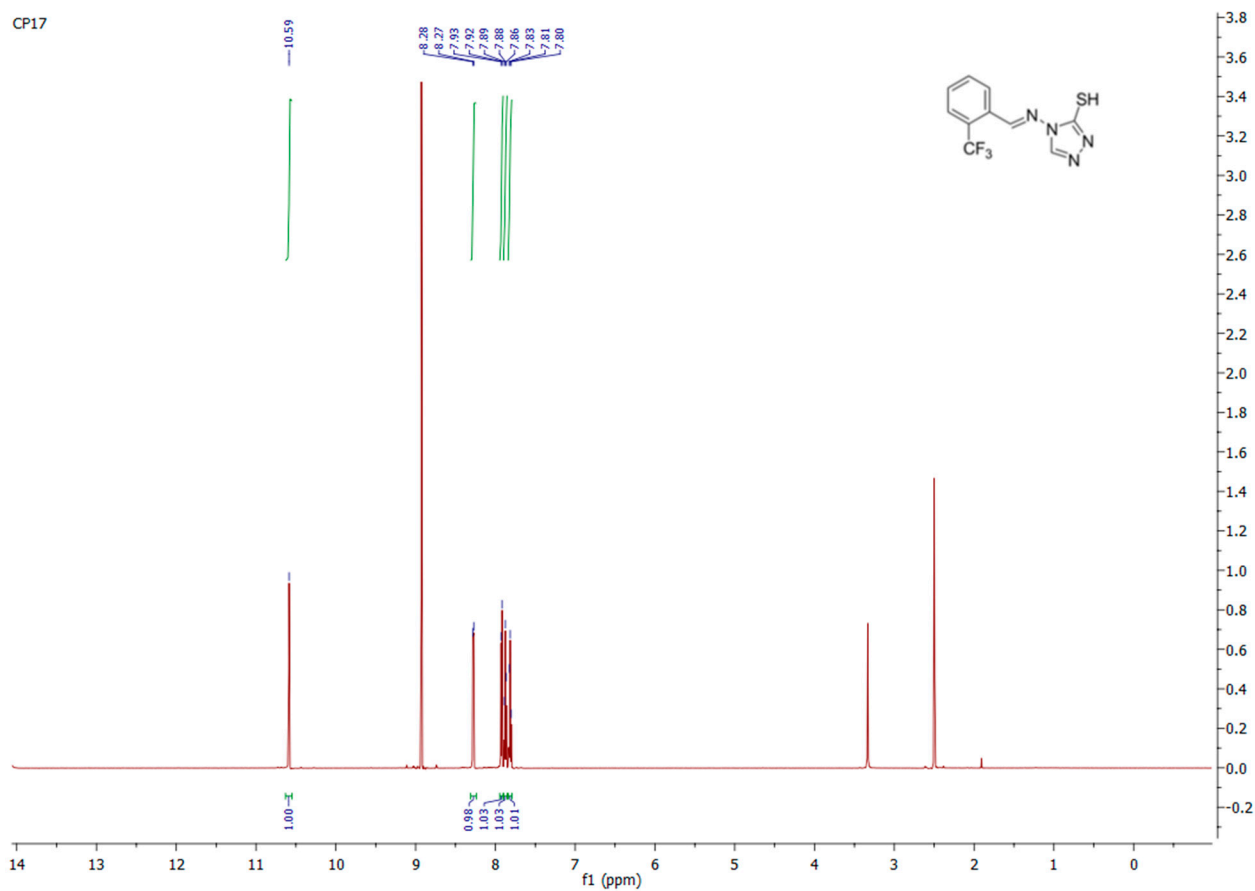
B

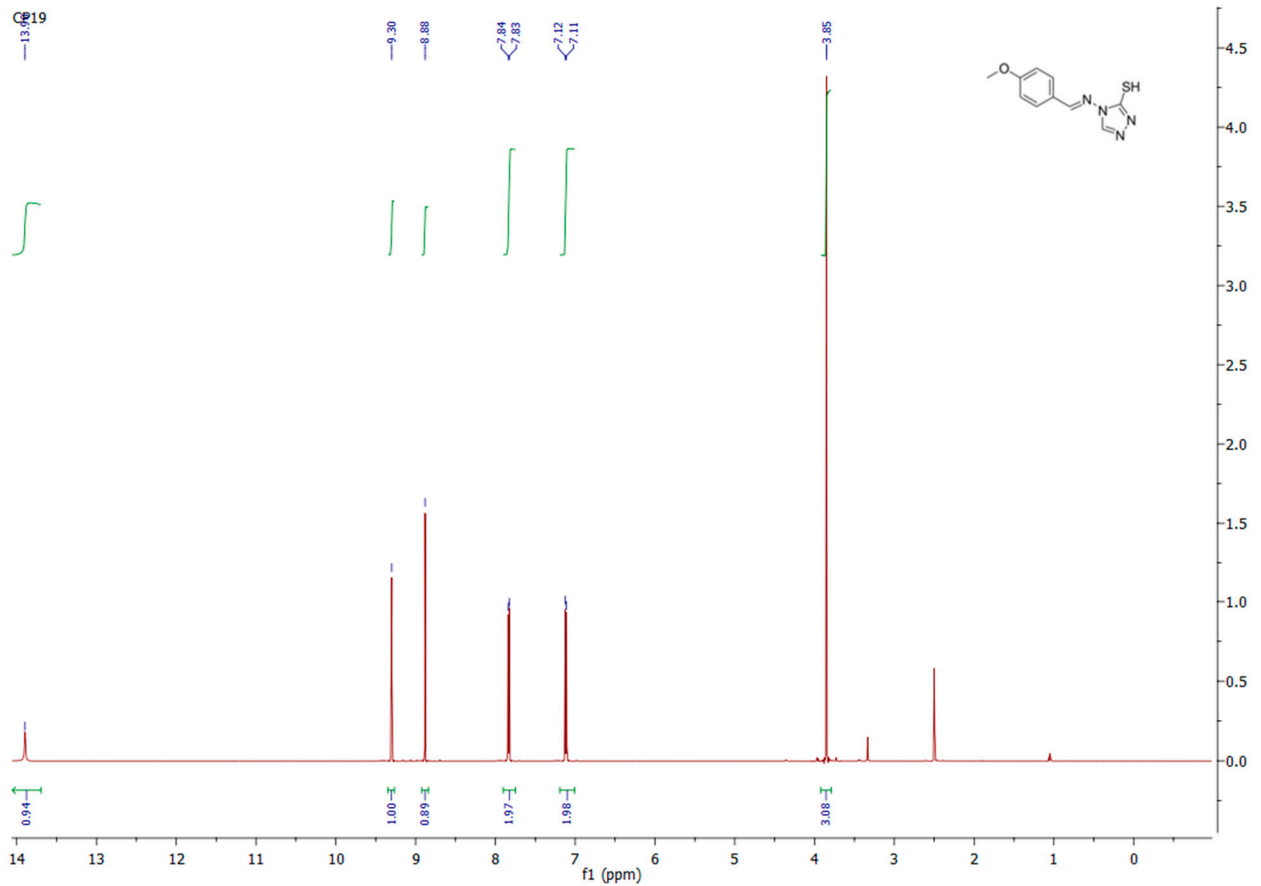
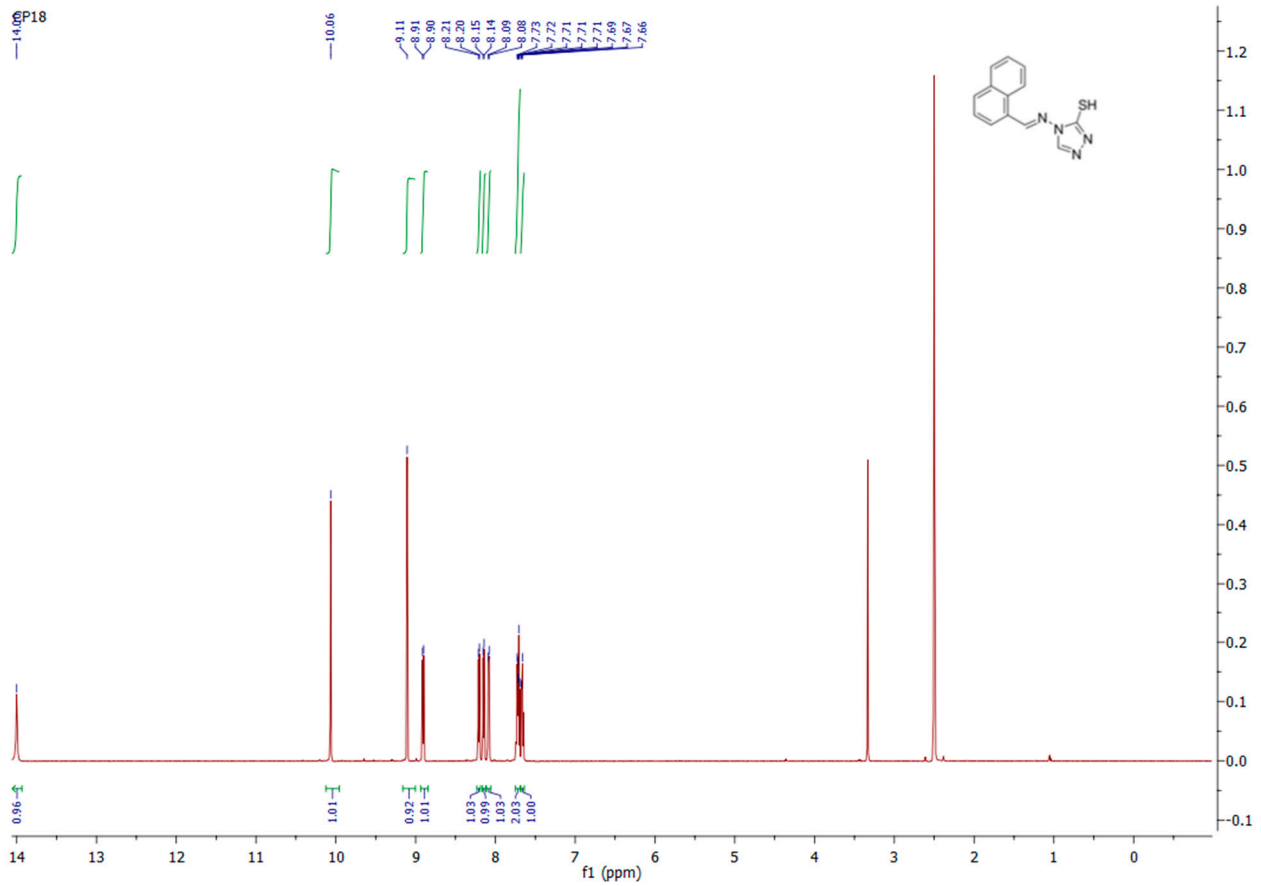


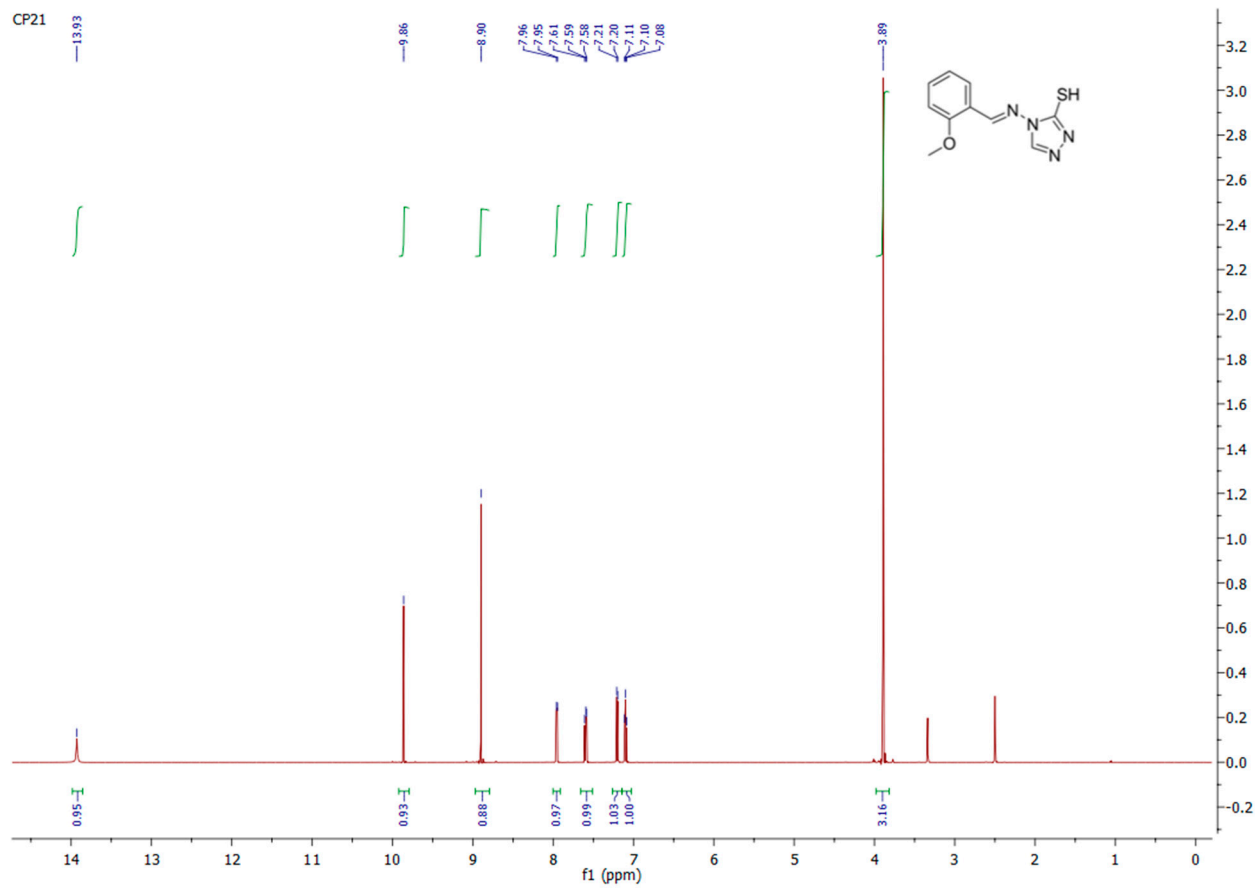
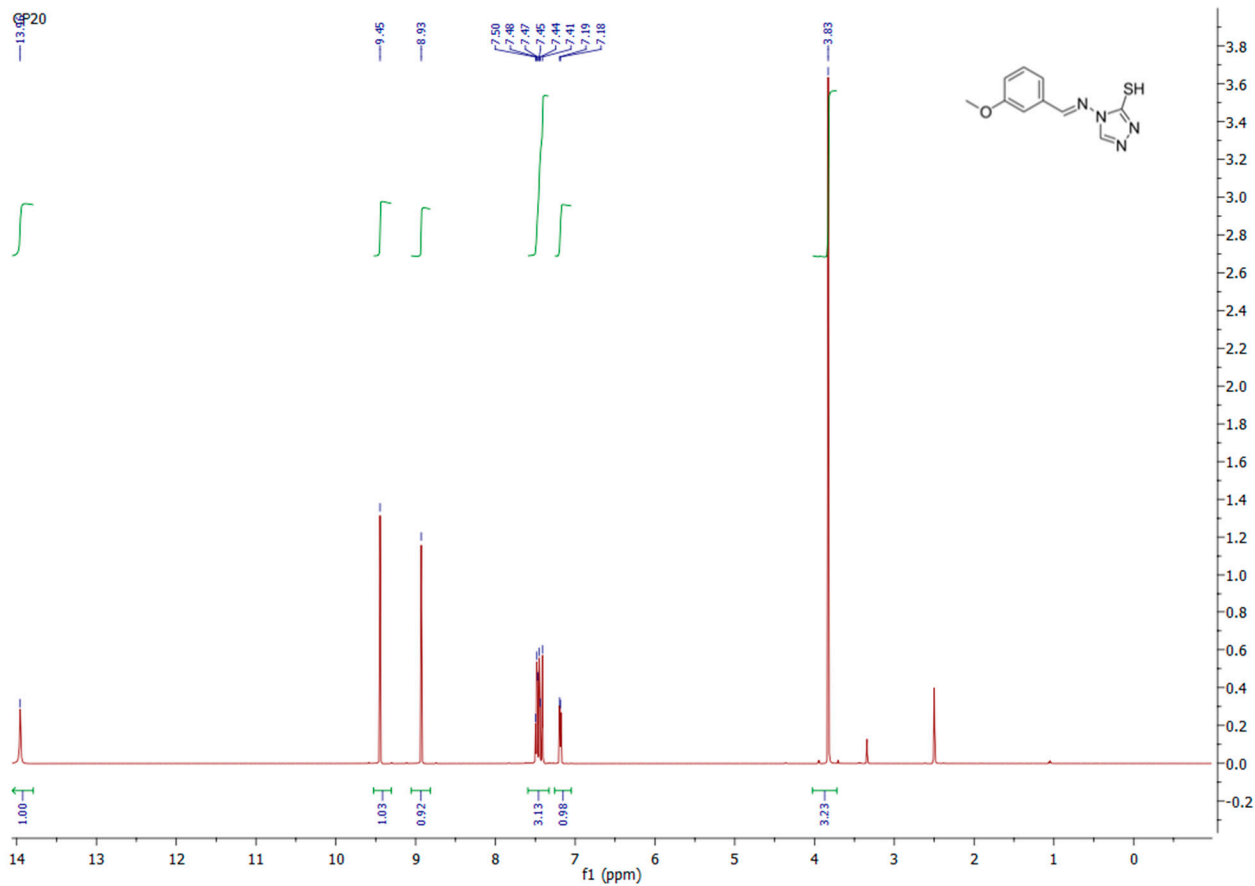
**Table S3.** X-Ray crystallographic data for NDM-1 in complex with **CP 35**, **CP 56** and **CP P57**.

	NDM-1/CP 35	NDM-1/CP 56	NDM-1/CP 57
PDB ID	8B1W	8B1Z	8B20
<b>Data collection statistics</b>			
Diffraction source	ID23-1 (ESRF)	ID23-1 (ESRF)	ID23-1 (ESRF)
Wavelength (Å)	0.873	0.873	0.873
Temperature (K)	100	100	100
Detector	Eiger2 XE 16M	Eiger2 XE 16M	Eiger2 XE 16M
Crystal-detector distance (mm)	167.1	144.9	167.1
Rotation range per image (°)	0.15	0.10	0.10
Exposure time per image (s)	0.013	0.016	0.019
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
No. of molecules/ASU	2	2	2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	70.19 73.70 77.28	68.61 73.96 77.11	77.07 70.44 73.8
Total no. of reflections	241383 (10302)	345446 (16227)	277691 (15290)
No. of unique reflections	44671 (2328)	52482 (2570)	39230 (2195)
Completeness (%)	99.7 (99.5)	100 (100)	100 (100)
Redundancy	5.4 (4.4)	6.6 (6.3)	7.1 (7.0)
$\langle I/\sigma(I) \rangle$	8.6 (2.2)	6.8 (2.1)	6.8 (2.4)
$R_{\text{mrg}}$	0.194 (0.531)	0.161 (0.920)	0.178 (0.956)
Overall <i>B</i> factor from Wilson plot (Å <sup>2</sup> )	19.1	19.7	22.3
<b>Refinement statistics</b>			
Resolution range (Å)	36.88 – 1.70	34.19 – 1.60	53.31 – 1.78
No. of reflections, working set	44139	52418	39169
No. of reflections, test set	2185	2595	1900
Final $R_{\text{cryst}}$	0.192	0.174	0.184
Final $R_{\text{free}}$	0.222	0.209	0.207
No. of non-H atoms			
Protein	3442	3452	3442
Zn ions	4	4	4
Inhibitor	38	54	27
Others	31	36	30
Water	153	189	134
Total	3668	3735	3637
R.m.s. deviations			
Bonds (Å)	0.009	0.009	0.008
Angles (°)	1.568	1.642	1.574
Average <i>B</i> factors (Å <sup>2</sup> )	22.0	23.0	26.0
Ramachandran plot			
Most favored (%)	98	98	98
Allowed (%)	2	1	2
RSCC inhibitor (Chain ID)	0.94 (A) ; 0.90 (B)	0.93 (A) ; 0.94 (B)	0.91 (A)

**Figure S2.**  $^1\text{H}$  NMR spectra of the reported final compounds (CP17-CP32, CP35, CP44-CP46, CP55-CP58).

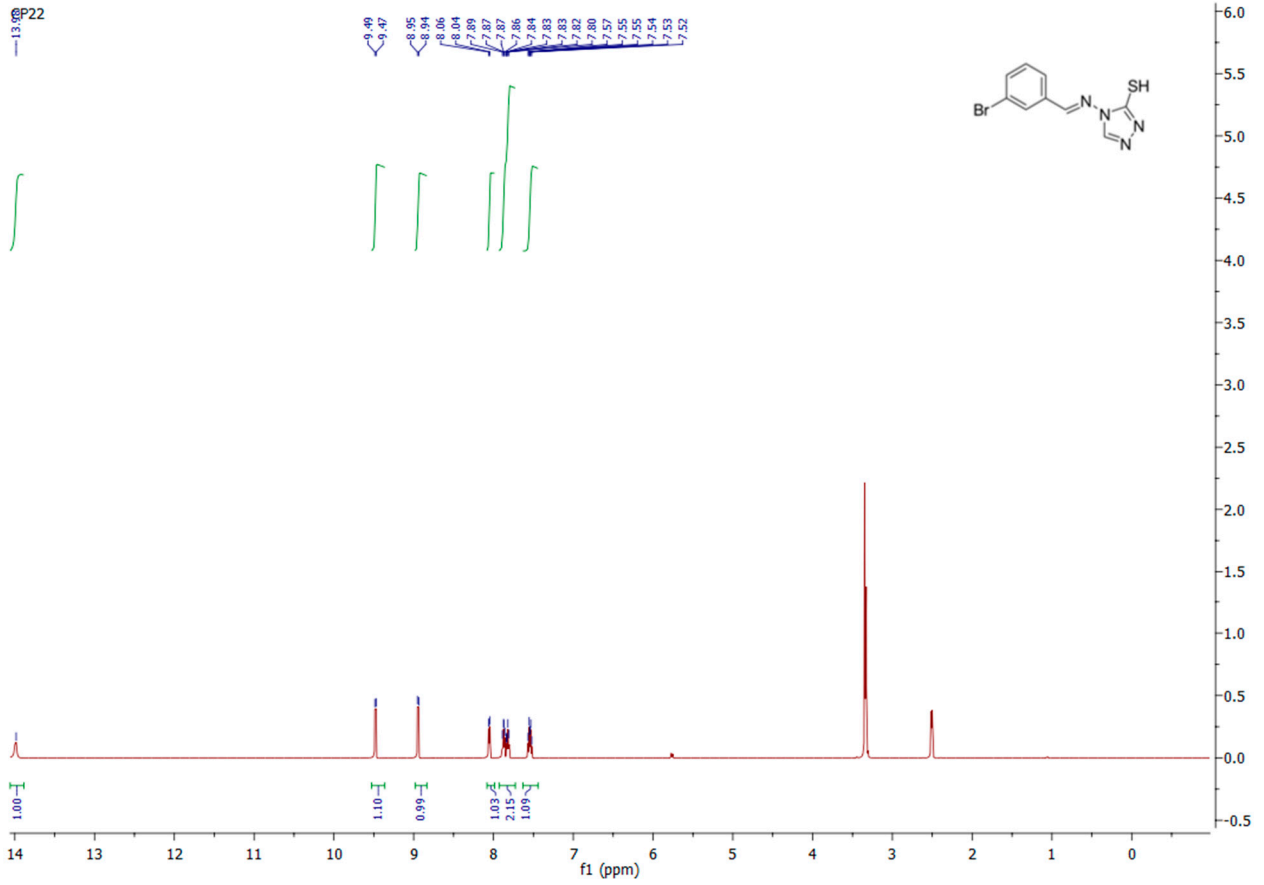




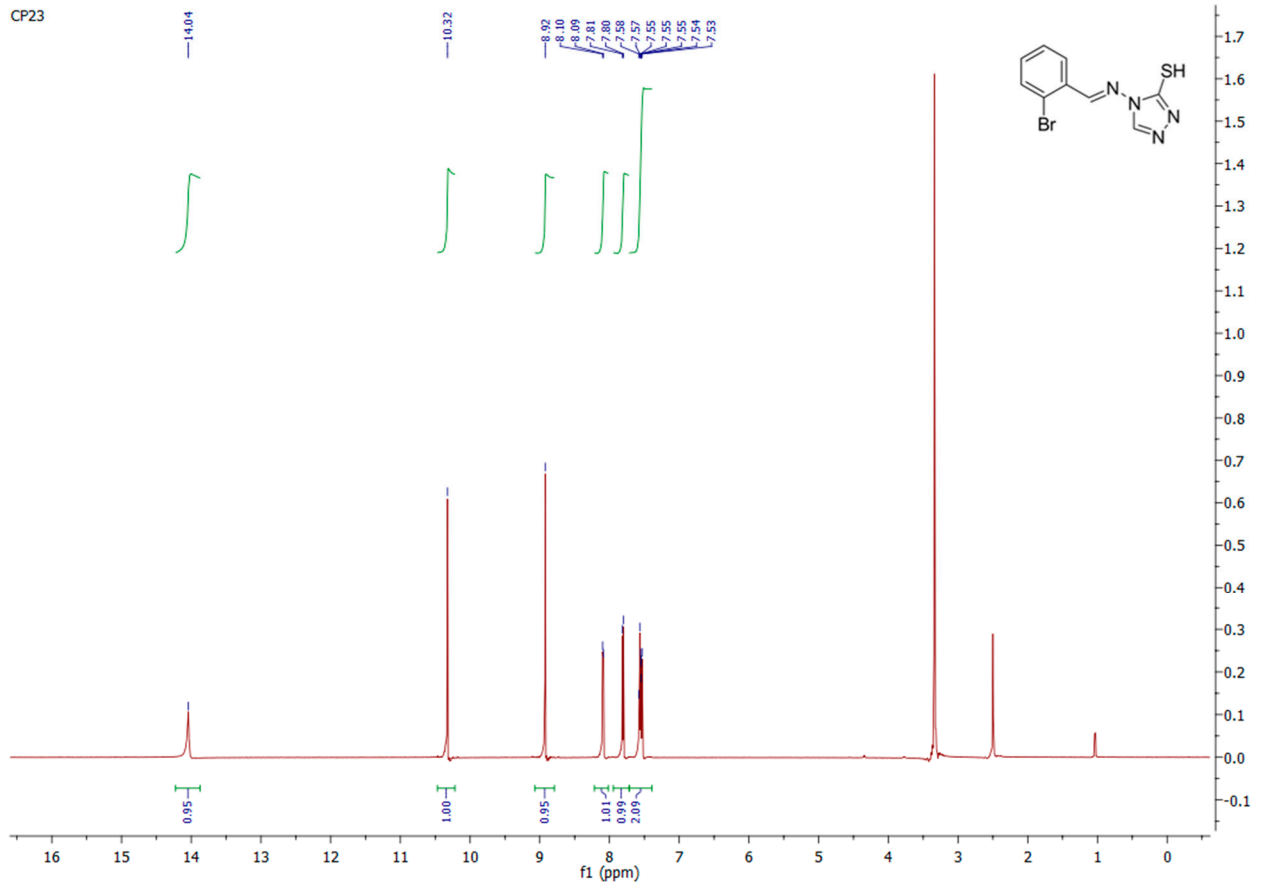




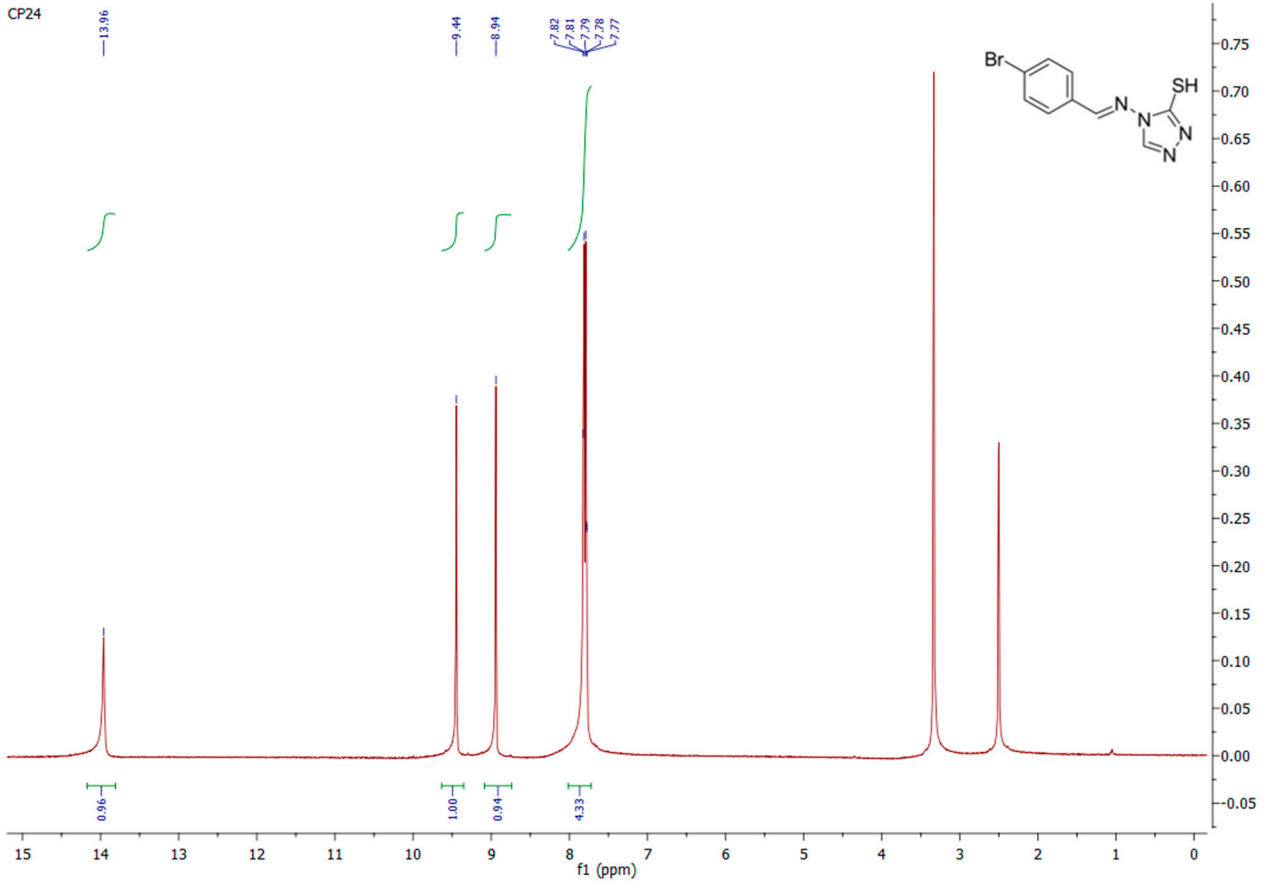
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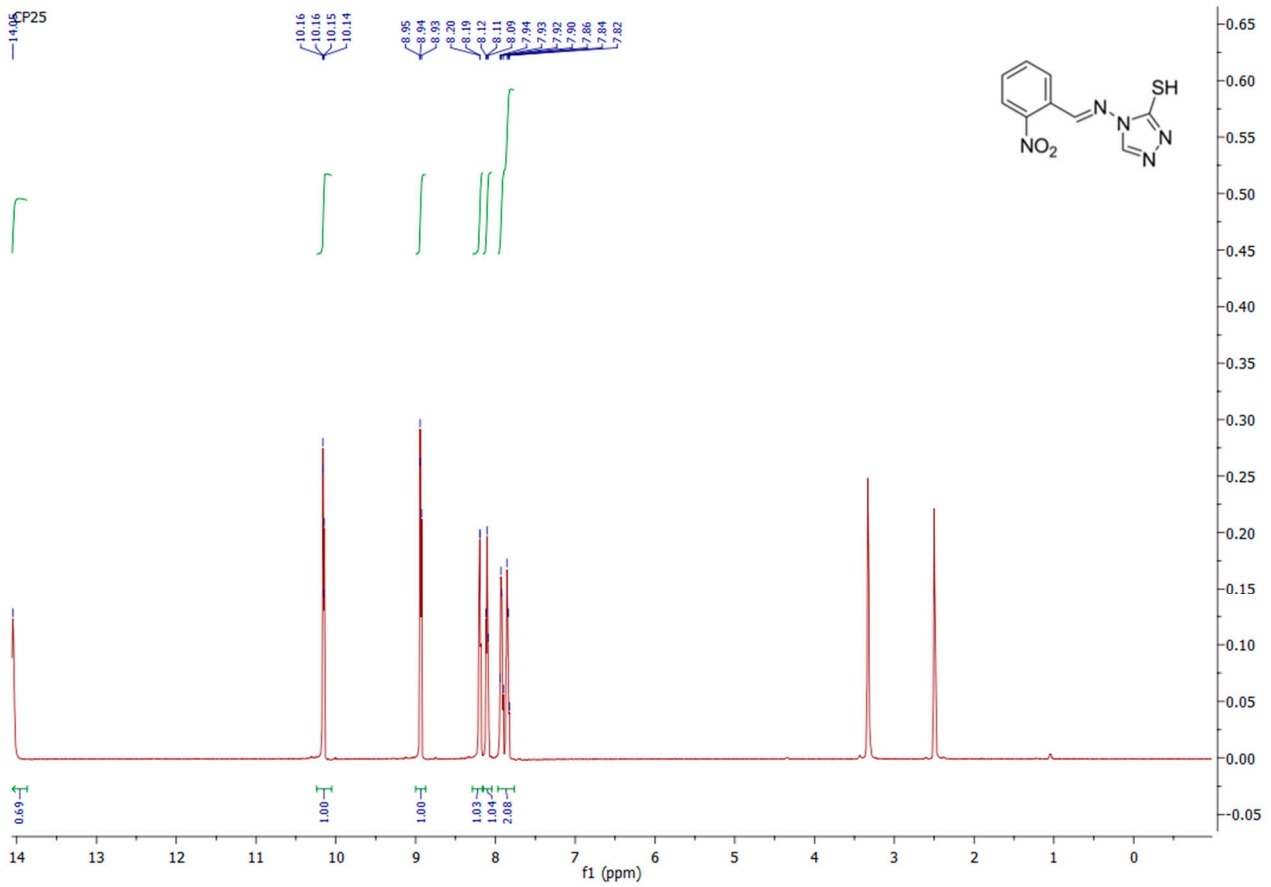
CP23



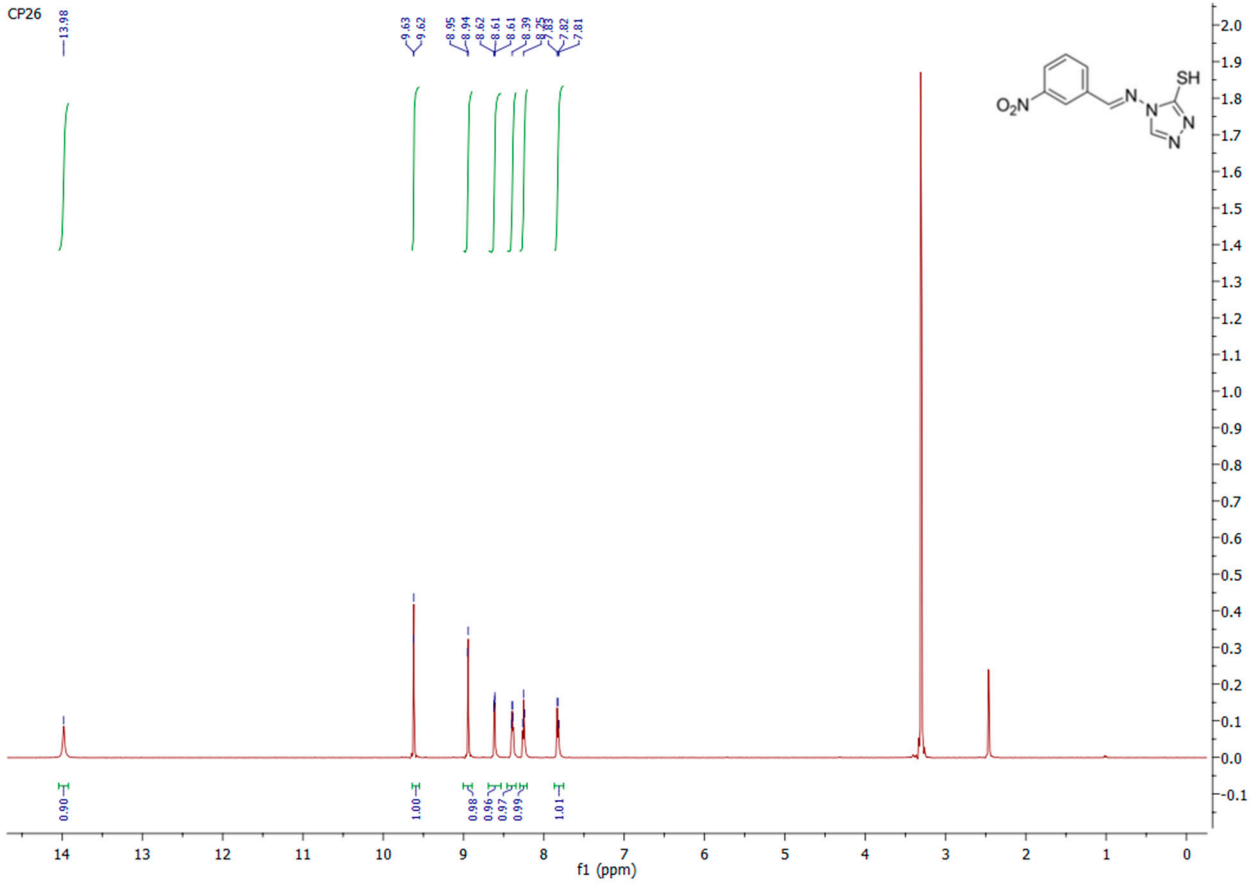
CP24



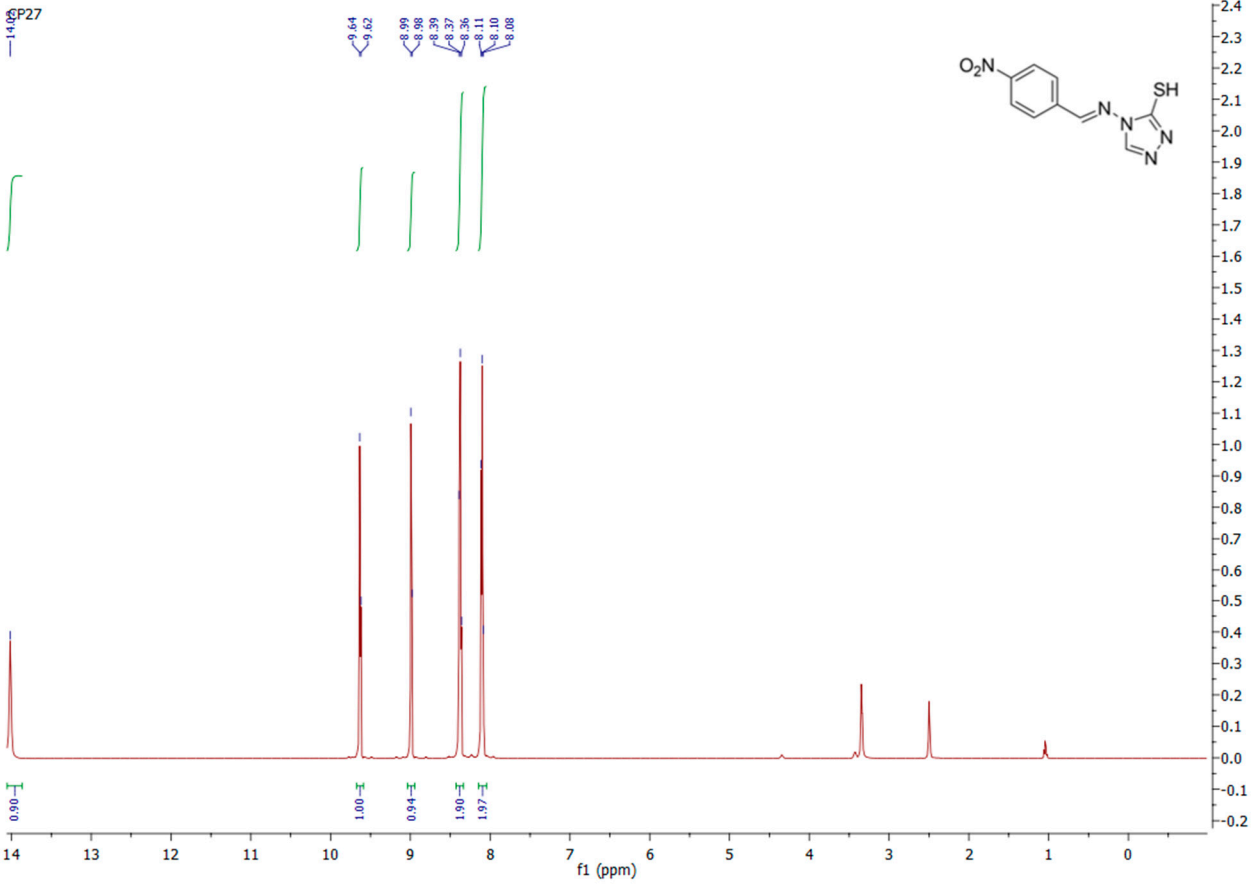
CP25

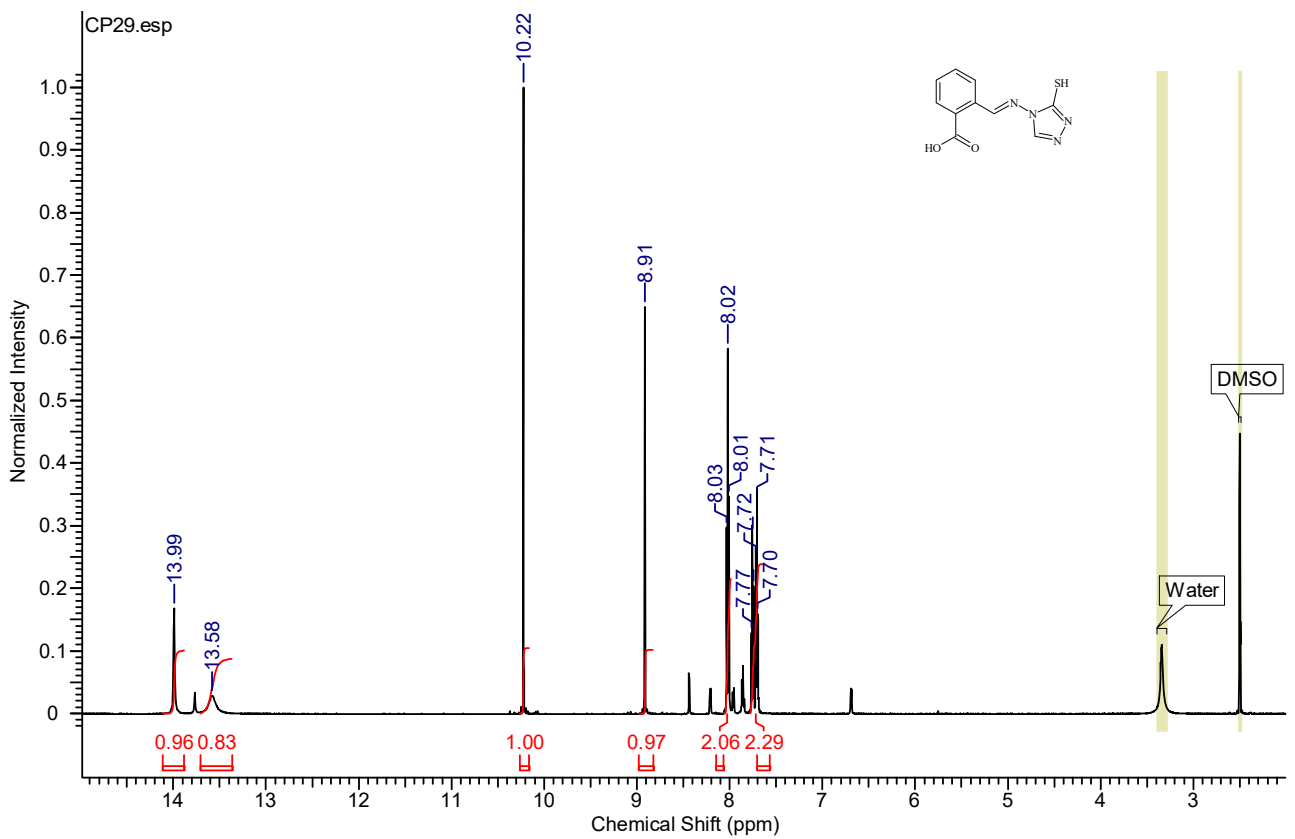
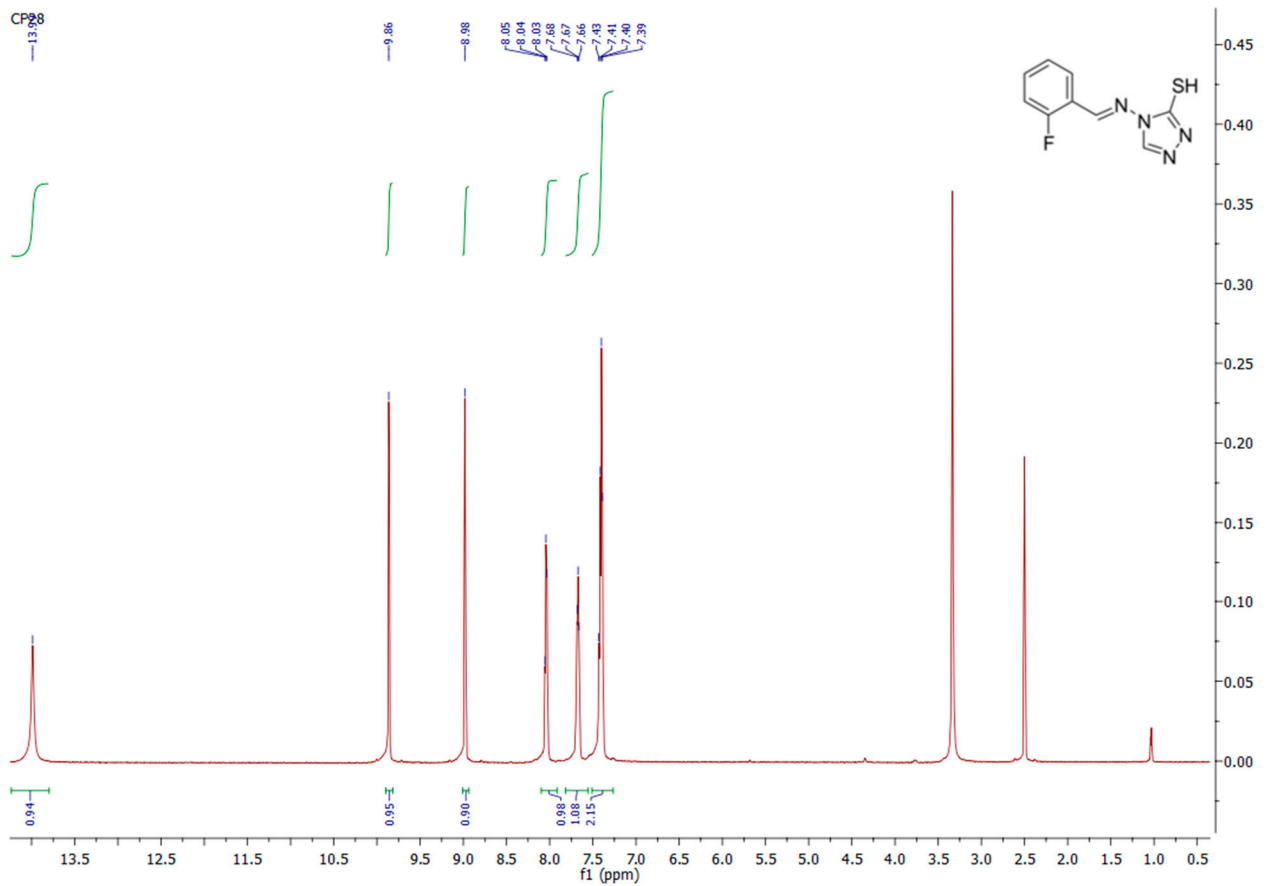


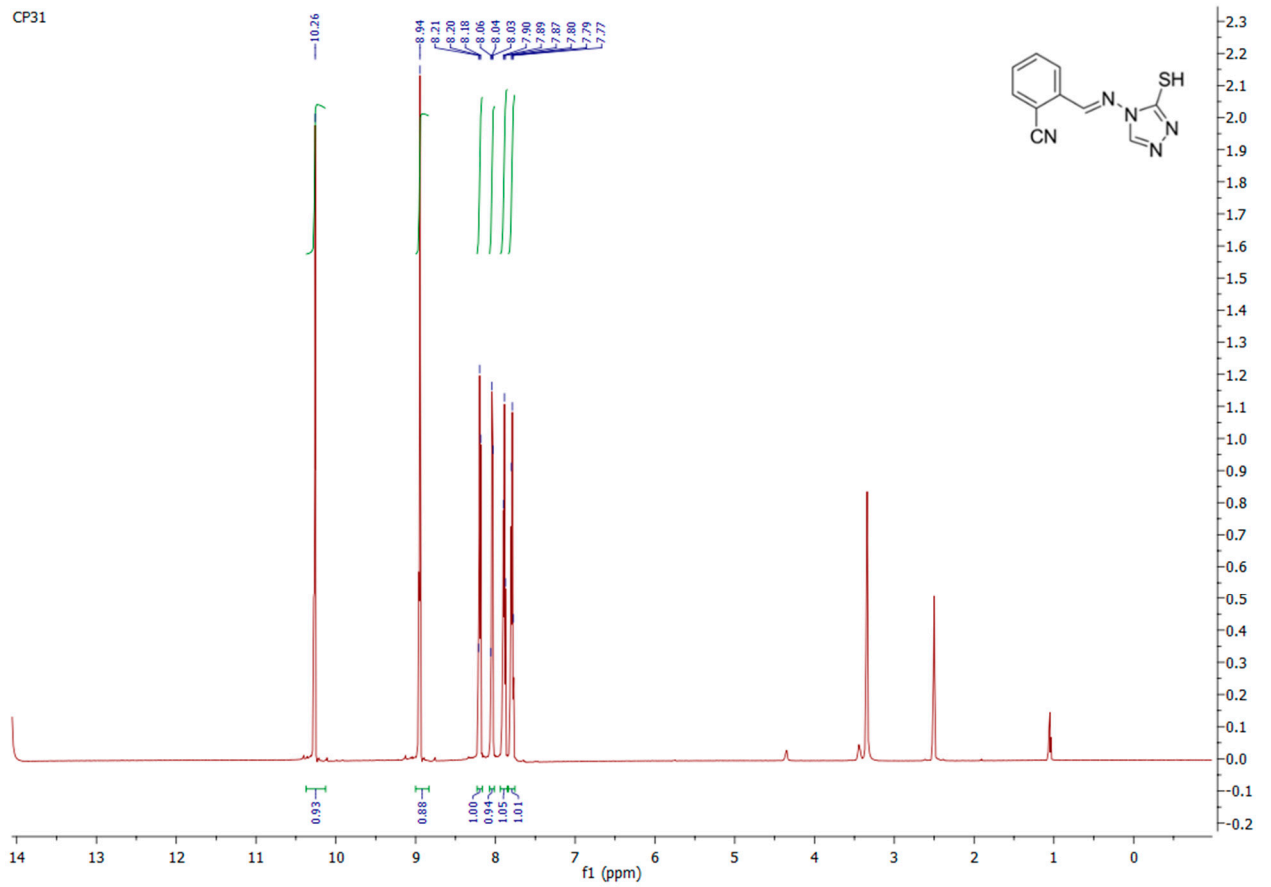
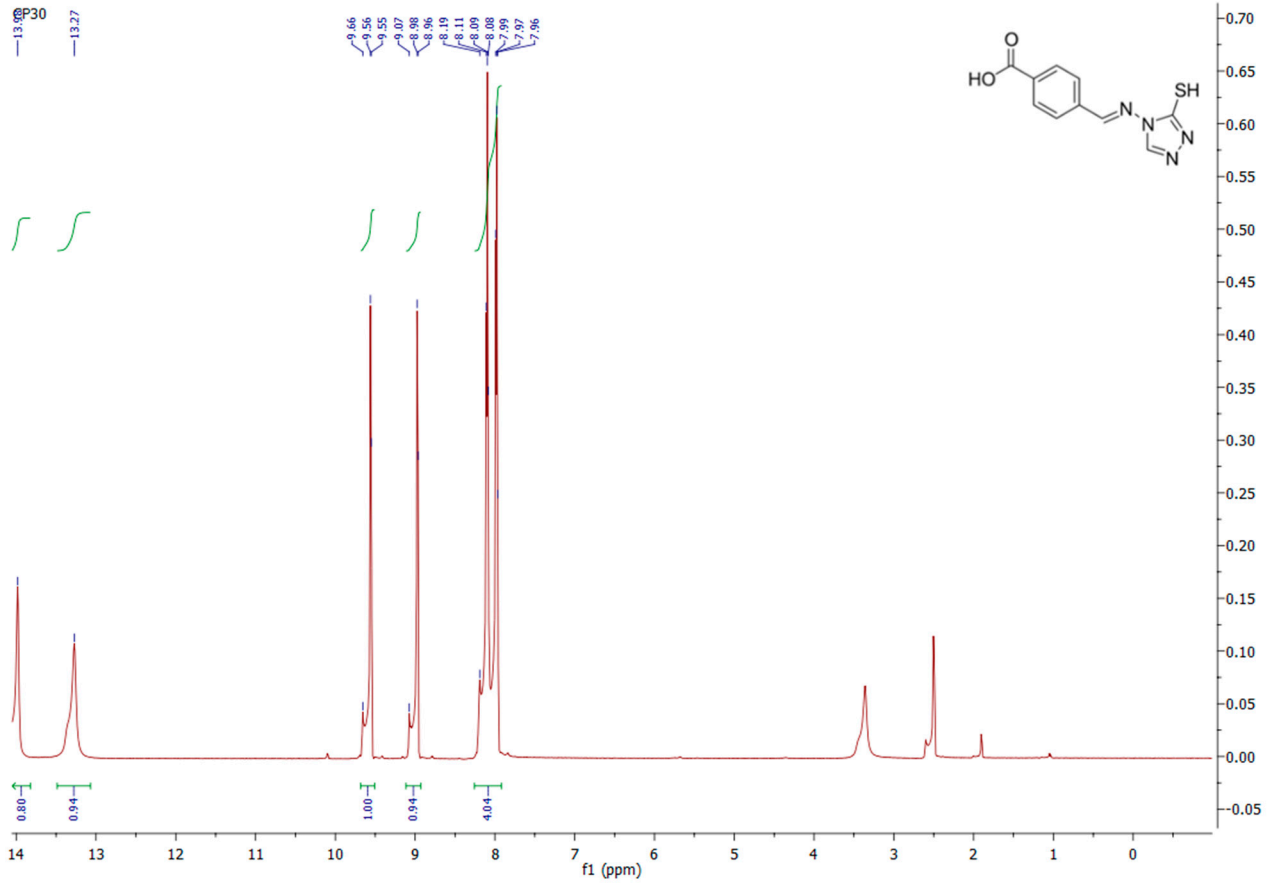
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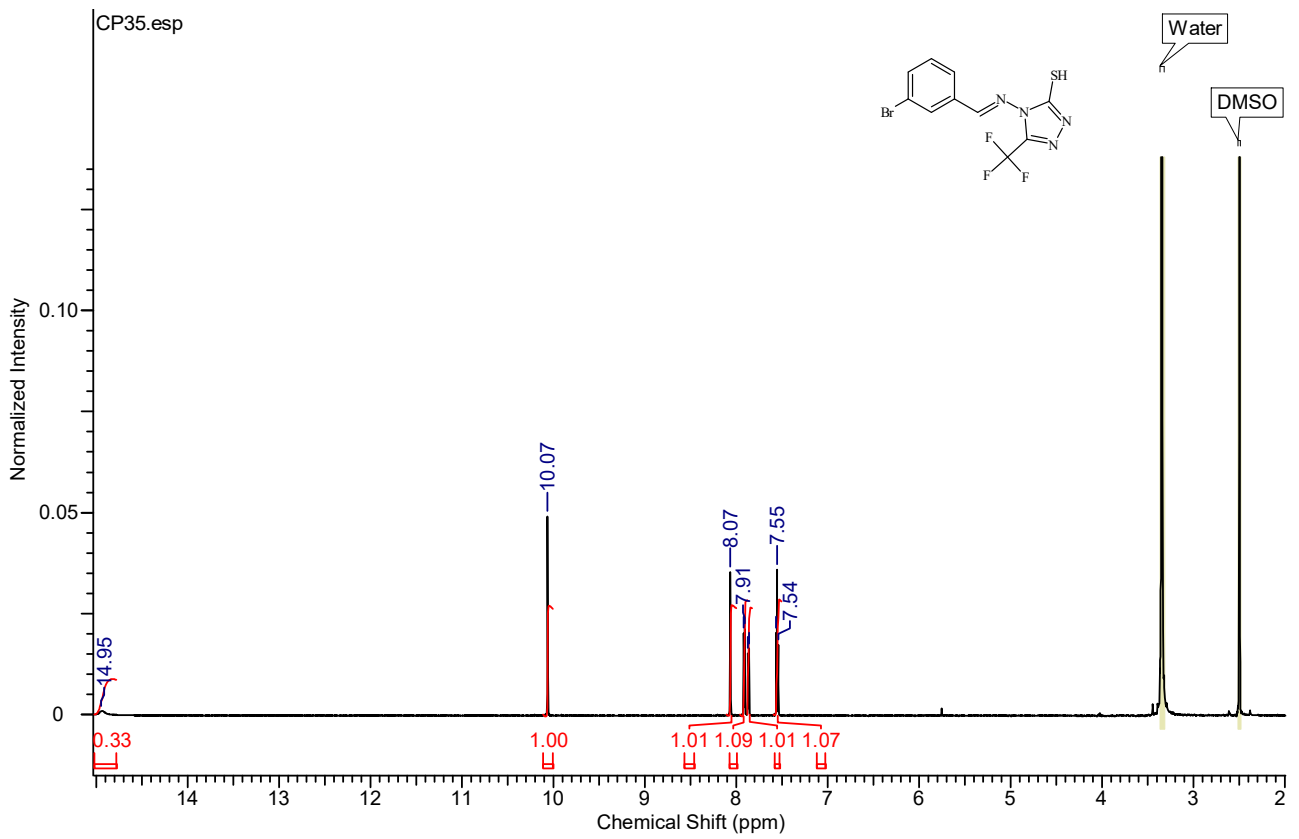
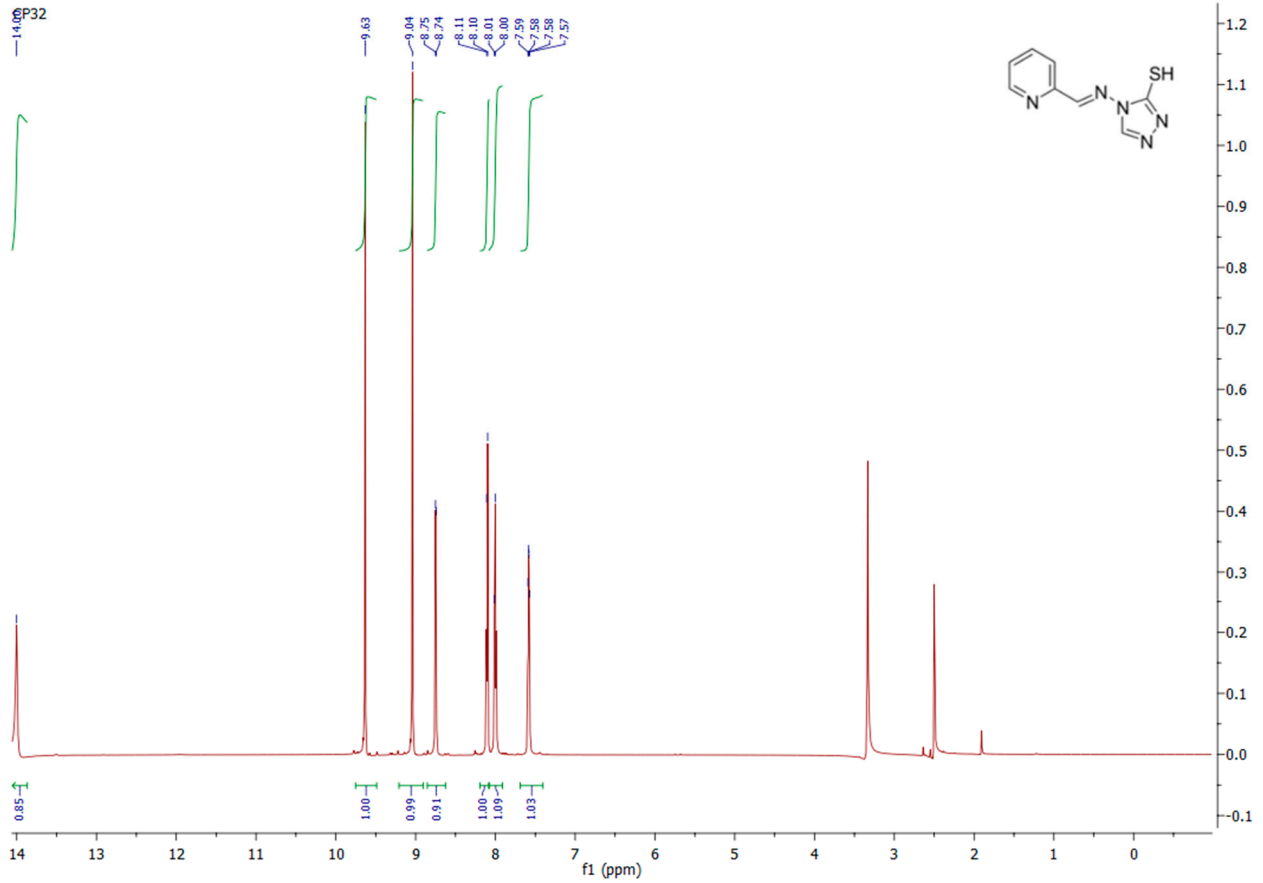


P27

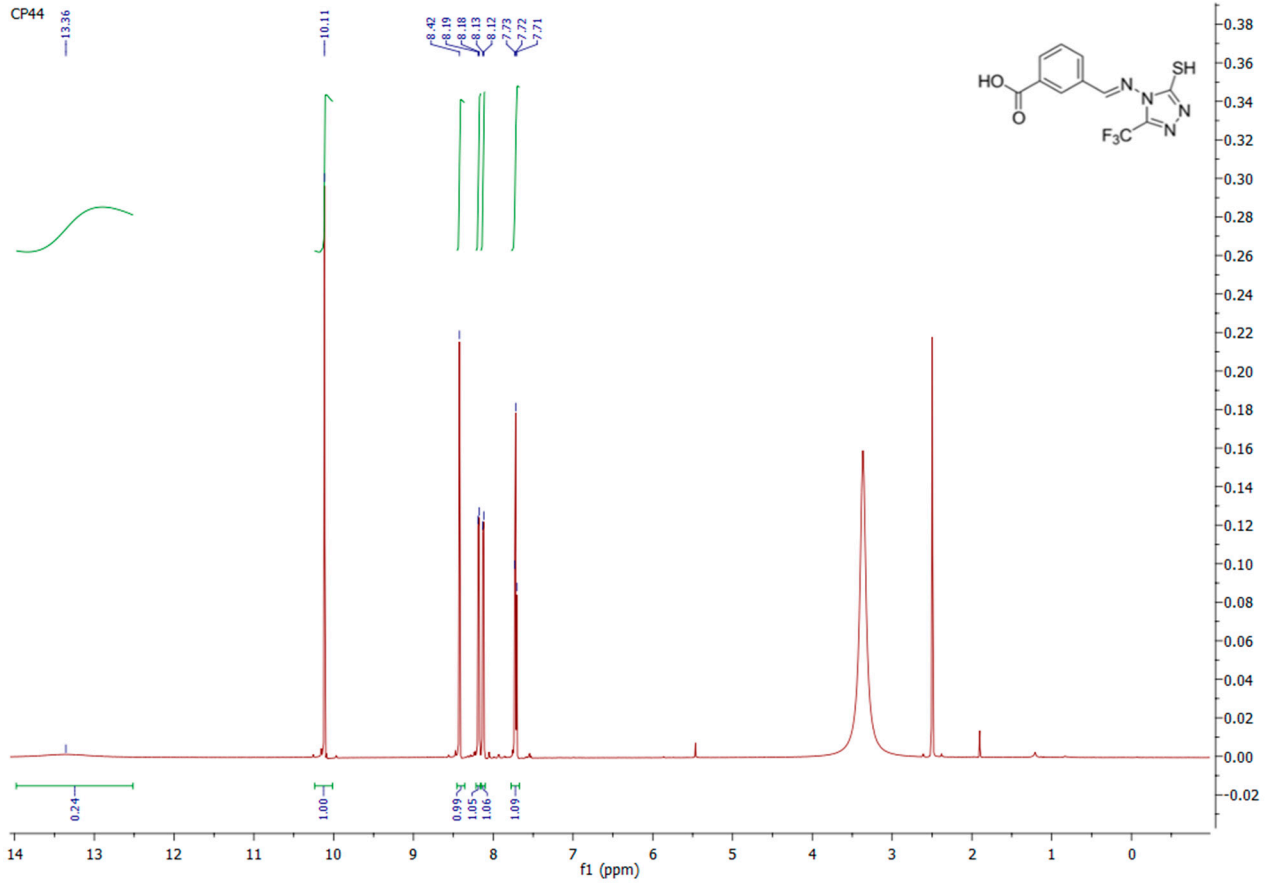




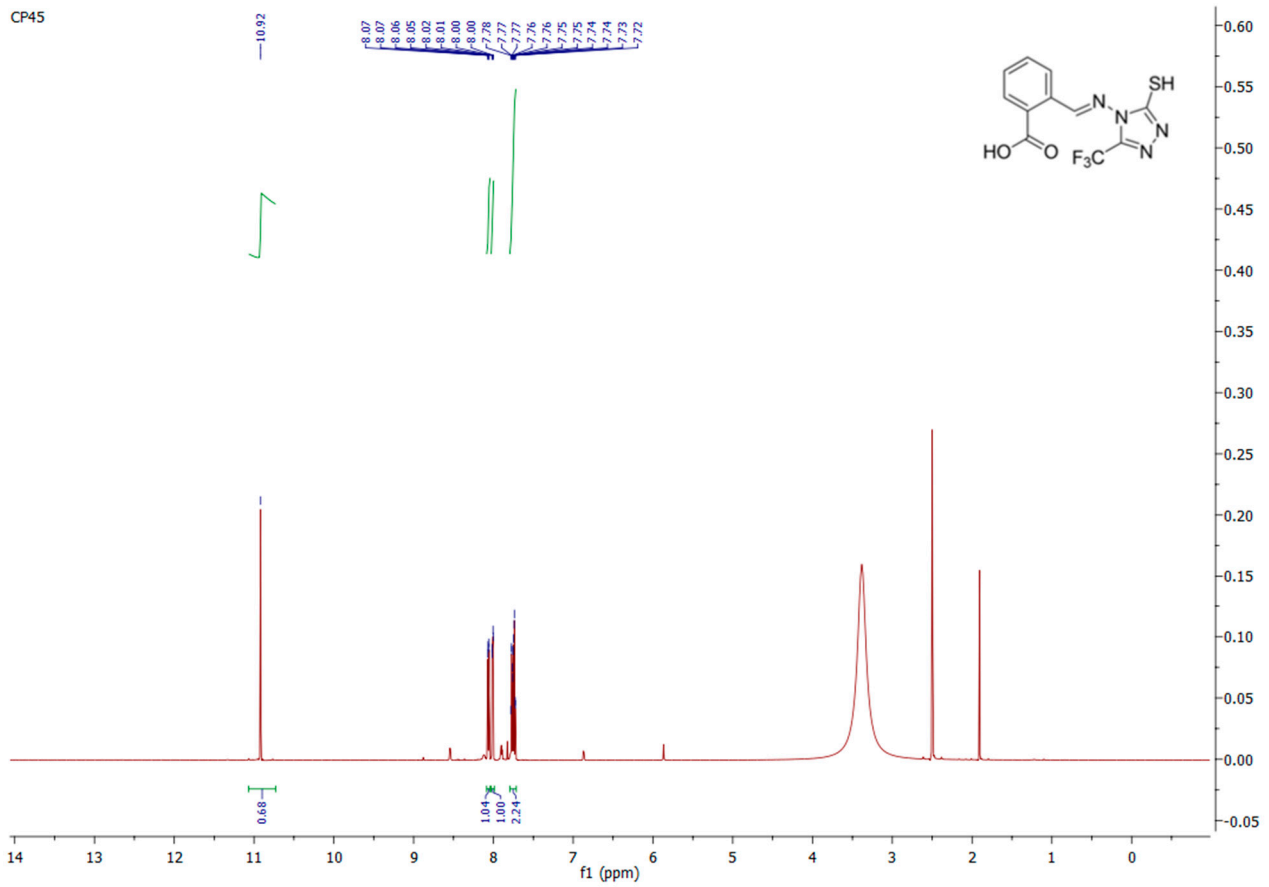




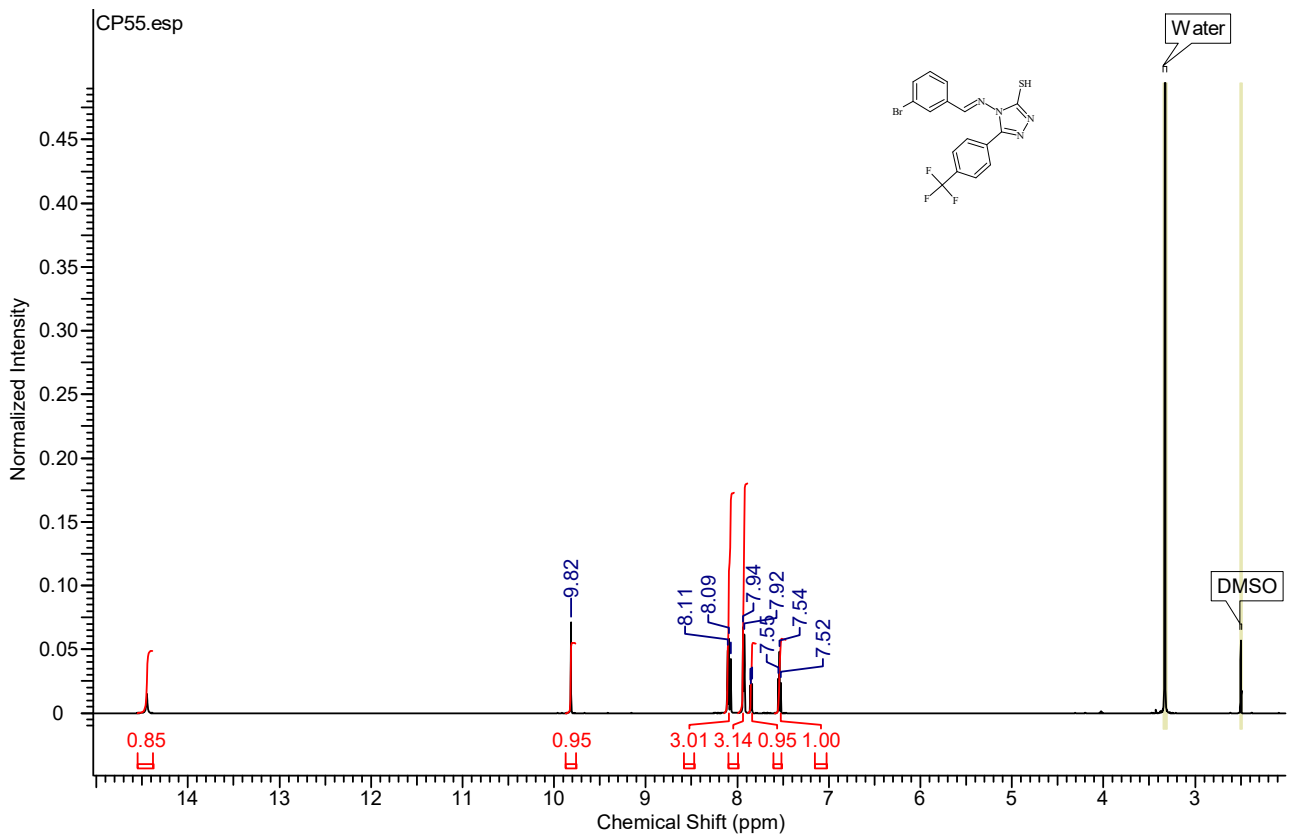
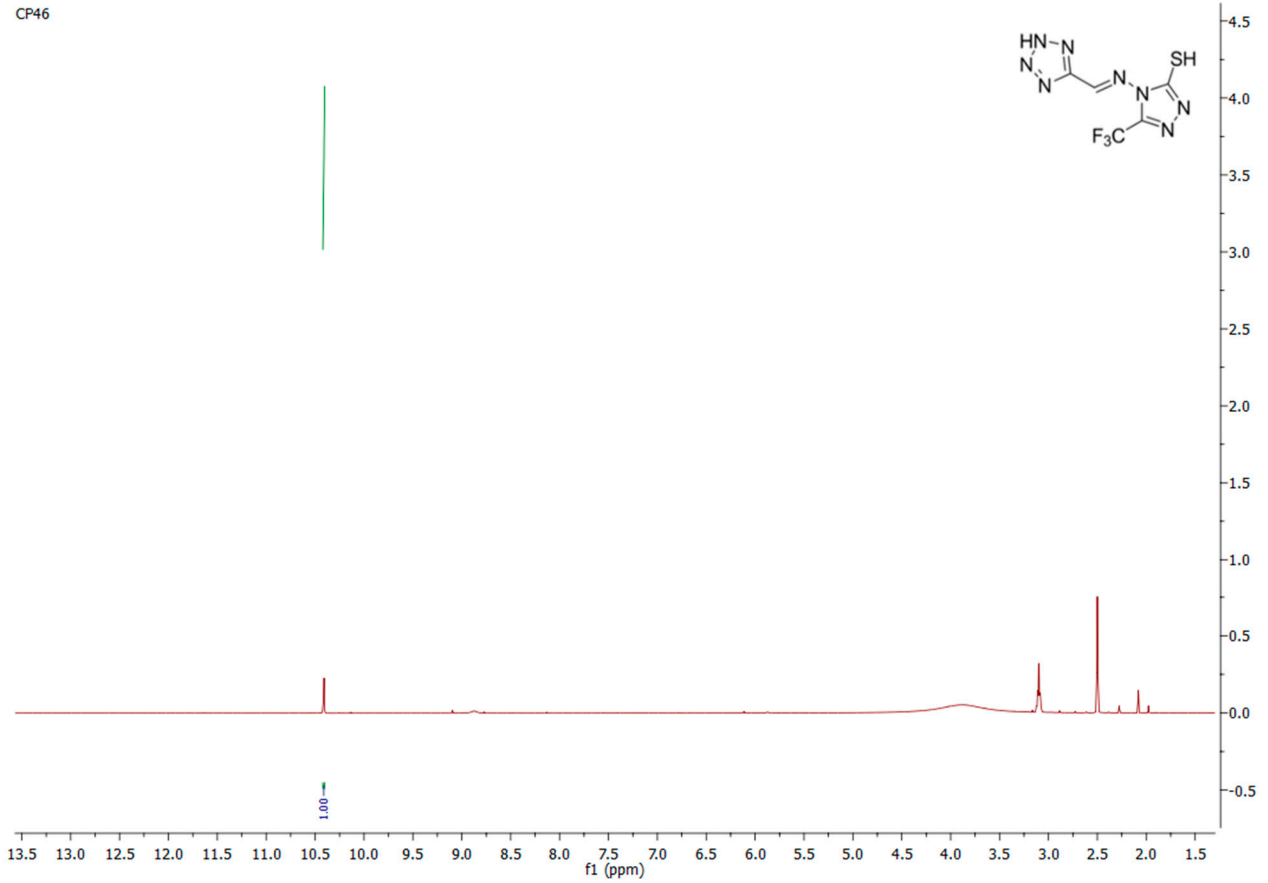
CP44



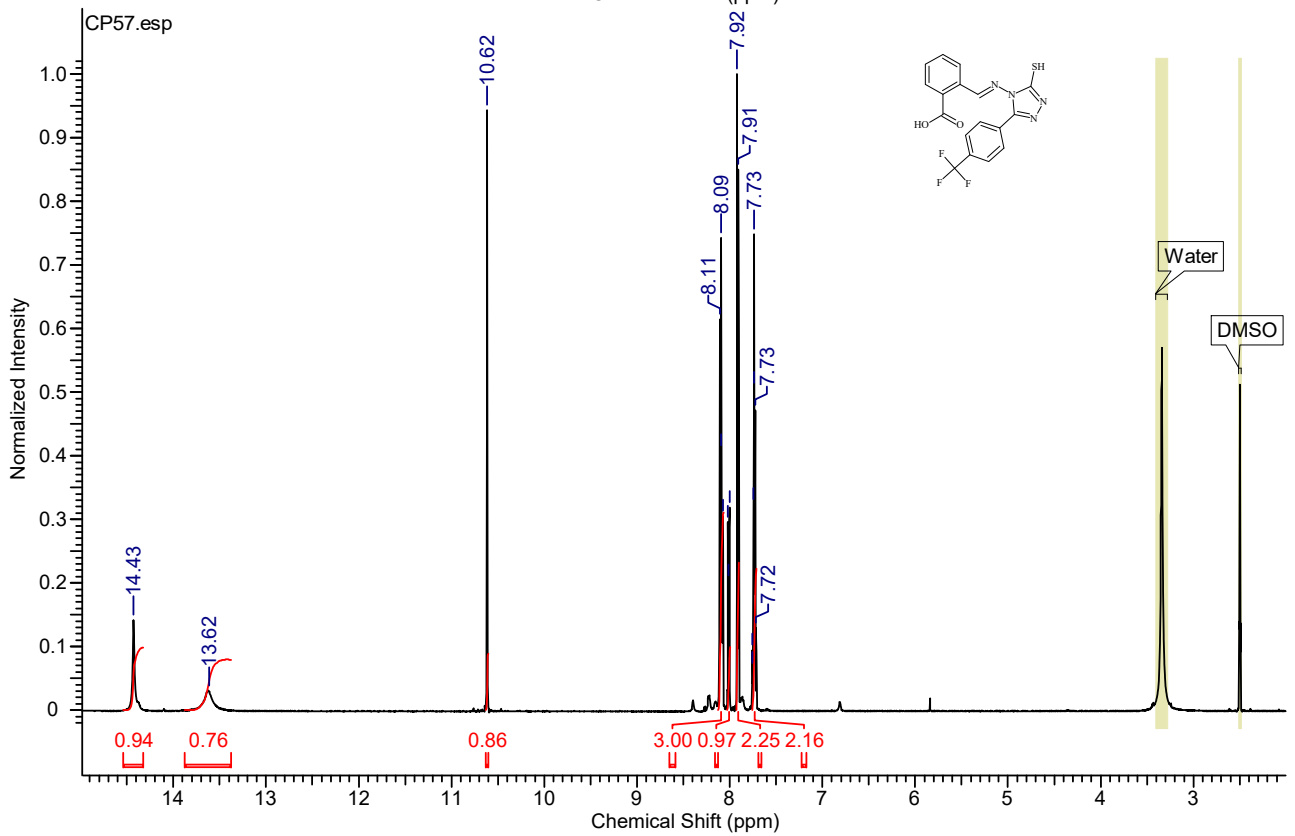
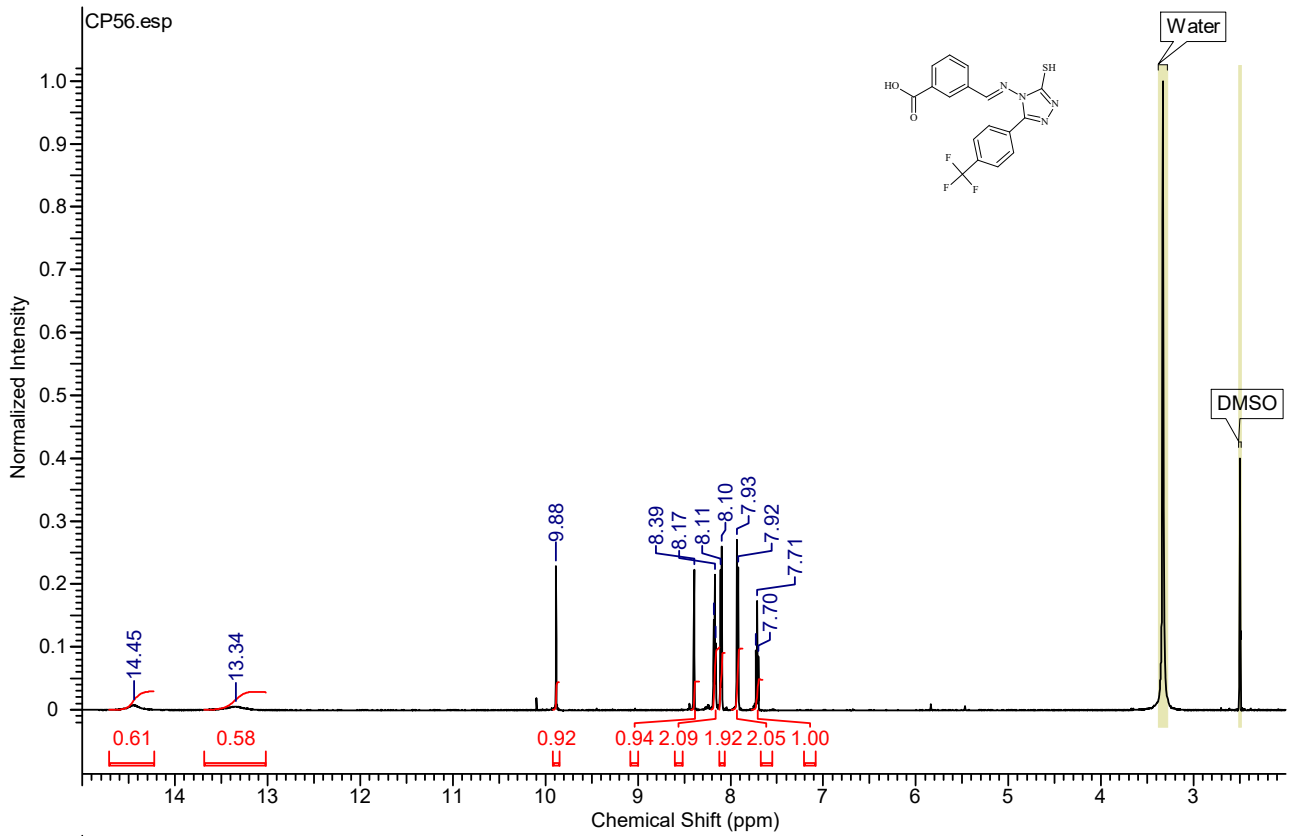
CP45

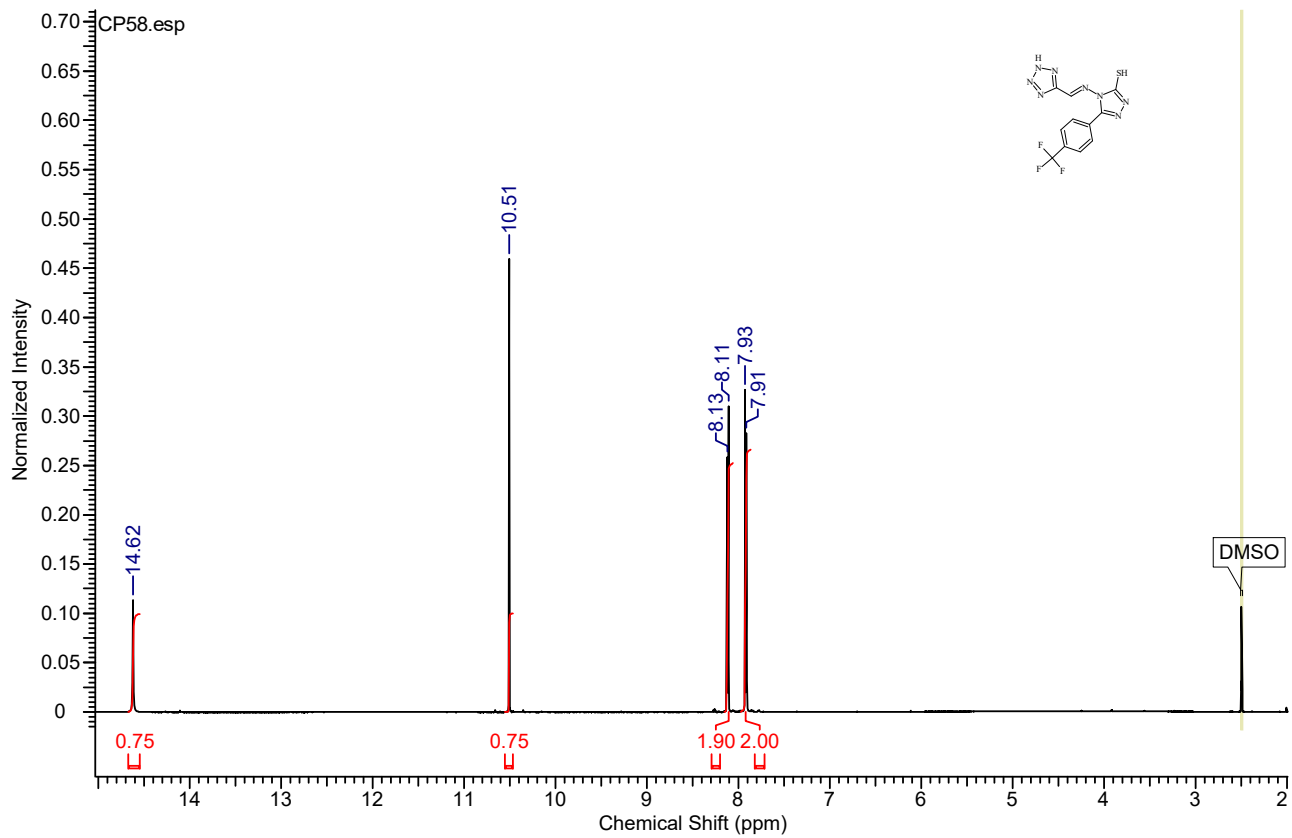


CP46

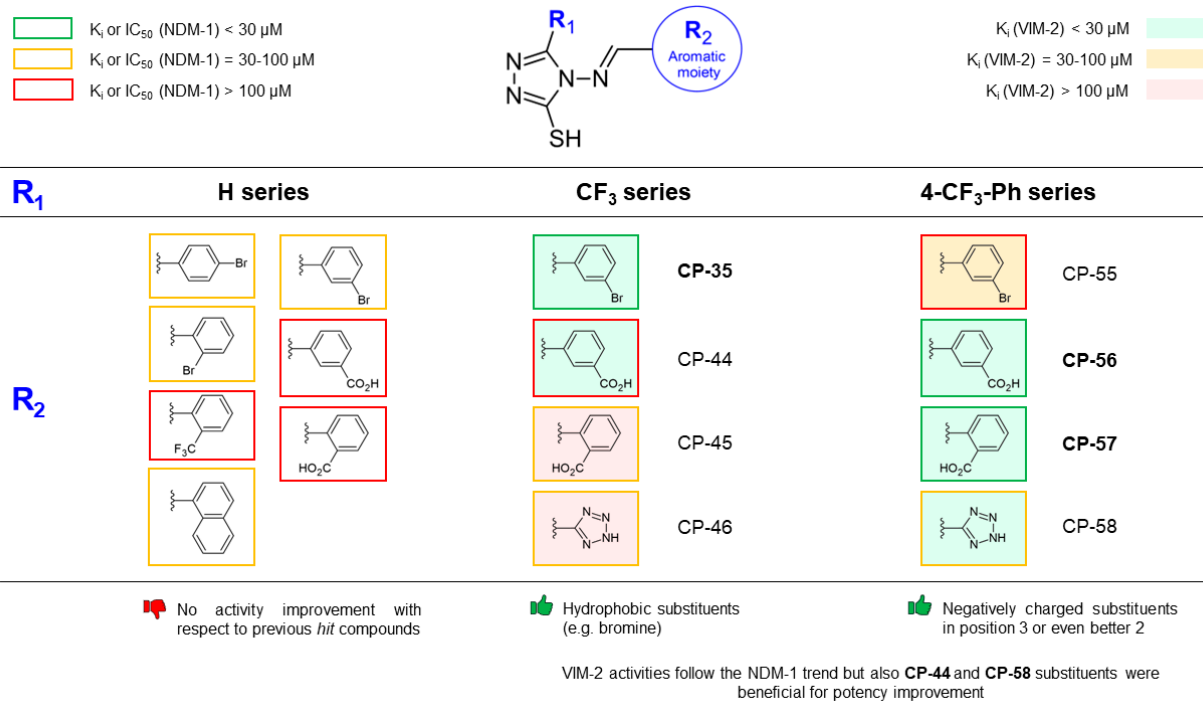








**Figure S3.** Structure-Activity relationship for synthesized compounds



**Figure S4.** Docking procedure validation result for compound OP31

