Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Masking thiol reactivity with thioamide, thiourea, and thiocarbamate-based MBPs

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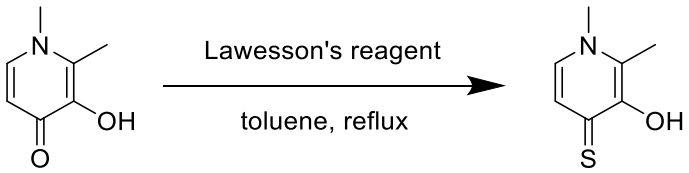
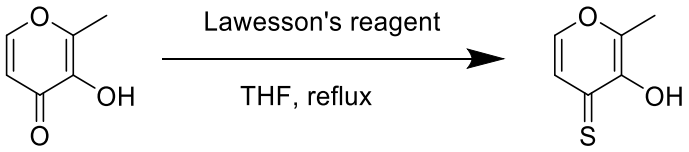
1. General Experimental Details

All reagents and solvents were obtained from commercial sources (Sigma Aldrich, Alfa Aesar, TCI, Combi-Blocks etc.) and used without further purification. Compounds **1, 2, 3, 4, 5, 6, 7, 8, 11, 12,** and **13** were purchased from commercial vendors. Compounds **5a**, **5b**, **9** and **10** were synthesized from widely available starting materials. [($Tp^{Ph,Me}$)Zn(**MBP**)] and [Zn(TPA)(**5**)]BPh_4 complexes were prepared according to literature methods. High resolution mass spectrometry (HR-MS) analysis was performed using an Agilent 6230 accurate-mass liquid chromatography time-of-flight mass spectrometry LC−TOFMS at the Molecular Mass Spectrometry Facility (MMSF) in the Department of Chemistry and Biochemistry at the University of California, San Diego. For HPLC analysis, Agilent 1200 series degasser and pump system with an Agilent Ecplise XDB-C18 (5 μm 150×4.6 mm) column was utilized in the Department of Chemistry and Biochemistry at the University of California, San Diego. ^{1}H NMR spectra were recorded at ambient temperature on 300 MHz Bruker, 400 MHz Jeol NMR instrument, or 500 MHz Varian NMR instrument in the Department of Chemistry and Biochemistry at the University of California, San Diego. Processing of the NMR data was performed using the MestReNova 14.2 program.

2. Preparation of Compounds

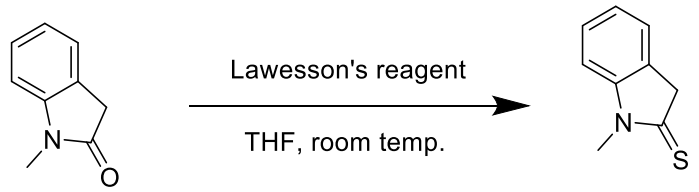


3-Hydroxy-1,2-dimethylpyridine-4(1*H*)-thione (9). The compound was synthesized by using a modified literature procedure.[1] To a stirred solution of 3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one (400 mg, 1 eq, 2.87 mmol) in toluene (50 mL), Lawesson's reagent (698 mg, 0.6 eq, 1.72 mmol) was added. The reaction mixture was left refluxing under nitrogen overnight followed by concentration in vacuo. The reaction mixture was diluted with $NaHCO_3$ (20 mL), extracted with EtOAc (3×20 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified via flash column chromatography (CH_2Cl_2:MeOH 0-5%) to give the purified product. Yield: 145 mg (32.5%). **^{1}H NMR** (300 MHz, DMSO-d_6): δ 8.73 (s, 1H), 7.66 (d, J = 6.6 Hz, 1H), 7.28 (d, J = 6.6 Hz, 1H), 3.82 (s, 3H), 2.42 (s, 3H). **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_7H_{10}NOS]^+$, 156.0478; found, 156.0479.

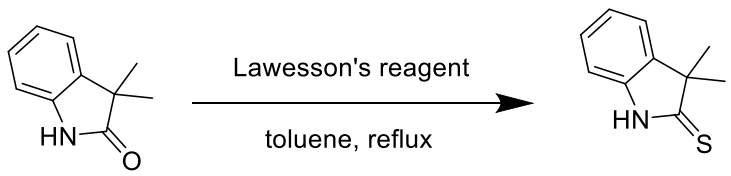


3-Hydroxy-2-methyl-4*H*-pyran-4-thione (10). The compound was synthesized by using a modified literature procedure.[2] To a stirred solution of 3-hydroxy-2-methyl-4*H*-pyran-4-one (500 mg, 1 eq, 3.96 mmol) in THF (30 mL), Lawesson's reagent (962 mg, 0.6 eq, 2.38 mmol) was added. The reaction mixture was heated to reflux under a nitrogen atmosphere for 4 h followed by concentration in vacuo. The crude product was purified via flash column chromatography (hexane:EtOAc 0-30%) to give the purified product as a yellow solid. Yield: 408 mg (72.4%). **^{1}H**

NMR (300 MHz, DMSO-d_6): δ 8.28 (s, 1H), 8.09 (d, J = 5.0 Hz, 1H), 7.34 (d, J = 5.0 Hz, 1H), 2.39 (s, 3H). **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_6H_7O_2S]^+$, 143.0161; found, 143.0161.



1-Methylindoline-2-thione (5a). The compound was synthesized by using a modified literature procedure.[3] To a stirred solution of 1-methylindolin-2-one (400 mg, 1 eq, 2.72 mmol) in THF (10 mL), Lawesson's reagent (660 mg, 0.6 eq, 1.63 mmol) was added. The mixture was stirred at room temperature for 16 h followed by concentration in vacuo. The residue was purified via flash column chromatography (hexane:EtOAc 0-50%) to give the purified product. Yield: 370 mg (83.4%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.37 – 7.25 (m, 2H), 7.16 (td, J = 7.5, 1.0 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 4.09 (s, 2H), 3.62 (s, 3H). **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_9H_{10}NS]^+$, 164.0528; found, 164.0530.



3,3-dimethylindoline-2-thione (5b). The compound was synthesized by using a modified literature procedure.[4] To a stirred solution of 3,3-dimethylindolin-2-one (400 mg, 1 eq, 2.48 mmol) in toluene (10 mL), Lawesson's reagent (552 mg, 0.55 eq, 1.36 mmol) was added. The reaction mixture was heated to reflux under a nitrogen atmosphere overnight followed by concentration in vacuo. The reaction mixture was diluted with H_2O (20 mL), extracted with EtOAc (3×20 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash

chromatography (hexane:EtOAc 0-20%) to give the purified product. Yield: 357 mg (81.2%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 11.11 (s, 1H), 7.29 – 7.22 (m, 2H), 7.14 (td, J = 7.5, 1.0 Hz, 1H), 7.08 (dd, J = 7.7, 0.9 Hz, 1H), 1.45 (s, 6H). **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{10}H_{12}NS]^+$, 178.0685; found, 178.0687.

3. Preparation of [(Tp^{Ph,Me})Zn(MBP)] and [Zn(TPA)(5)]BPh$_4$ complexes

[(Tp^{Ph,Me})Zn(**MBP**)]. The complex was synthesized by using a modified literature procedure.[5] To obtain an X-ray structure of [(Tp^{Ph,Me})Zn(**MBP**)], [(Tp^{Ph,Me})K] and [(Tp^{Ph,Me})ZnOH] was prepared according to literature methods.[6] [(Tp^{Ph,Me})ZnOH] (50 mg, 0.09 mmol) was dissolved in 15 mL of CH_2Cl_2 in a 50 mL round-bottom flask. Each compound (0.09 mmol, 1 eq) in 10 mL of MeOH was added, and the reaction mixture was stirred overnight under a nitrogen atmosphere. The resulting mixture was evaporated to dryness and subsequently dissolved in a minimal amount (~1 mL) of benzene. The solution was filtered using a syringe filter to remove any undissolved solids. The resulting complex in benzene was recrystallized using vapor diffusion with pentane. Crystals typically formed within a few days. The resulting crystals are characterized by X-ray crystallography and HR-MS. [(Tp^{Ph,Me})Zn(**1**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{37}H_{33}BN_7OSZn]^+$, 698.1853; found, 698.1859. [(Tp^{Ph,Me})Zn(**2**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{38}H_{36}BN_8SZn]^+$, 711.2169; found, 711.2172. [(Tp^{Ph,Me})Zn(**3**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{37}H_{34}BN_8SZn]^+$, 697.2013; found, 697.2009. [(Tp^{Ph,Me})Zn(**4**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{37}H_{33}BN_7S_2Zn]^+$, 714.1624; found, 714.1624. [(Tp^{Ph,Me})Zn(**5**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{38}H_{35}BN_7SZn]^+$, 696.2060; found, 696.2054. [(Tp^{Ph,Me})Zn(**7**)]: **HR-MS (ESI)** (*m/z*): $[M+H]^+$ calcd. for $[C_{39}H_{35}BN_7S_2Zn]^+$, 740.1781; found, 740.1778.

[Zn(TPA)(**5**)]BPh_4. The complex was synthesized by using a modified literature procedure.[7] Zinc(II) acetate dihydrate (23 mg, 1 Eq, 0.10 mmol) was dissolved in MeOH (3 mL). Tris(pyridin-2-ylmethyl)amine (30 mg, 1 Eq, 0.10 mmol) was then dissolved in the vessel (yellowish transparent), sonicated and stirred for 10min. Indoline-2-thione (15 mg, 1 Eq, 0.10 mmol) was added to the vessel and stirred for 10min. Ammonium tetraphenylborate (35 mg, 1 Eq, 0.10 mmol) was added and stirred for 3h. After filtration and washing with methanol, the green precipitate precipitates were crystalized by vapor diffusion of Et_2O into a CH_3CN solution of the complex. The resulting crystal was characterized by X-ray crystallography.

4. DTNB Reactivity Analysis

Tested compounds (compounds **1** – **13**) were prepared as 50 mM DMSO stock solution and the stock solution was further diluted to 5, 2, and 1 mM. 0.5 mM DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid)) was prepared in 50 mM tris sulfate buffer (pH 8). 10 μL of each compound was incubated with 200 μL of 0.5 mM DTNB for 90 min at 25° C and the absorbance at 412 nm was measured. All tested compounds were also incubated with buffer solution without DNTB for 90 min and no background signal at 412 nm was observed. Each measurement was triplicated, and their background absorbance was subtracted from the measurement. Their reactivity with DTNB was quantified based on the standard calibration curve of L-cysteine methyl ester.

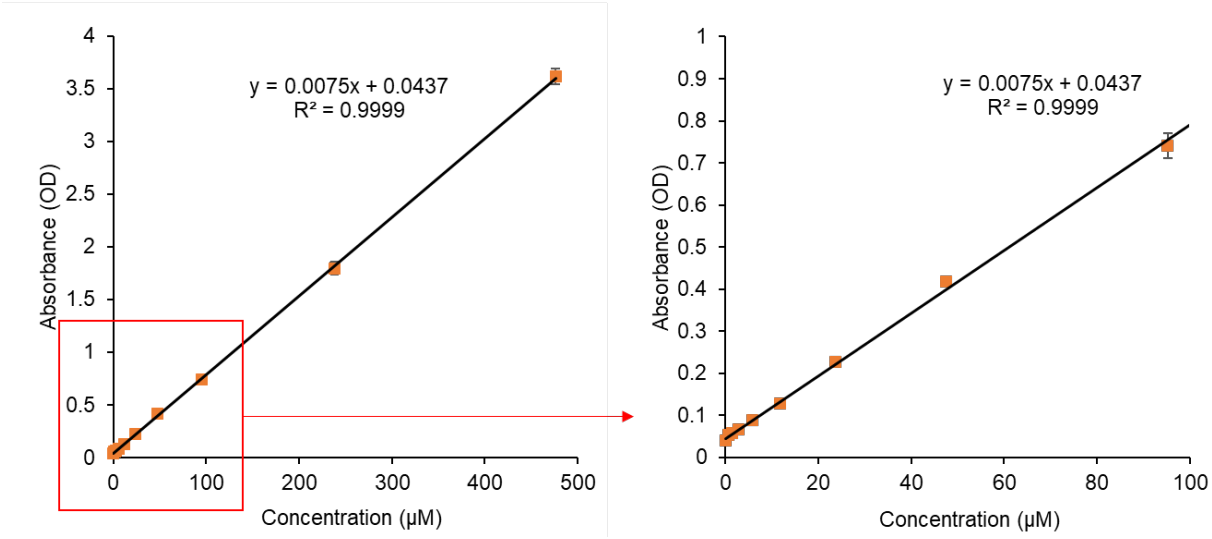


Figure S1. Standard calibration curve of L-cysteine methyl ester. The final concentration range of L-cysteine methyl ester was between 1 and 500 μM with the fixed concentration of DTNB (500 μM). The whole concentration range (*left*) and the lower concentration range (*right*) of the standard calibration curve.

Table S1. Percent reactivity of the proposed compounds with DTNB. Excess amount of DTNB was used with different molar ratios (1:2, 1:5, 1:10). 2-Mercapophenol (**11**), captopril (**12**), and L-cysteine methyl ester (**13**) were used as representative free thiol groups and showed almost 100% reactivity with DTNB.

	Molar ratio (Compound/DTNB)		
Compound	1:2	1:5	1:10
1,2,3,4,6, and 8	2 (0)	3 (0)	5 (0)
5	35 (1)	36 (1)	33 (0)
7	23 (2)	19 (1)	19 (0)
9	50 (2)	50 (4)	52 (2)
10	51 (6)	74 (1)	64 (7)
11	98 (5)	98 (1)	97 (1)
12	101 (11)	104 (1)	105 (0)
13	100 (7)	100 (2)	100 (1)

5. Cysteine Reactivity Analysis

HPLC Analysis. 25 μL of 50 mM compounds **1**–**11** dissolved in DMSO were mixed with 25 μL of 50 mM L-cysteine methyl (1:1 molar ratio), and then the mixture were dissolved in 950 μL of 10% ACN/ 90% H_2O (final concentration of each compound: 1.25 mM). The pH of the solution was adjusted to 7.4 and the mixture incubated for 24 h. The HPLC spectra were measured at 254 nm/280nm. The solvents (HPLC grade) were Millipore water (solvent A) and acetonitrile (solvent B). The following solvent gradient was used: 0-12 min: linear gradient from 95% A (5% B) to 5% A (95% B); 12-14 min: isocratic 5% A (95% B); 14-15 min: linear gradient from 5% A (95% B) to 95% A (5% B); 15-20 min: isocratic 95% A (5% B).

^{1}H NMR Analysis. Compounds **1**–**11** were incubated in a 1:1 molar ratio with L-cysteine methyl ester in DMSO-d_6 for 24 h (final concentration of each compound was 25 mM). ^{1}H NMR spectra were measured for each compound, L-cysteine methyl ester, and the mixture after 24 h incubation.

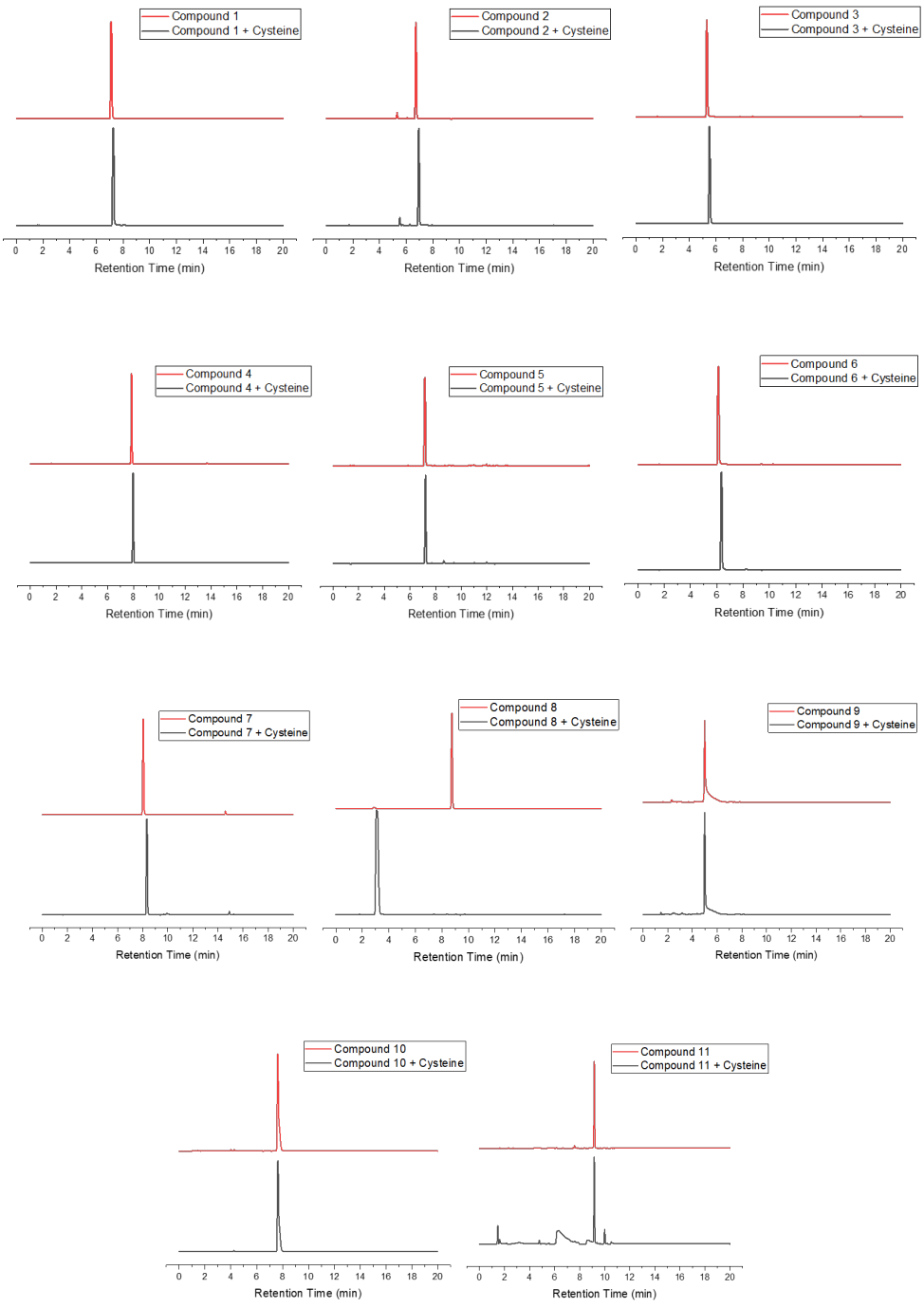


Figure S2. HPLC spectra of the proposed thioamide, thiourea, and thiocarbamate compounds (*top*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

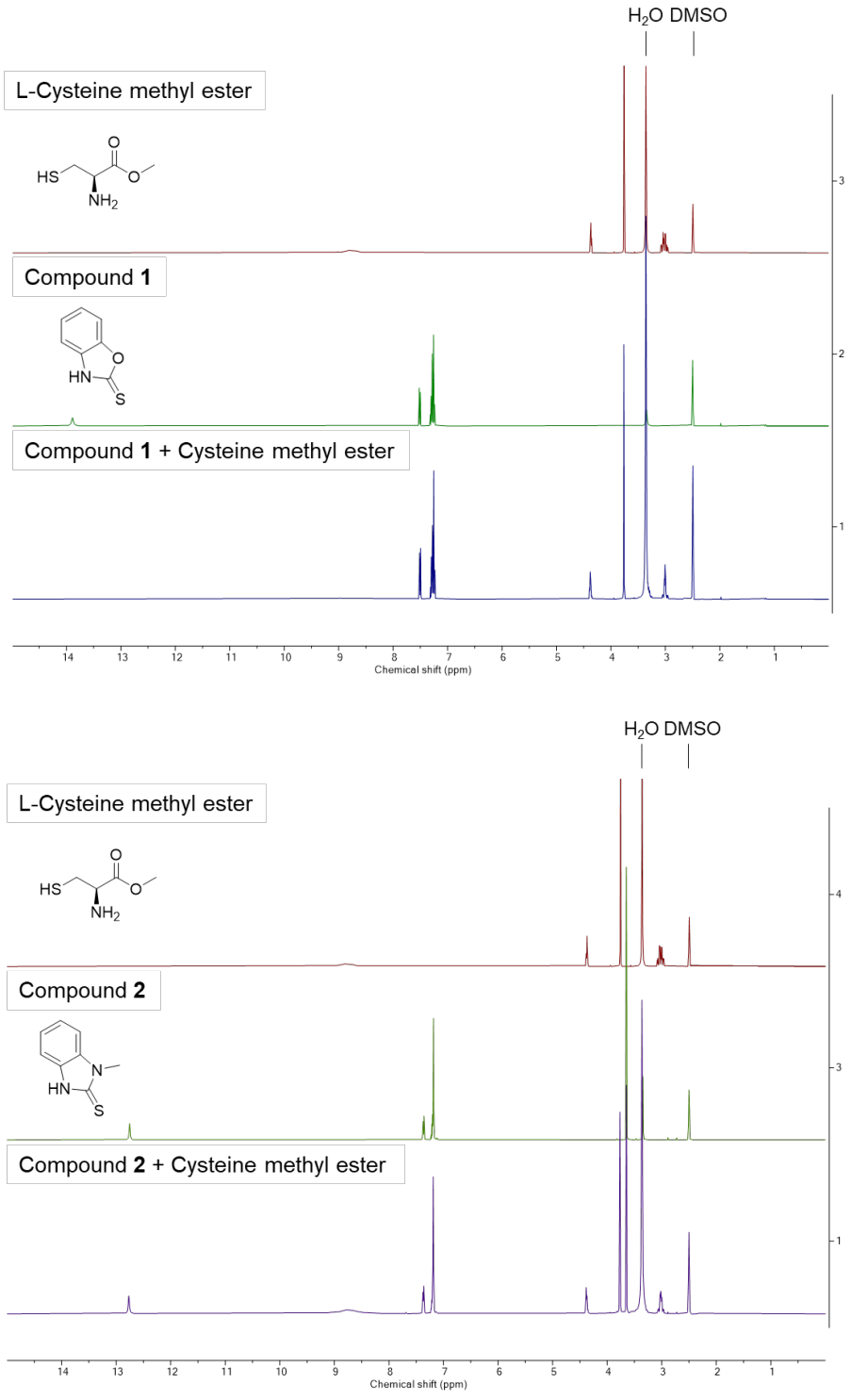


Figure S3. ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

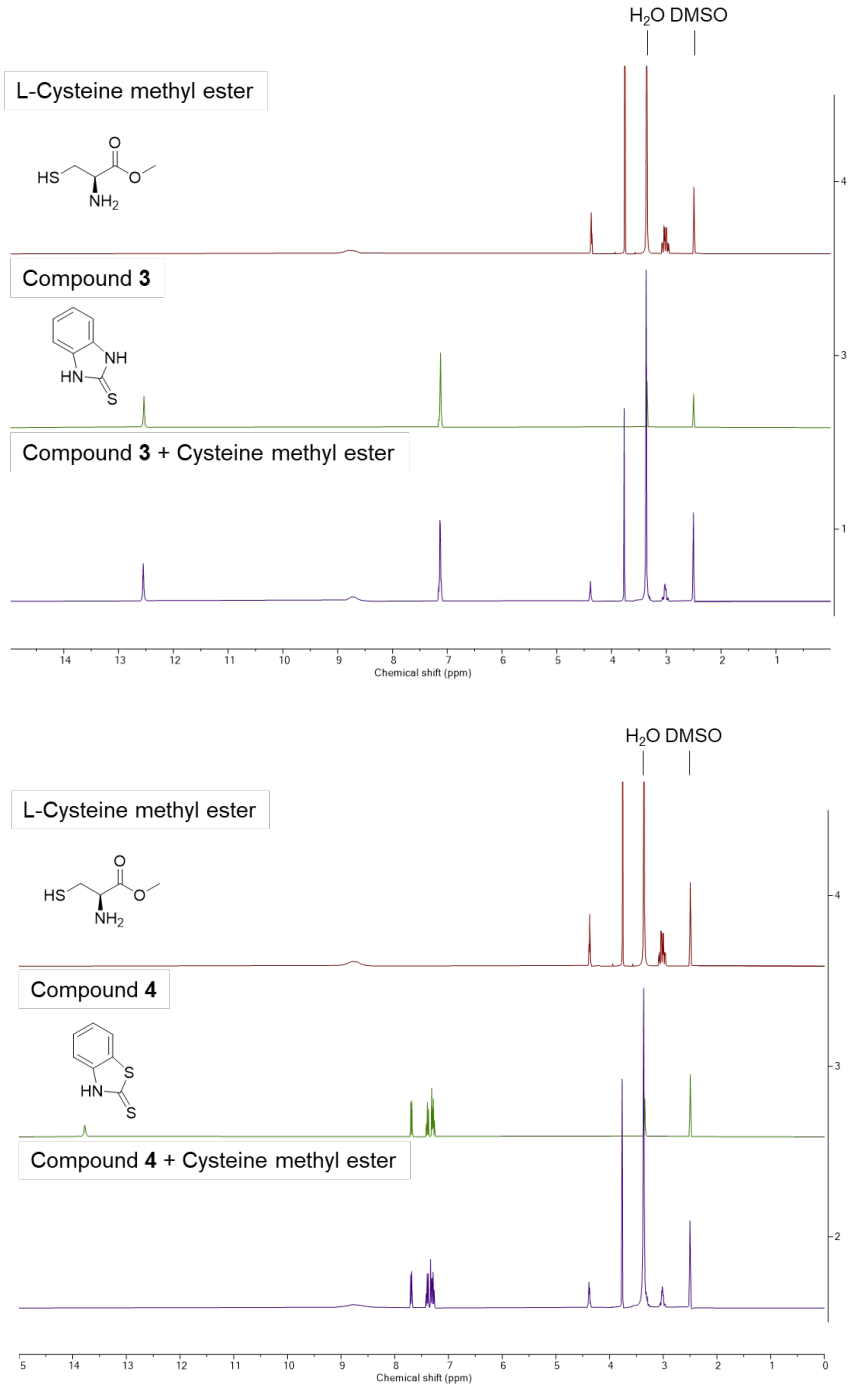


Figure S3 (continued). ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

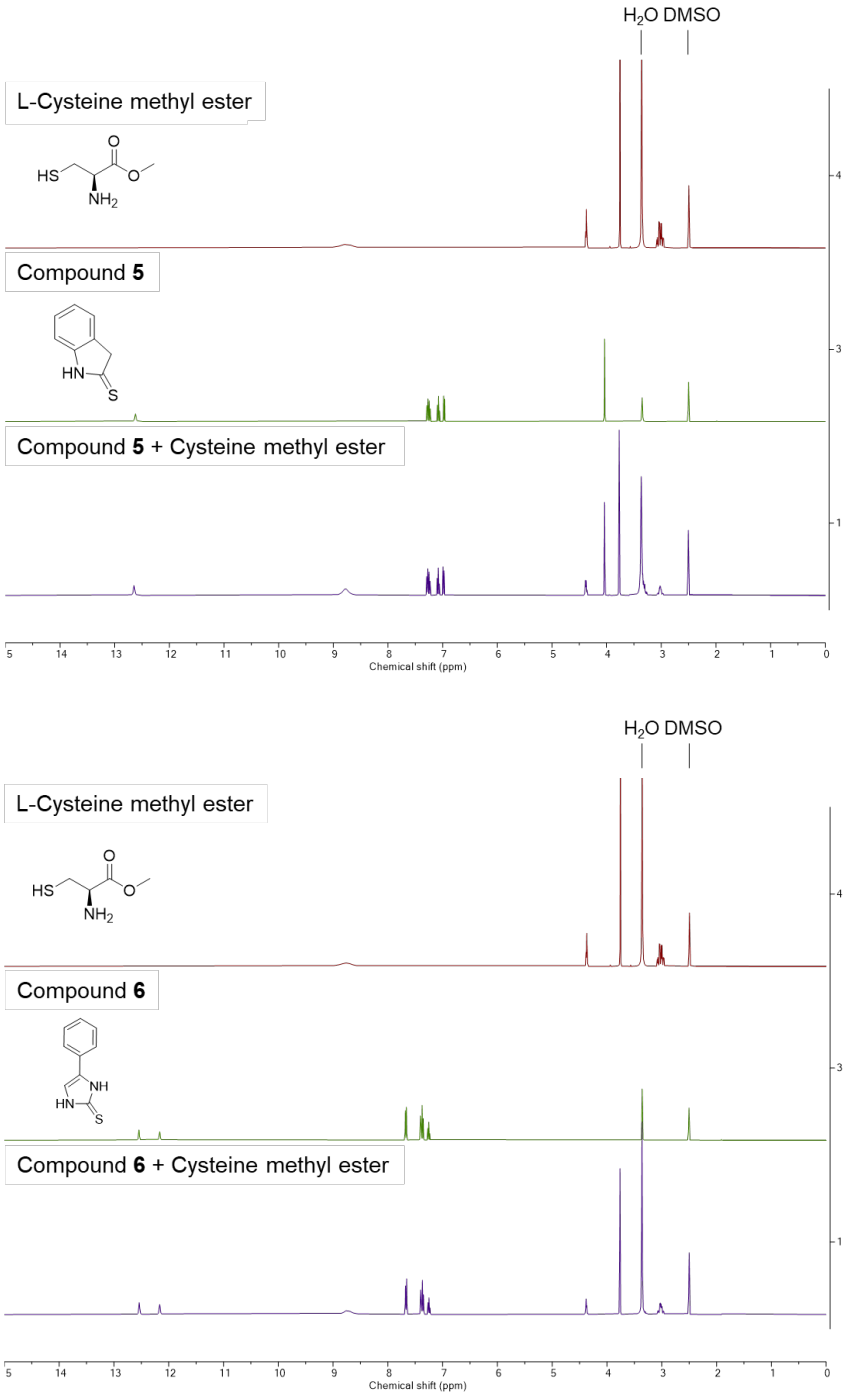


Figure S3 (continued). ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

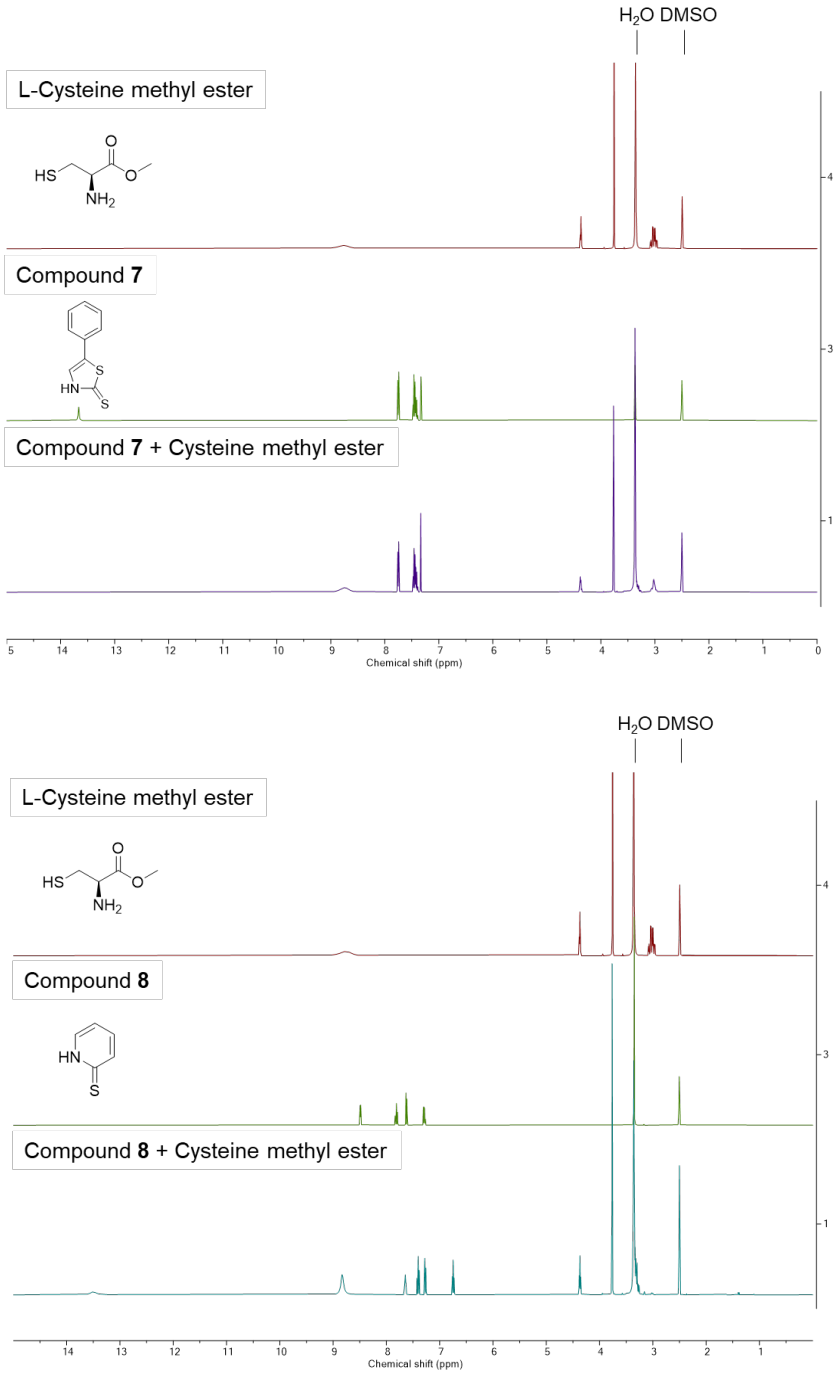


Figure S3 (continued). ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

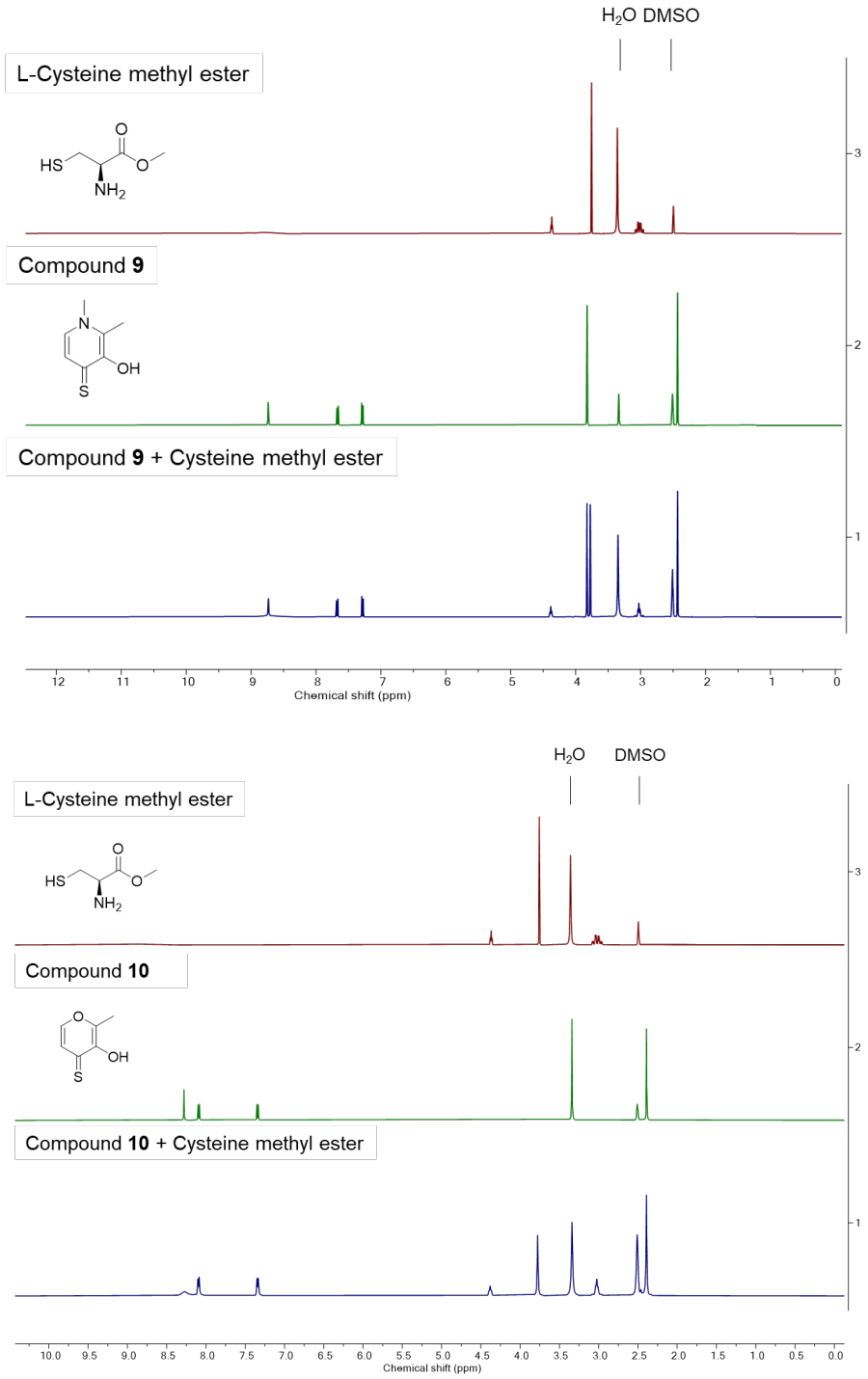


Figure S3 (continued). ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

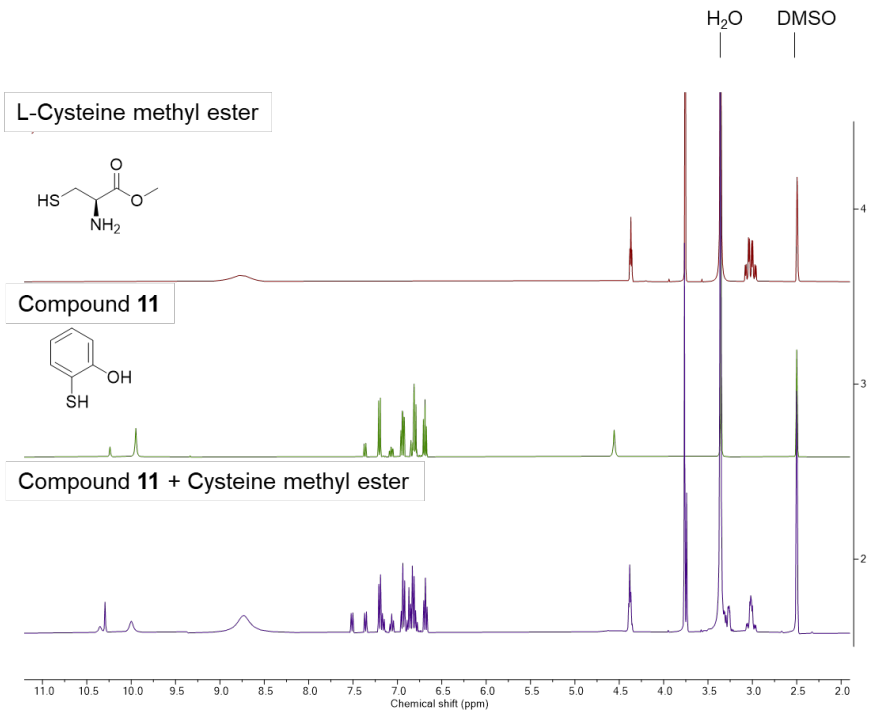


Figure S3 (continued). ^{1}H NMR spectra of L-cysteine methyl ester (*top*), the proposed thioamide, thiourea, and thiocarbamate compounds (*middle*), and 1:1 molar mixture of the compounds and L-cysteine methyl ester after 24 h incubation (*bottom*).

6. Single Crystal X-ray Diffraction

Suitable crystals of [($Tp^{Ph,Me}$)Zn(**MBP**)] ($Tp^{Ph,Me}$ = hydrotris(3,5-phenylmethylpyrazolyl)borate) were selected and data was collected at 100 K on a Bruker APEX-II Ultra diffractometer with a Mo-Kα Microfocus Rotating Anode and a APEX-II CCD area detector or a Bruker Kappa diffractometer equipped with a Bruker X8 APEX II Mo sealed tube and a Bruker APEX-II CCD. The data were integrated using the Bruker SAINT Software program and scaled using the SADABS software program. The structure was solved with the ShelXT[8] structure solution program using direct methods and refined with the XL[9] refinement package using least squares minimization using Olex2.[10] The crystal data files were deposited into the Cambridge Crystallographic Data Centre (CCDC).

Table S2. Selected bond lengths and angles of [$Tp^{Ph,Me}$Zn(**MBP**)] complexes.

*Previously reported structure. Refcodes (entry ID) for the reported compounds are listed below.

	C−S (Å)	Adjacent C-N (Å)	Zn-S (Å)
1	1.7168 (3)	1.313 (2)	2.2831(4)
2	1.733 (2)	1.318 (3)	2.2438(7)
3	1.6908 (18)	1.364 (2) 1.357 (2)	- -
4	1.7284(16)	1.302 (2)	2.2915(4)
5	1.7654 (15)	1.3834 (19)	2.2513(4)
7	1.723 (11) 1.741 (14)	1.296 (15) 1.319 (16)	2.267 (3) 2.275 (3)
8* (LUZVEH)	1.754	1.340	2.351
9* (BIBDEW)	1.711 1.732 1.736	-	2.287 2.297 2.329
10* (BIBDIA)	1.707	-	2.340
11* (KEXPUZ)	1.770 1.777	-	2.279 2.248

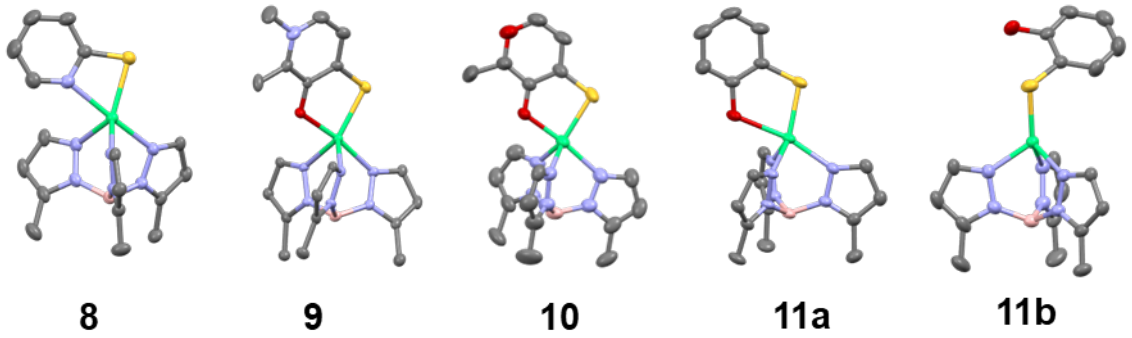


Figure S4. Reported crystal structure of [$Tp^{Ph,Me}$Zn(**MBP**)] complexes (ORTEP, 50% probability ellipsoids). Hydrogen atoms and phenyl groups from the $Tp^{Ph,Me}$ ligand were removed for clarity. Color scheme: carbon = gray, nitrogen = blue, oxygen = red, sulfur = yellow, boron = pink, and zinc = green.

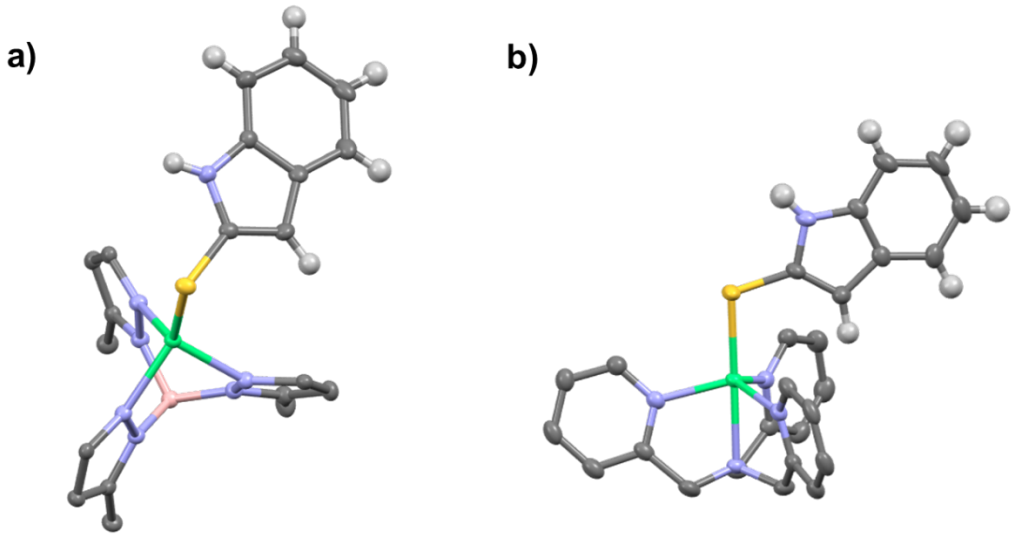


Figure S5. Crystal structure of a) [($Tp^{Ph,Me}$)Zn(**5**)] b) [Zn(TPA)(**5**)]BPh_4 complexes (ORTEP, 50% probability ellipsoids). Hydrogen atoms and phenyl groups from the $Tp^{Ph,Me}$ ligand were removed for clarity. Color scheme: carbon = gray, nitrogen = blue, sulfur = yellow, boron = pink, and zinc = green.

Table S3. Crystal data and structure refinement for [($Tp^{Ph,Me}$)Zn(**MBP**)] complexes.

Compound	[($Tp^{Ph,Me}$)Zn(**1**)]	[($Tp^{Ph,Me}$)Zn(**2**)]
Identification code	2211573	2211575
Empirical formula	$C_{37}H_{32}BN_7OSZn$	$C_{38}H_{35}BN_8SZn$
Formula weight	698.93	711.98
Temperature/K	100	100
Crystal system	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/c$
a/Å	16.0769(5)	13.2565(10)
b/Å	12.0606(4)	16.8902(11)
c/Å	17.4588(5)	16.1386(13)
α/°	90	90
β/°	101.3210(10)	107.479(2)
γ/°	90	90
Volume/Å^3	3319.34(18)	3446.7(4)
Z	4	4
ρ_{calc}g/cm^3	1.399	1.372
μ/mm^{-1}	0.845	0.814
F(000)	1448	1480
Crystal size/mm^3	0.1 × 0.06 × 0.06	0.1 × 0.1 × 0.1
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.252 to 52.74	4.252 to 52.862
Index ranges	-20 ≤ h ≤ 20, -13 ≤ k ≤ 15, -21 ≤ l ≤ 21	-16 ≤ h ≤ 16, -20 ≤ k ≤ 21, -20 ≤ l ≤ 20
Reflections collected	81571	157697
Independent reflections	6749 [R_{int} = 0.0739, R_{sigma} = 0.0290]	7086 [R_{int} = 0.1189, R_{sigma} = 0.0407]
Data/restraints/parameters	6749/0/436	7086/0/446
Goodness-of-fit on F^2	1.043	1.054
Final *R* indexes [I>=2σ (I)]	R_1 = 0.0281, wR_2 = 0.0658	R_1 = 0.0378, wR_2 = 0.0918
Final *R* indexes [all data]	R_1 = 0.0333, wR_2 = 0.0698	R_1 = 0.0496, wR_2 = 0.1006
Largest diff. peak/hole / e Å^{-3}	0.33/-0.27	0.32/-0.67

Table S3 (continued). Crystal data and structure refinement for [($Tp^{Ph,Me}$)Zn(**MBP**)] complexes.

Compound	[($Tp^{Ph,Me}$)Zn(**3**)]	[($Tp^{Ph,Me}$)Zn(**4**)]
Identification code	2211570	2211572
Empirical formula	$C_{43}H_{39}BN_8SZn$	$C_{37}H_{32}BN_7S_2Zn$
Formula weight	776.06	714.99
Temperature/K	100	100
Crystal system	triclinic	monoclinic
Space group	P-1	$P2_1/n$
a/Å	12.2415(9)	16.2876(11)
b/Å	12.4061(9)	12.0580(8)
c/Å	14.3641(10)	17.5877(12)
α/°	84.4340(10)	90
β/°	67.5970(10)	101.4370(10)
γ/°	68.4550(10)	90
Volume/Å^3	1873.6(2)	3385.6(4)
Z	2	4
ρ_{calc}g/cm^3	1.376	1.403
μ/mm^{-1}	0.755	0.888
F(000)	808	1480
Crystal size/mm^3	0.1 × 0.1 × 0.1	0.1 × 0.1 × 0.1
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.07 to 56.816	3.114 to 54.252
Index ranges	-16 ≤ h ≤ 16, -16 ≤ k ≤ 16, -17 ≤ l ≤ 19	-20 ≤ h ≤ 20, -14 ≤ k ≤ 15, -21 ≤ l ≤ 22
Reflections collected	24447	43464
Independent reflections	9398 [R_{int} = 0.0332, R_{sigma} = 0.0377]	7493 [R_{int} = 0.0409, R_{sigma} = 0.0263]
Data/restraints/parameters	9398/0/490	7493/0/436
Goodness-of-fit on F^2	1.016	1.03
Final *R* indexes [I>=2σ (I)]	R_1 = 0.0362, wR_2 = 0.1215	R_1 = 0.0271, wR_2 = 0.0650
Final *R* indexes [all data]	R_1 = 0.0429, wR_2 = 0.1288	R_1 = 0.0348, wR_2 = 0.0688
Largest diff. peak/hole / e Å^{-3}	0.81/-0.66	0.35/-0.19

Table S3 (continued). Crystal data and structure refinement for [($Tp^{Ph,Me}$)Zn(**MBP**)] complexes.

Compound	[($Tp^{Ph,Me}$)Zn(**5**)]	[($Tp^{Ph,Me}$)Zn(**7**)]	[Zn(TPA)(**5**)]BPh_4
Identification code	2211571	2211576	2211574
Empirical formula	$C_{38}H_{34}BN_7SZn$	$C_{42}H_{37}BN_7S_2Zn$	$C_{50}H_{44}BN_5SZn$
Formula weight	696.96	780.08	823.14
Temperature/K	273.15	273.15	273.15
Crystal system	triclinic	monoclinic	monoclinic
Space group	P-1	$P2_1/n$	$P2_1/c$
a/Å	12.0736(3)	16.078(2)	11.7128(7)
b/Å	12.1394(4)	20.118(3)	9.8315(5)
c/Å	12.7540(4)	23.900(4)	35.780(2)
α/°	103.5020(10)	90	90
β/°	112.5280(10)	98.684(4)	95.106(2)
γ/°	92.3260(10)	90	90
Volume/Å^3	1661.25(9)	7642(2)	4103.9(4)
Z	2	8	4
ρ_{calc}g/cm^3	1.393	1.356	1.332
μ/mm^{-1}	0.842	0.793	0.692
F(000)	724	3240	1720
Crystal size/mm^3	0.1 × 0.1 × 0.1	0.1 × 0.1 × 0.1	0.1 × 0.1 × 0.1
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.994 to 53.444	4.96 to 50.526	5.378 to 52.988
Index ranges	-15 ≤ h ≤ 15, -15 ≤ k ≤ 15, -15 ≤ l ≤ 16	-19 ≤ h ≤ 19, -24 ≤ k ≤ 23, -28 ≤ l ≤ 28	-14 ≤ h ≤ 14, -12 ≤ k ≤ 12, -44 ≤ l ≤ 44
Reflections collected	24789	176725	80358
Independent reflections	6997 [R_{int} = 0.0370, R_{sigma} = 0.0409]	13706 [R_{int} = 0.3439, R_{sigma} = 0.0969]	8464 [R_{int} = 0.0965, R_{sigma} = 0.0478]
Data/restraints/parameters	6997/0/436	13706/0/961	8464/0/531
Goodness-of-fit on F^2	1.053	1.152	1.028
Final *R* indexes [I>=2σ (I)]	R_1 = 0.0276, wR_2 = 0.0675	R_1 = 0.1325, wR_2 = 0.3099	R_1 = 0.0368, wR_2 = 0.0803
Final *R* indexes [all data]	R_1 = 0.0297, wR_2 = 0.0688	R_1 = 0.1546, wR_2 = 0.3223	R_1 = 0.0574, wR_2 = 0.0874
Largest diff. peak/hole / e Å^{-3}	0.32/-0.28	1.21/-1.69	0.37/-0.36

7. hCAII Assay

The plasmid for recombinant expression of hCAII with a T7 RNA polymerase promoter and ampicillin resistance gene (pACA) was a gift from Thomas R. Ward (U. Basel, Switzerland). The protein for activity assays was expressed in BL21 Escherichia coli cells and purified as reported previously.[11] The Zn content of expressed hCAII was measured by inductively coupled plasma-mass spectrometry (ICP-MS). The metal-to-protein (Zn:protein) molar ratio was determined to be 0.951±0.006, indicating the holo enzyme (fully metalated) is was isolated (data not shown). Assays were carried out in clear-bottom Costar 96-well plates (catalog # 07-200-706) with a total volume of 100 μL per well. The assay buffer was comprised of 50 mM HEPES pH 8.0 and 100 mM $NaSO_4$. MBIs were added from a 50 mM DMSO stock to a final concentration of 200 μM and incubated with hCAII (40 nM final concentration) for 15 min at room temperature. *p*-Nitrophenyl acetate was used as the substrate (500 μM final concentration), and the absorbance at 405 nM was monitored for 20 min at 1 min intervals using BioTek Synergy H4 plate reader. Percent inhibition was determined by comparing the activity of wells. A positive control (acetazolamide inhibitor, 50 μM final concentration) showed complete inhibition under the assay conditions described above. Dose-response curves were generated, analyzed, and fitted to obtain IC_{50} values of compounds using a concentration range of between 0.01 μM and 1000 μM depending on compound solubility.

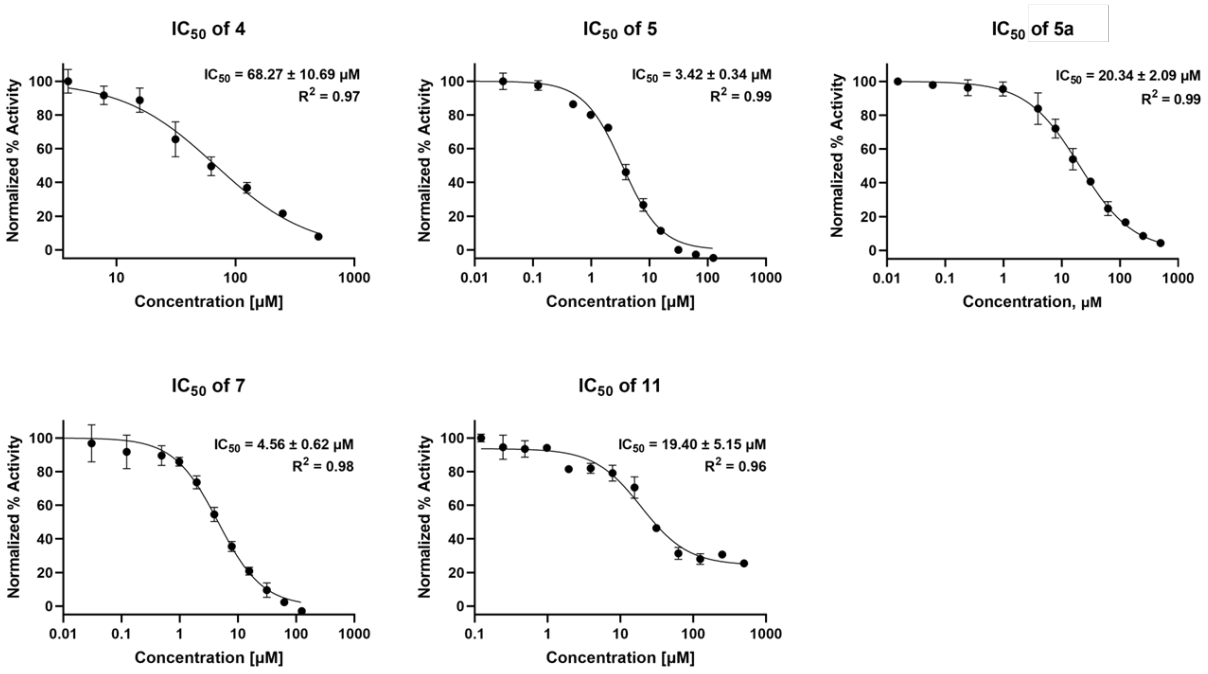


Figure S6. IC_{50} curves of compounds **4**, **5**, **5a**, **7**, and **11** against hCAII. The range of concentration of the compounds is between 0.01 and 1000 µM. IC_{50} values reported in µM with the 95% confidence interval indicated.

8. MMP-2 Assay

Human recombinant MMP-2 catalytic domain was purchased from ENZO Life Sciences (catalog # BML-SE237-0010). Assays were carried out in clear Costar 96-well, half-area, flat-bottom assay plates (catalog # 80-2404). Each well contained a total volume of 100 μL including buffer (50 mM MES, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5), MMP-2 (1.16 U), and the fragment solution (200 μM final concentration). After a 30 min incubation period at 37 °C, the reaction was initiated by the addition of 10 μL of fluorogenic MMP-2 substrate (4 μM final concentration, Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2·AcOH, ENZO Life Sciences, catalog # BML-P126-0001). Fluorescence was monitored at E_x/E_m=328/420 nm using BioTek Synergy H4 plate reader, and measurements were recorded every minute for 10 min. The rate of fluorescence increase was compared for samples versus negative controls (no inhibitor, arbitrarily set as 100% activity). A positive control (NNGH as inhibitor, 50 μM final concentration) showed complete inhibition under the assay conditions described above. Dose-response curves were generated, analyzed, and fitted to obtain IC_{50} values of compounds using a concentration range of between 1 μM and 1000 μM depending on compound solubility.

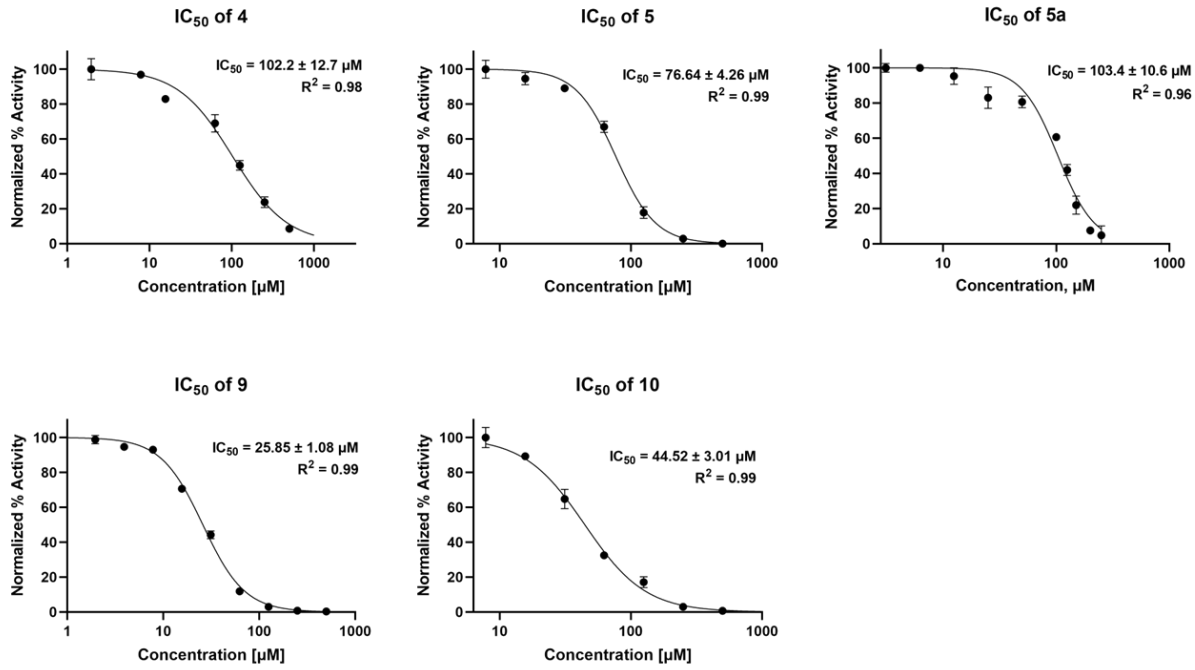


Figure S7. IC_{50} curves of compounds **4**, **5**, **5a**, **9**, and **10** against MMP-2. The range of concentration of the compounds is between 1 and 1000 µM. IC_{50} values reported in µM with the 95% confidence interval indicated.

9. hCAII Expression & Purification

Plasmids encoding human carbonic anhydrase isozyme II and containing a T7 RNA polymerase promoter and an ampicillin resistance gene (pACA) were a generous gift from Carol A. Fierke, University of Michigan Medical School. Plasmids were introduced into BL21(DE3) *Escherichia coli* cells via heat-shock (45 s at 42 °C) and incubated in sterile SOC medium for 1 h at 37 °C with gentle shaking. The cells were then placed onto agar plates containing 60 μg /mL ampicillin and 34 μg/mL chloramphenicol and incubated overnight at 37 °C. Single colonies were transferred to 30 mL of autoclaved LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) containing 100 μg /mL ampicillin and 34 μg/mL chloramphenicol and incubated overnight at 37 °C. Six pre-cultures were used to inoculate 6 L of autoclaved induction media (20 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, 0.36×M9 salts solution, 0.4% glucose, 60 μM $ZnSO_4$, 100 μg/mL ampicillin and 34 μg/mL chloramphenicol). Cells were shaken (250 rpm) in induction media at 37 °C until OD_{600} = 0.6 – 0.8. Addition of isopropyl-β-D-thiogalactopyranoside (IPTG, 250 μM final concentration) and $ZnSO_4$ (450 μM final concentration) induced protein expression and the temperature was lowered to 30 °C. The protease inhibitors phenylmethanesulfonyl fluoride (PMSF, 8 μg/mL) and *Nα-p*-tosyl-L-arginine methyl ester hydrochloride (TAME, 1 μg/mL) were added to the induction media after 3 h. The cells were shaken for an additional 3 h after addition of protease inhibitors (for a total of 6 h induction) and pelleted via centrifugation (4,400 rpm at 4 °C) for 15 min. Carbonic anhydrase expression was confirmed via SDS-PAGE and Coomassie staining. Cell paste was flash frozen and stored at -80 °C prior to lysis. Cell paste was thawed in batches on ice for 2 h and resuspended in an equal volume of lysis buffer (1% Triton-X, 200 μM ZnSO4, 2 mM DTT, 10-100 μg/mL DNAse-1, 1 mg/mL lysozyme, and 1% glycerol with three EDTA free protease inhibitor tablets (Roche) added prior to resuspension) to cell pellet. Cells

were lysed using a probe sonicator (Fisherbrand model 120) with cycles of 25 second pulses and 59 second rest at 60% amplitude. Cell debris was then pelleted by centrifugation (10,000 rpm at 4 °C) for 45 min. The supernatant was decanted from the pellet and dialyzed against 4 L of activity buffer (50 mM Tris-sulfate (pH 8.0) and 0.5 mM $ZnSO_4$). Cell lysates were slowly mixed with DEAE-Sephacel ion exchange resin equilibrated with activity buffer (50 mM Tris-sulfate (pH 8.0) and 0.5 mM $ZnSO_4$) and 1 mM DTT for 1 h at 4 °C. The mixture was filtered using a Nalgene filter flask (0.45 μm) and the resin was washed three times with activity buffer. The eluent was combined and dialyzed in activity buffer overnight at 4 °C. The protein is then purified by affinity chromatography with 25 mL of 4-aminomethylbenzene sulfonamide agarose resin packed in a XK 16/20 column. Briefly, the column was equilibrated with 5 CV of activity buffer (50 mM Tris-sulfate (pH 8.0) and 0.5 mM $ZnSO_4$) or until absorbance reached a steady baseline. Dialyzed protein was loaded to the column from a 150 mL Superloop at a flow rate of 0.5 mL/min. The column was then washed with 5 CV of wash buffer (50 mM Na_2PO_4H, 50 mM KSCN, and 25 mM Tris at pH 8.8). The bound protein was then eluted with 10 CV of elution buffer (200 mM KSCN and 50 mM Na_2PO_4H at pH 5.6) at a flow rate of 2 mL/min collecting 2 mL fractions. hCAII eluted between fractions 13-41. SDS-PAGE analysis showed a band corresponding to hCAII running at ~29 kDa with only one small impurity at ~50 kDa. Pure fractions were pooled and dialyzed against 4 L of activity buffer for 24 h at 4 °C. A portion of this protein was then either concentrated to 80 nM for activity assays or 20 mg/ml for crystallography and flash frozen. The remaining protein was dialyzed against 4 L of DI water for 24 h at 4 °C and mQ water for 24 h at 4 °C and then lyophilized. All protein samples are stored at -80 °C.

10. hCAII Crystallography

Crystals of hCAII were obtained by the hanging-drop vapor diffusion method using 24-well pre-greased plates (Hampton) with siliconized glass slides (Hampton). The protein solution consisted of 20 mg/mL hCAII and 1 mM *p*-chloromercuribenzoic acid in 50 mM Tris-SO_4 (pH 8.0) supplemented with 0.5 mM ZnSO4. Protein solution was incubated on ice with 1 mM compound for 1 h prior to setting the crystallization drops. The precipitant solution contained 2.6-3.0 M $(NH_4)_2SO_4$ in 50 mM Tris-SO_4 (pH 8). Drops consisting of 3 µL of protein solution and 2.5-4.0 µL of precipitant solution were equilibrated at room temperature against 500 µL of precipitant solution. Colorless crystals roughly 0.3×0.3×0.3 mm in size appeared after 7-10 days. Crystals formation occurred spontaneously, but streak crystal seeding with natural or artificial cat whiskers produced larger and more abundant single crystals. Crystals soaked by transferring crystals to a 5 µL drop of the corresponding reservoir solution that was supplemented with 5 µL of a 50mM DMSO compound stock for 4-48 h. Collected crystals were cryoprotected with perfluoroether (Hampton) prior to flash freezing and were stored in liquid nitrogen until data collection. All structures include a 4-mercuribenzoic acid ligand bound to Cys206. X-ray diffraction studies were carried out on a Bruker Microfocus Rotating Anode (MicroStar FR- 592) X-ray generator with a Bruker APEX II CCD detector at wavelength 1.54178 Å. Data was integrated, scaled, and merged using the Bruker APEX3 software package (Bruker, 2017). All crystals belong to the monoclinic space group $P2_1$. The data were phased by molecular replacement using a previously reported hCAII structure (PDB: 4E49), with water molecules removed, using PHASER. All structures were refined with Phenix version 1.19.2 and model building and visualized using Coot version 0.9.5.

Table S4. X-ray crystallographic data collection and refinement statistics.

Compound	**4**	**7**
PDB	8FAL	8FAU
Resolution range	35.54- 1.37 (1.42-1.37)	35.53- 1.44 (1.49-1.44)
Space group	$P2_1$	$P2_1$
Cell dimensions a, b, c, (Å) α, β, γ (°)	42.009 41.143 71.915 90 104.556 90	42.144 41.316 71.919 90 104.541 90
Unique reflections	88780	75411
Completeness (%)	99	92.4
Mean I/sigma(I)	11 (1.5)	15 (2.2)
R-merge	0.060 (0.355)	0.045 (0.225)
R-measured	0.067 (0.476)	0.050 (0.312)
R-work	0.1903 (0.2997)	0.1756 (0.2544)
R-free	0.2224 (0.2772)	0.2086 (0.3072)
RMS(bonds)	0.012	0.007
RMS(angles)	1.25	1.02
Ramachandran favored (%)	95.69	96.06
Ramachandran outliers (%)	0	0
Average B-factor	18.1	19.6
Redundancy	4	4.1
CC1/2	0.999 (0.803)	0.999 (0.892)

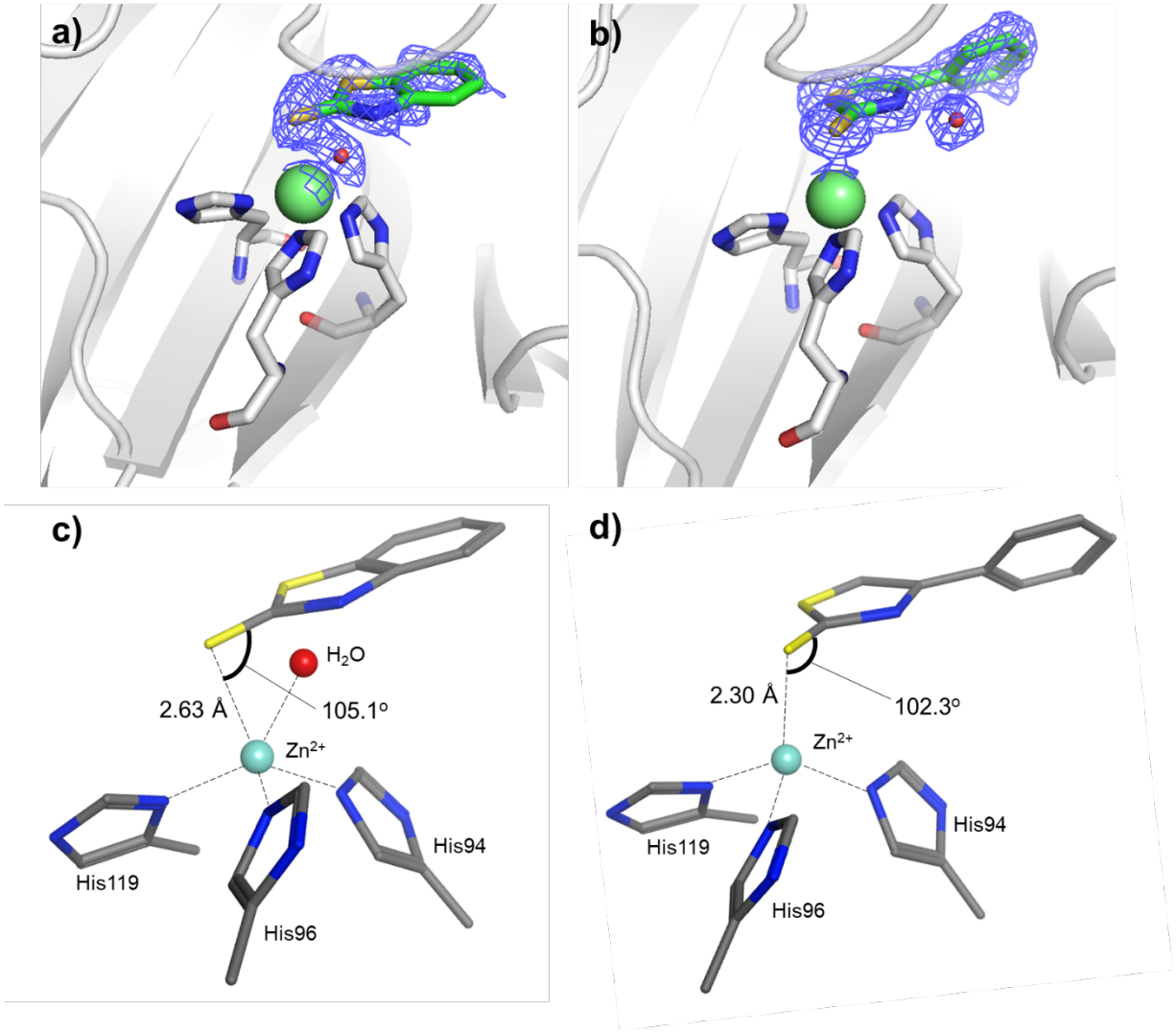


Figure S8. Co-crystal structures of hCAII with a) **4** and b) **7**. Zn(II) ion and water molecules are shown as green and red spheres, respectively. Residues coordinating the Zn^{2+} ion is shown in sticks. Atom colors are: carbon (green for compound, gray for protein), oxygen (red), nitrogen (blue) and sulfur (yellow). Details of the coordination modes of c) **4** and d) **7** bound to the hCAII active site. Zn(II) coordination is represented by dashed lines.

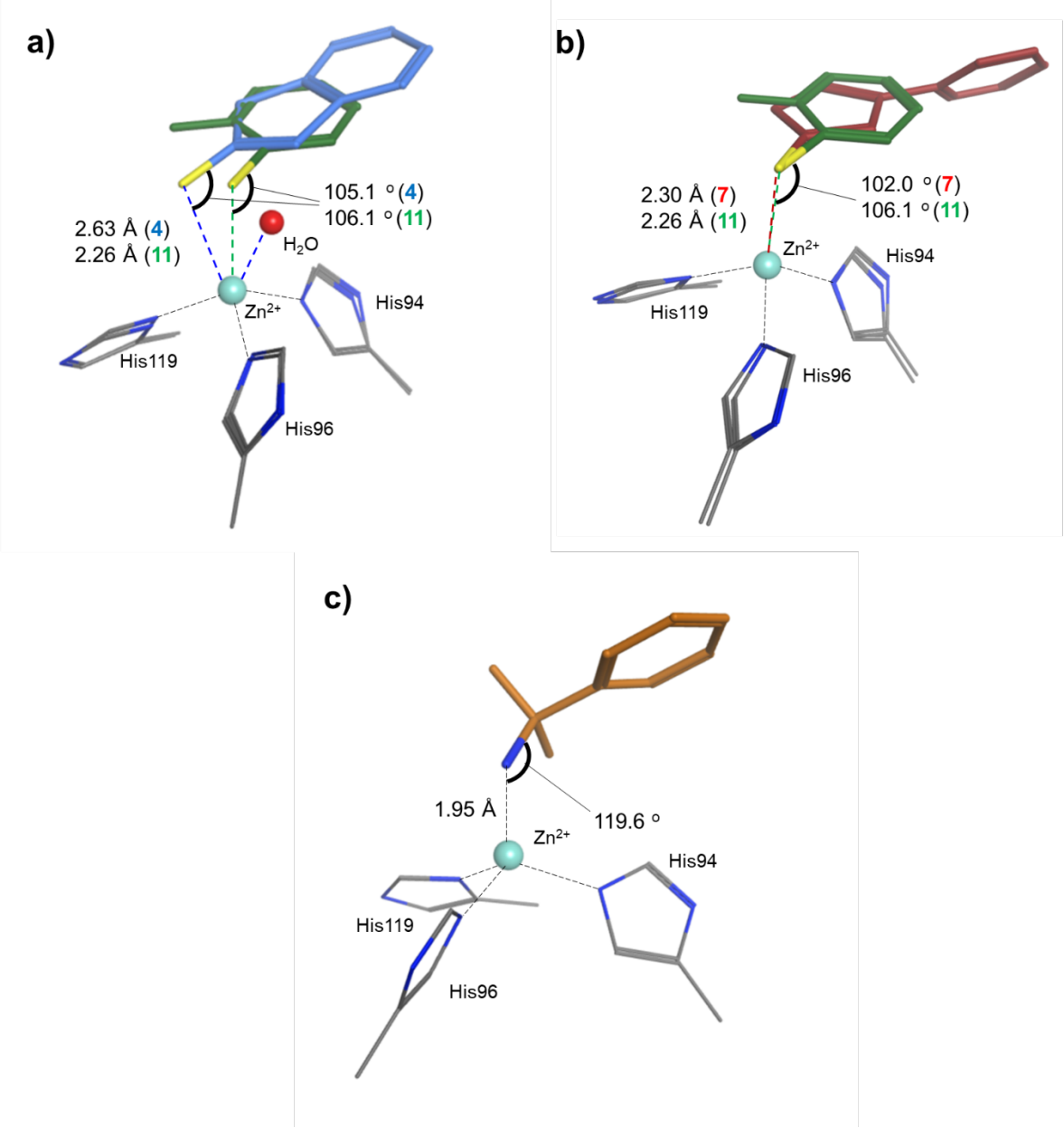


Figure S9. Structural superposition between compounds a) **4** (blue) and **11** (green) (PDB: 2OSM), b) **7** (red) and **11** (green) bound to the hCAII active site. c) Coordination mode of benzenesulfonamide (orange) bound to the hCAII active site (PDB: 2WEJ). Atoms participating in the Zn(II) coordination are colored: sulfur (yellow) and nitrogen (blue). Zn(II)-ligand bonds are represented by dashed lines.

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