Supplemental Data

Title: "Synthetic metallochaperone ZMC1 rescues mutant p53 conformation by transporting zinc into cells as an ionophore"

Authors: Adam R. Blanden, Xin Yu, Aaron J. Wolfe, John A. Gilleran, David J. Augeri, Ryan S. O'Dell, Eric C. Olson, S. David Kimball, Thomas J. Emge, Liviu Movileanu, Darren R. Carpizo, and Stewart N. Loh

Journal Title: Molecular Pharmacology.

Supplemental Data Contains:

SUPPLEMENTAL METHODS

Liposome Preparation

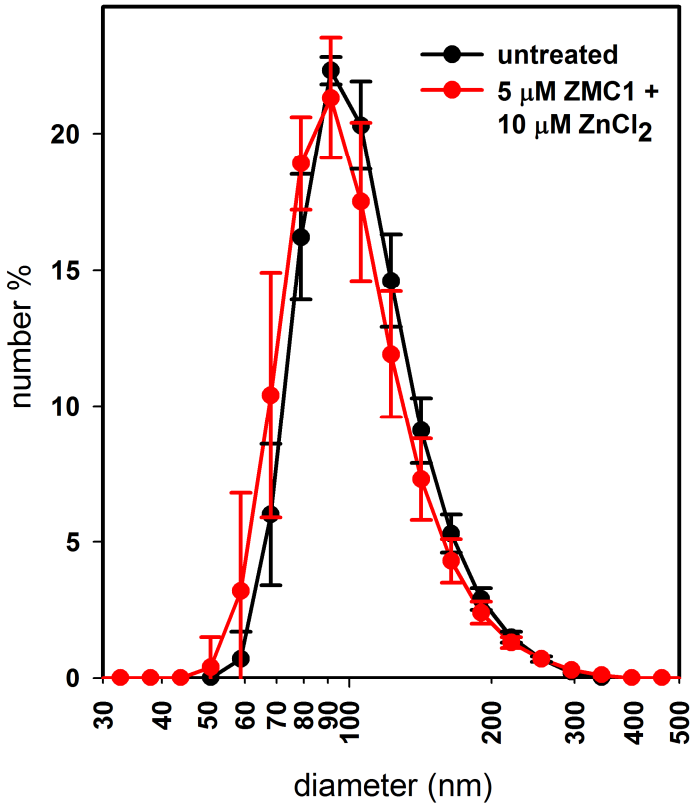
25 mg of 1,2-diphytanoyl-*sn*-glycero-3-phosohocholine (Avanti Polar Lipids, Alabaster, USA) was dissolved in chloroform. Solvent was evaporated in a round bottom flask by a steady stream of nitrogen. The flask was further dried in a vacuum overnight. The resulting film was hydrated with buffer (50 mM Tris pH 7.2, 100 mM NaCl) and fluorophore. Calcein and RhodZin-3 were

dissolved in 1 mL buffer to final concentrations of 10 mM and 10 μM, respectively (Hee Dong, 2005). The lipid suspensions were extruded 15 times through a 100-nm polycarbonate filter using Avanti-mini extruder (MacDonald *et al.*, 1991). The solution was subjected to 5 cycles of freezing and thawing using liquid nitrogen, followed by an additional 15 cycles of extrusion (Hope *et al.*, 1985; Hee Dong, 2005). The fluorophore encapsulated liposomes were separated from free fluorophore by elution over a buffer equilibrated Sephadex-G25 column, and diluted to an OD_{600} = 0.06 for use.

Synthesis and Crystallization of [Zn(ZMC1)$_2$] complex

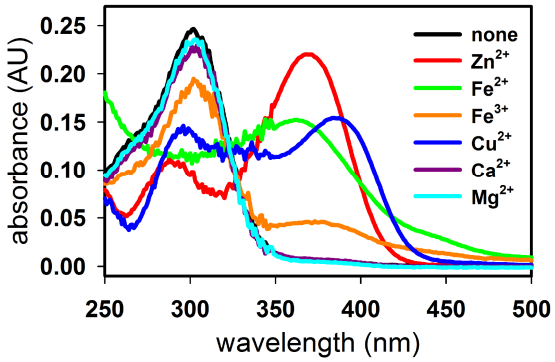
To a suspension of ZMC1 (190.8 mg, .814 mmol, 1 equiv.) in EtOH (20 ml) was added $ZnCl_2$ (55.5 mg, 0.407 mmol, 0.5 equiv.). After 5 minutes, TEA (0.80 ml, excess) was added and the mixture was heated for 2 hours at reflux under nitrogen. Upon cooling to ambient temperature, a solid precipitated that was collected by filtration and washed with EtOH followed by Et_2O. The solids were dried under high vacuum to give [Zn(ZMC1)$_2$] (215 mg, 0.404 mmol, 99%) as a bright yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6, C2 symmetric complex, protons are doubled to account for two ZMC1 molecules in complex δ 2.26 (overlapping tt, J = 7.48Hz, 7.40Hz, 4H), 2.58 (s, 6H), 4.05 (m, 8H), 7.29 (dd, J = 7.28 Hz, 5.60 Hz, 2H), 7.75 (m, 4H), 7.88 (dt, J = 8.04 Hz, 1.52 Hz, 2H). Slow evaporation of [Zn(ZMC1)$_2$]from a 1:1 mixture of DCM/MeOH afforded yellow crystals that were suitable for X-ray crystallography.

SUPPLEMENTAL FIGURES AND TABLES



Supplemental Figure 1. Liposome size distribution measured by Dynamic Light Scattering.

Points are mean ± SD for 6 sequential measurements. Cumulant mean diameters are 141.59 ± 1.73 nm (untreated) and 141.44 ± 0.63 nm (10 μM $ZnCl_2$ + 5 μM ZMC1) (mean ± SEM, n=6).



Supplemental Figure 2. Absorbance spectra of ZMC1-metal ion complexes. Concentrations were 100 μM metal salt and 10 μM ZMC1. Spectra are blanked against the corresponding metal salt solution.

Supplemental Table 1. Crystal data and structure refinement for [Zn(ZMC1)$_2$].

Identification code	[Zn(ZMC1)$_2$]	
Empirical formula	C23 H28.5 Cl1.5 N8 O0.25 S2 Zn	
Formula weight	603.70	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C 2/c	
Unit cell dimensions	a = 14.2570(16) Å	α= 90°.
	b = 11.4474(13) Å	β= 108.496(2)°.
	c = 17.1560(19) Å	γ = 90°.
Volume	2655.3(5) Å^3	
Z	4	
Density (calculated)	1.510 Mg/m^3	
Absorption coefficient	1.264 mm^{-1}	
F(000)	1248	
Crystal size	0.39 x 0.09 x 0.05 mm^3	
Theta range for data collection	2.331 to 30.546°.	
Index ranges	-20<=h<=20, -16<=k<=16, -24<=l<=24	
Reflections collected	14552	
Independent reflections	4036 [R(int) = 0.0519]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7461 and 0.6266	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4036 / 1 / 174	
Goodness-of-fit on F^2	1.049	
Final R indices [I>2sigma(I)]	R1 = 0.0494, wR2 = 0.1063	
R indices (all data)	R1 = 0.0666, wR2 = 0.1129	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.740 and -0.591 e.Å^{-3}	

Supplemental Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for [Zn(ZMC1)$_2$]. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Zn(1)	0	6167(1)	2500	11(1)
S(1)	1024(1)	7548(1)	3501(1)	17(1)
N(1)	-1128(2)	4781(2)	2082(1)	14(1)
N(2)	-585(1)	5913(2)	3487(1)	11(1)
N(3)	-262(2)	6509(2)	4214(1)	14(1)
N(4)	764(2)	7914(2)	4924(1)	23(1)
C(1)	-1418(2)	4299(2)	1336(2)	19(1)
C(2)	-2215(2)	3538(2)	1084(2)	25(1)
C(3)	-2709(2)	3244(2)	1630(2)	25(1)
C(4)	-2404(2)	3737(2)	2407(2)	20(1)
C(5)	-1609(2)	4520(2)	2619(2)	13(1)
C(6)	-1262(2)	5127(2)	3421(1)	13(1)
C(7)	-1660(2)	4852(2)	4107(2)	22(1)
C(8)	455(2)	7280(2)	4233(2)	15(1)
C(9)	1448(2)	8893(2)	5169(2)	24(1)
C(10)	1069(3)	9072(3)	5909(2)	34(1)
C(11)	354(2)	8038(3)	5601(2)	26(1)
C(12A)	0	-186(4)	-2500	25(1)
Cl(1A)	1008(1)	-1064(1)	-1946(1)	31(1)
O(1B)	-241(15)	-931(19)	-2615(14)	50
C(12B)	580(20)	-460(30)	-1889(16)	50

Supplemental Table 3. Bond lengths [Å] and angles [°] for [Zn(ZMC1)$_2$].

Zn(1)-N(2)#1	2.1312(18)	C(5)-C(6)	1.479(3)
Zn(1)-N(2)	2.1312(18)	C(6)-C(7)	1.494(3)
Zn(1)-N(1)	2.210(2)	C(7)-H(7A)	0.9600
Zn(1)-N(1)#1	2.210(2)	C(7)-H(7B)	0.9600
Zn(1)-S(1)	2.4474(7)	C(7)-H(7C)	0.9600
Zn(1)-S(1)#1	2.4474(7)	C(9)-C(10)	1.543(4)
S(1)-C(8)	1.726(2)	C(9)-H(9A)	0.9700
N(1)-C(1)	1.333(3)	C(9)-H(9B)	0.9700
N(1)-C(5)	1.346(3)	C(10)-C(11)	1.543(4)
N(2)-C(6)	1.298(3)	C(10)-H(10A)	0.9700
N(2)-N(3)	1.367(3)	C(10)-H(10B)	0.9700
N(3)-C(8)	1.342(3)	C(11)-H(11A)	0.9700
N(4)-C(8)	1.340(3)	C(11)-H(11B)	0.9700
N(4)-C(9)	1.458(3)	C(12A)-Cl(1A)#2	1.764(3)
N(4)-C(11)	1.464(3)	C(12A)-Cl(1A)	1.764(3)
C(1)-C(2)	1.388(4)	C(12A)-H(12A)	0.9700
C(1)-H(1)	0.9300	C(12A)-H(12B)	0.9700
C(2)-C(3)	1.382(4)	O(1B)-C(12B)	1.510(10)
C(2)-H(2)	0.9300	O(1B)-H(1B)	0.8200
C(3)-C(4)	1.384(4)	C(12B)-H(12C)	0.9600
C(3)-H(3)	0.9300	C(12B)-H(12D)	0.9600
C(4)-C(5)	1.400(3)	C(12B)-H(12E)	0.9600
C(4)-H(4)	0.9300		

N(2)#1-Zn(1)-N(2)	164.32(10)	N(2)#1-Zn(1)-S(1)#1	80.08(5)
N(2)#1-Zn(1)-N(1)	94.12(7)	N(2)-Zn(1)-S(1)#1	110.40(5)
N(2)-Zn(1)-N(1)	74.48(7)	N(1)-Zn(1)-S(1)#1	91.46(6)
N(2)#1-Zn(1)-N(1)#1	74.48(7)	N(1)#1-Zn(1)-S(1)#1	154.46(5)
N(2)-Zn(1)-N(1)#1	94.12(7)	S(1)-Zn(1)-S(1)#1	99.48(3)
N(1)-Zn(1)-N(1)#1	88.25(11)	C(8)-S(1)-Zn(1)	94.68(9)
N(2)#1-Zn(1)-S(1)	110.40(5)	C(1)-N(1)-C(5)	119.7(2)
N(2)-Zn(1)-S(1)	80.08(5)	C(1)-N(1)-Zn(1)	125.53(17)
N(1)-Zn(1)-S(1)	154.46(5)	C(5)-N(1)-Zn(1)	114.40(16)
N(1)#1-Zn(1)-S(1)	91.46(6)	C(6)-N(2)-N(3)	116.85(19)

C(6)-N(2)-Zn(1)	119.55(15)	N(3)-C(8)-S(1)	129.03(18)
N(3)-N(2)-Zn(1)	123.56(14)	N(4)-C(9)-C(10)	87.9(2)
C(8)-N(3)-N(2)	112.58(19)	N(4)-C(9)-H(9A)	114.0
C(8)-N(4)-C(9)	132.4(2)	C(10)-C(9)-H(9A)	114.0
C(8)-N(4)-C(11)	130.7(2)	N(4)-C(9)-H(9B)	114.0
C(9)-N(4)-C(11)	95.5(2)	C(10)-C(9)-H(9B)	114.0
N(1)-C(1)-C(2)	122.3(2)	H(9A)-C(9)-H(9B)	111.2
N(1)-C(1)-H(1)	118.9	C(11)-C(10)-C(9)	89.0(2)
C(2)-C(1)-H(1)	118.9	C(11)-C(10)-H(10A)	113.8
C(3)-C(2)-C(1)	119.0(3)	C(9)-C(10)-H(10A)	113.8
C(3)-C(2)-H(2)	120.5	C(11)-C(10)-H(10B)	113.8
C(1)-C(2)-H(2)	120.5	C(9)-C(10)-H(10B)	113.8
C(2)-C(3)-C(4)	118.8(2)	H(10A)-C(10)-H(10B)	111.0
C(2)-C(3)-H(3)	120.6	N(4)-C(11)-C(10)	87.6(2)
C(4)-C(3)-H(3)	120.6	N(4)-C(11)-H(11A)	114.0
C(3)-C(4)-C(5)	119.5(2)	C(10)-C(11)-H(11A)	114.0
C(3)-C(4)-H(4)	120.2	N(4)-C(11)-H(11B)	114.0
C(5)-C(4)-H(4)	120.2	C(10)-C(11)-H(11B)	114.0
N(1)-C(5)-C(4)	120.7(2)	H(11A)-C(11)-H(11B)	111.2
N(1)-C(5)-C(6)	116.1(2)	Cl(1A)#2-C(12A)-Cl(1A)	110.6(3)
C(4)-C(5)-C(6)	123.2(2)	Cl(1A)#2-C(12A)-H(12A)	109.5
N(2)-C(6)-C(5)	115.2(2)	Cl(1A)-C(12A)-H(12A)	109.5
N(2)-C(6)-C(7)	122.4(2)	Cl(1A)#2-C(12A)-H(12B)	109.5
C(5)-C(6)-C(7)	122.4(2)	Cl(1A)-C(12A)-H(12B)	109.5
C(6)-C(7)-H(7A)	109.5	H(12A)-C(12A)-H(12B)	108.1
C(6)-C(7)-H(7B)	109.5	C(12B)-O(1B)-H(1B)	109.5
H(7A)-C(7)-H(7B)	109.5	O(1B)-C(12B)-H(12C)	109.5
C(6)-C(7)-H(7C)	109.5	O(1B)-C(12B)-H(12D)	109.5
H(7A)-C(7)-H(7C)	109.5	H(12C)-C(12B)-H(12D)	109.5
H(7B)-C(7)-H(7C)	109.5	O(1B)-C(12B)-H(12E)	109.5
N(4)-C(8)-N(3)	114.3(2)	H(12C)-C(12B)-H(12E)	109.5
N(4)-C(8)-S(1)	116.65(19)	H(12D)-C(12B)-H(12E)	109.5

Symmetry transformations used to generate equivalent atoms: #1 -x,y,-z+1/2 #2 -x,y,-z-1/2

Supplemental Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for $[Zn(ZMC1)_2]$. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Zn(1)	12(1)	12(1)	10(1)	0	6(1)	0
S(1)	18(1)	19(1)	14(1)	-3(1)	7(1)	-7(1)
N(1)	15(1)	13(1)	13(1)	-1(1)	3(1)	-2(1)
N(2)	11(1)	12(1)	11(1)	1(1)	4(1)	1(1)
N(3)	16(1)	15(1)	10(1)	-2(1)	4(1)	-1(1)
N(4)	31(1)	26(1)	16(1)	-9(1)	12(1)	-12(1)
C(1)	25(1)	18(1)	15(1)	-3(1)	8(1)	-5(1)
C(2)	30(2)	23(1)	20(1)	-8(1)	4(1)	-8(1)
C(3)	26(1)	20(1)	28(2)	-6(1)	6(1)	-10(1)
C(4)	19(1)	18(1)	25(1)	1(1)	10(1)	-6(1)
C(5)	14(1)	11(1)	15(1)	2(1)	6(1)	1(1)
C(6)	14(1)	13(1)	12(1)	1(1)	5(1)	1(1)
C(7)	24(1)	25(1)	19(1)	-2(1)	12(1)	-8(1)
C(8)	16(1)	14(1)	13(1)	0(1)	4(1)	1(1)
C(9)	28(1)	21(1)	21(1)	-7(1)	7(1)	-7(1)
C(10)	49(2)	33(2)	25(2)	-16(1)	16(2)	-14(1)
C(11)	36(2)	28(2)	18(1)	-8(1)	14(1)	-7(1)
C(12A)	29(3)	11(2)	33(3)	0	9(2)	0
Cl(1A)	27(1)	34(1)	31(1)	9(1)	6(1)	5(1)

Supplemental Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for [Zn(ZMC1)$_2$].

	x	y	z	U(eq)
H(1)	-1074	4479	973	23
H(2)	-2413	3230	554	30
H(3)	-3237	2724	1479	30
H(4)	-2725	3549	2785	24
H(7A)	-1126	4640	4588	32
H(7B)	-2119	4214	3950	32
H(7C)	-1993	5526	4225	32
H(9A)	1300	9537	4781	28
H(9B)	2139	8673	5319	28
H(10A)	1567	8935	6437	41
H(10B)	740	9815	5904	41
H(11A)	482	7376	5973	31
H(11B)	-340	8254	5428	31
H(12A)	-198	311	-2122	30
H(12B)	198	311	-2878	30
H(1B)	-251	-1646	-2591	75
H(12C)	1072	-98	-2079	75
H(12D)	872	-1094	-1525	75
H(12E)	313	101	-1602	75

Supplemental Table 6. Torsion angles [°] for [Zn(ZMC1)$_2$].

C(6)-N(2)-N(3)-C(8)	178.3(2)	C(4)-C(5)-C(6)-N(2)	-173.1(2)
Zn(1)-N(2)-N(3)-C(8)	0.7(3)	N(1)-C(5)-C(6)-C(7)	-174.5(2)
C(5)-N(1)-C(1)-C(2)	0.8(4)	C(4)-C(5)-C(6)-C(7)	7.0(4)
Zn(1)-N(1)-C(1)-C(2)	-171.8(2)	C(9)-N(4)-C(8)-N(3)	-173.9(3)
N(1)-C(1)-C(2)-C(3)	-1.8(4)	C(11)-N(4)-C(8)-N(3)	-10.7(4)
C(1)-C(2)-C(3)-C(4)	1.3(4)	C(9)-N(4)-C(8)-S(1)	6.5(4)
C(2)-C(3)-C(4)-C(5)	0.2(4)	C(11)-N(4)-C(8)-S(1)	169.6(2)
C(1)-N(1)-C(5)-C(4)	0.8(4)	N(2)-N(3)-C(8)-N(4)	177.5(2)
Zn(1)-N(1)-C(5)-C(4)	174.12(18)	N(2)-N(3)-C(8)-S(1)	-2.9(3)
C(1)-N(1)-C(5)-C(6)	-177.8(2)	Zn(1)-S(1)-C(8)-N(4)	-177.3(2)
Zn(1)-N(1)-C(5)-C(6)	-4.5(3)	Zn(1)-S(1)-C(8)-N(3)	3.1(2)
C(3)-C(4)-C(5)-N(1)	-1.2(4)	C(8)-N(4)-C(9)-C(10)	168.2(3)
C(3)-C(4)-C(5)-C(6)	177.3(2)	C(11)-N(4)-C(9)-C(10)	0.9(2)
N(3)-N(2)-C(6)-C(5)	178.62(19)	N(4)-C(9)-C(10)-C(11)	-0.9(2)
Zn(1)-N(2)-C(6)-C(5)	-3.7(3)	C(8)-N(4)-C(11)-C(10)	-168.5(3)
N(3)-N(2)-C(6)-C(7)	-1.5(3)	C(9)-N(4)-C(11)-C(10)	-0.9(2)
Zn(1)-N(2)-C(6)-C(7)	176.21(18)	C(9)-C(10)-C(11)-N(4)	0.9(2)
N(1)-C(5)-C(6)-N(2)	5.4(3)		

Symmetry transformations used to generate equivalent atoms: #1 -x,y,-z+1/2 #2 -x,y,-z-1/2

LEGEND FOR SUPPLEMENTAL MOVIES 1-10

Supplemental Movies 1-10. Zn^{2+} Import Measurement by Time Lapse Microscopy. HEK293 (S1-S5) and TOV112D (S6-S10) were incubated with 1 μM FluoZin-3-AM at 37 °C for 40 min, exchanged into Ca^{2+}- and Mg^{2+}-free EBSS, and monitored by confocal microscopy. A baseline was recorded for ~90 s, at which point the cells were exchanged into EBSS containing the indicated treatments. 50 μM PYR/$ZnCl_2$ and 100 μM TPEN were used as positive controls and negative controls, respectively, in movies S1 and S6. DMSO (0.2%) was used as a vehicle control. Images were captured using a 10x (NA = 0.3) air objective. Quantification seen in Fig. 3A. The movies are: **1)** HEK293, 50 μM PYR/$ZnCl_2$ then 100 μM TPEN. **2)** HEK293, 1 μM ZMC1. **3)** HEK293, 10 μM $ZnCl_2$ + 0.2% DMSO. **4)** HEK293, 1 μM ZMC1 + 10 μM $ZnCl_2$. **5)** HEK293, 1 μM ZMC1 + 1 μM $ZnCl_2$. **6)** TOV112D, 50 μM PYR/$ZnCl_2$ then 100 μM TPEN. **7)** TOV112D, 1 μM ZMC1. **8)** TOV112D, 10 μM $ZnCl_2$ + 0.2% DMSO. **9)** TOV112D, 1 μM ZMC1 + 10 μM $ZnCl_2$. **10)** TOV112D, 1 μM ZMC1 + 1 μM $ZnCl_2$. We recommend VLC media player for viewing movies available at: http://www.videolan.org/vlc/index.html.

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