Supplementary Information (SI) for Dalton Transactions.

In Medio Stat Virtus: Asymmetric Salphen Metal Complexes with Improved Biological Properties

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Supporting Information

1. SYNTHESIS AND CHARACTERIZATION

Materials. Chemicals and solvents of commercial grade (Sigma Aldrich and Thermo Fisher Scientific) were used directly from freshly opened containers without further purification. NMR spectra were recorded on Bruker AC-E series 300 MHz and 400 MHz spectrometers. Mass spectrometry experiments were conducted using an Agilent 6540 QTOF LC/MS system and Thermo Fisher Exploris Orbitrap 120. IR analysis was carried out by FT-IR Bruker Vertex 70v spectrometer. Elemental analyses were performed using an Eurovector EA3000 elemental analyzer at the Microanalytical Laboratory of the University of Vienna. X-ray intensity data measured on STOE STADIVARI diffractometer equipped with multilayer monochromator, Mo K/α and Cu K/α micro focus sealed tube and Oxford cooling system at the University of Vienna. Buffers were prepared using MilliQ water, and the final pH values were measured with a CREASON pH meter-GLP.

L1. *Synthesis in solution*: a solution of 1,2-phenylendiamine (108.14 mg, 1 mmol) and 3,5-dichlorosalicylaldehyde (191.01 mg, 1 mmol) in EtOH and under inert atmosphere, was refluxed for 3 hours. The bright red precipitate was filtrated, washed with cold EtOH and diethyl ether and dried under vacuum (yield: 70,24%). *Mechanochemistry*: using a ball mill with spherical grinding elements, the amine (59.47 mg, 0.55 mmol) was ground until a fine powder was obtained. The aldehyde (95.51 mg, 0.5 mmol) was added and the mixture was milled for 10 minutes while monitoring the reaction by TLC (hexane/ethyl acetate 8:2). A bright red precipitate was obtained with a yield of 97%.

^{1}H NMR (400 MHz, DMSO) δ 8.89 (s, 1H), 7.74 (d, J = 2.6 Hz, 1H), 7.70 (d, J = 2.6 Hz, 1H), 7.17 (dd, J = 8.0, 1.4 Hz, 1H), 7.05 (ddd, J = 8.1, 7.2, 1.5 Hz, 1H), 6.81 (dd, J = 8.1, 1.3 Hz, 1H), 6.65 (ddd, J = 7.9, 7.2, 1.4 Hz, 1H), 5.20 (s, 2H).; HR ESI-MS (m/z) calculated for $[M + H]^+$ 281.0243, 281.0248 found.

L2. A solution of 1,2-phenylendiamine (540.7 mg, 5 mmol) and 3,5- dichlorosalicylaldehyde (477.8 mg, 2.5 mmol) in EtOH was refluxed for 4 hours. The red precipitate was filtrated, washed with cold water, EtOH and diethyl ether and dried under vacuum. In the filtrate, the formation of a yellow precipitate was observed, which was further filtered and then re-crystallized in a mixture of water, acetonitrile, and diethyl ether (1:1.5:2.5).

^{1}H NMR (300 MHz, DMSO) δ 8.17 (d, J = 2.5 Hz, 1H), 7.72 (q, J = 3.3 Hz, 3H), 7.35 (m, 2H).; HR ESI-MS (m/z) calculated for $[M + H]^+$ 279.0086, 279.009 found;

Zn1. The complex **Zn1** was synthesized following the method previously reported by us.[1] Specifically, a solution of 3,5-dichlorosalicylaldehyde and 1,2-phenylenediamine in ethanol was stirred for 30 minutes at room temperature. Then, $Zn(CH_3COO)_2{\cdot}2H_2O$, previously dissolved in a minimal amount of H_2O, was added. The mixture was stirred for 4 hours. The resulting precipitate was washed with cold water, ethanol, and diethyl ether, yielding the compound as a yellow solid.

Zn2. To an orange suspension of **L1** (39.36 mg, 0.14 mmol) in MeOH, 5-(triethyl-ammoniummethyl)salicylaldehyde chloride (49.92 mg, 0.18 mmol) was added, without any observable change in color. Subsequently, a methanolic solution of $Zn(CH_3COOH)_2{\cdot}2H_2O$ (52.50 mg, 0.21 mmol) was added to the mixture resulting in the precipitation of a substantial amount of bright yellow solid. The mixture was filtered, the solid was washed with cold methanol and dried under vacuum. Yield: 53.3 %.

^1H NMR (400 MHz, DMSO) δ 8.97 (s, 1H, “m”), 8.96 (s, 1H, “e”), 7.95 – 7.80 (m, 2H, “b,c”), 7.54 – 7.46 (m, 3H, “f,g,l”), 7.46 – 7.38 (m, 2H, “a,d”), 7.28 (dd, J = 8.9, 2.6 Hz, 1H, “i”), 6.72 (d, J = 8.8 Hz, 1H, , “h”), 4.31 (s, 2H, “n”), 3.14 (q, J = 7.1 Hz, 6H, “o”), 1.31 (t, J = 7.1 Hz, 9H, “p”).

^{13}C NMR (101 MHz, DMSO) δ 172.97 “q_{ar}”, 164.73 “q_{ar}”, 162.42 “e”, 161.53 “m”, 140.81 “l”, 139.47 “q_{ar}”, 137.15 “i”, 133.23 “f”, 132.13 “g”, 127.87 “a”, 127.50 “d”, 126.73 “q_{ar}”, 123.73 “h”, 120.62 “q_{ar}”, 119.51 “q_{ar}”, 117.00 “b”, 116.76 “c”, 114.21 “q_{ar}”, 113.97 “q_{ar}”, 110.54 “q_{ar}”, 59.64 “n”, 51.20 “o”, 7.45 “p”.

IR: ν (C=N) 1615 cm^{-1}; ν (O–Zn) 504 cm^{-1}; ν (N–Zn) 349 cm^{-1}; HR ESI-MS (m/z) calculated for $[(M)_2]^{2+}$ (where M = $C_{27}H_{28}Cl_2N_3O_2Zn$) 562.0823; found 562.0821; $C_{27}H_{28}Cl_3N_3O_2Zn \cdot 2.5\ H_2O$ Anal. Calcd: C 50.41, H 5.17, N 6.53; found C 50.39, H 4.65, N 6.39.

Zn3. The complex **Zn3** was synthesized as already described by us.[2] 5-(trimethylammonium methyl)salicylaldehyde, 1,2-phenylenediamine, and $Zn(CH_3COOH)_2 \cdot 2H_2O$ were mixed in a 2:1.1:1 molar ratio in doubly distilled water. The precipitate, obtained after adding an excess $NaClO_4$, has been filtrated and dried under vacuum.

NMR

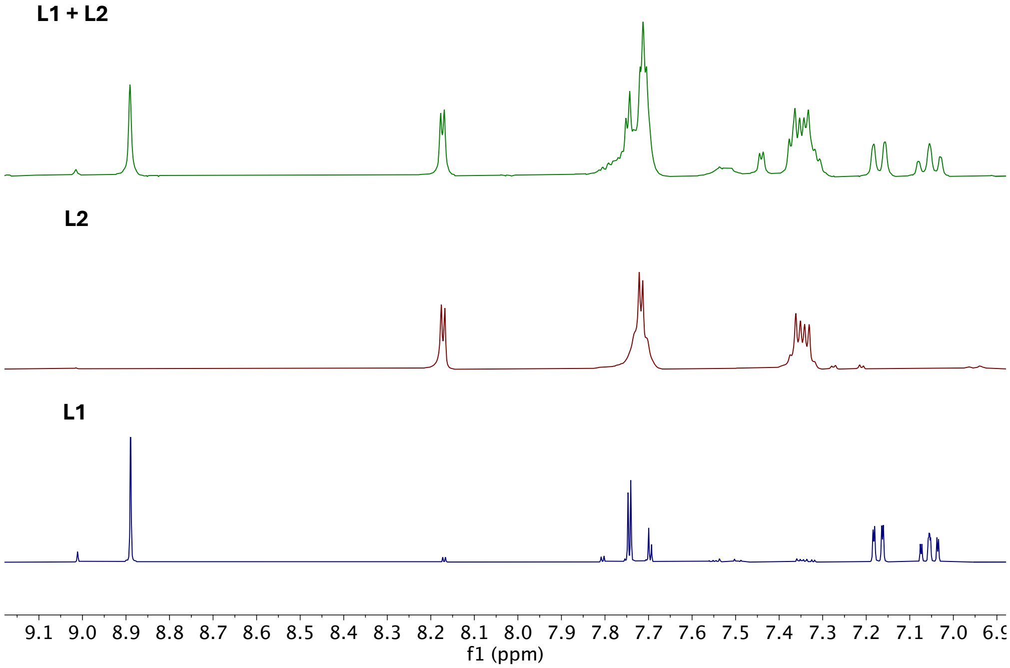


Figure S1. ^{1}H-NMR in DMSO of **L1**-**L2** mixture, **L2** and **L1**, respectively.

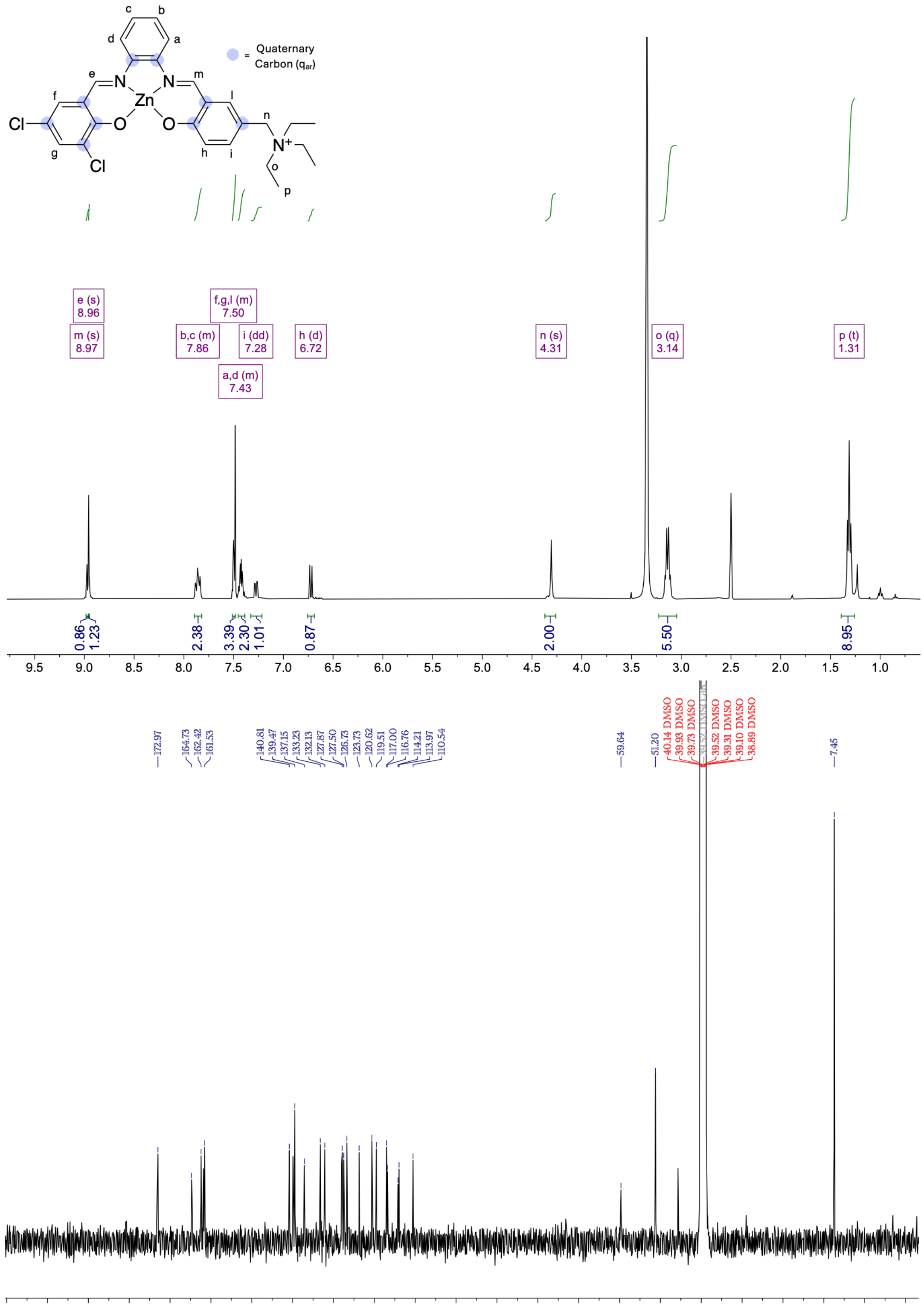


Figure S2. ^{1}H-NMR (up) with signals assigment and ^{13}C-NMR (down) in DMSO of **Zn2**

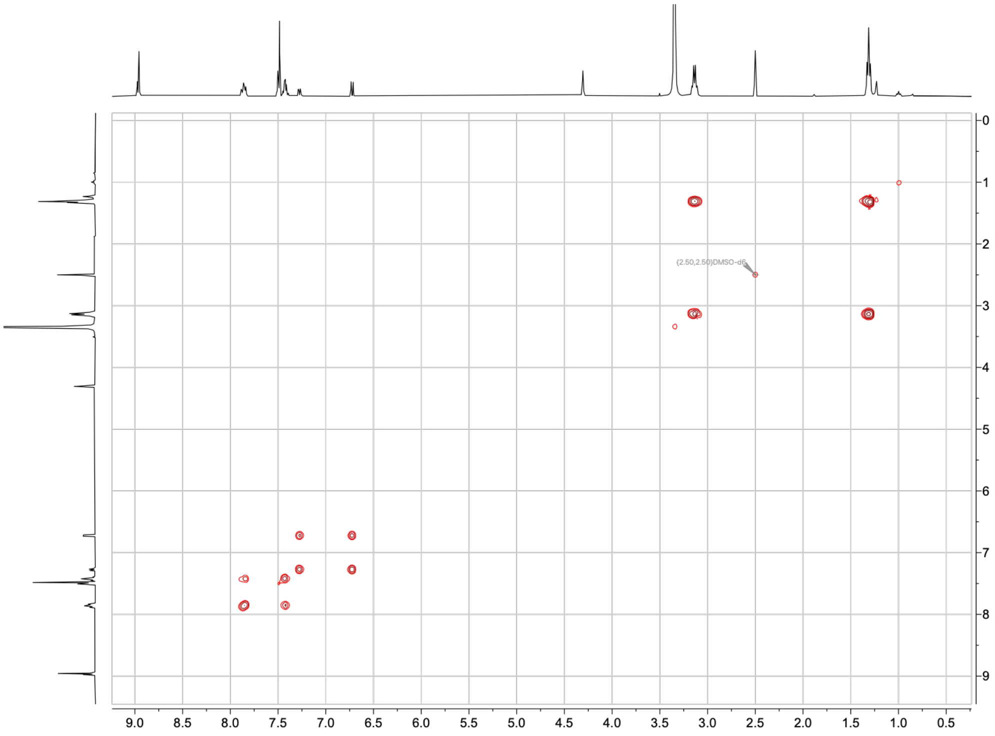


Figure S3. COSY in DMSO of **Zn2**

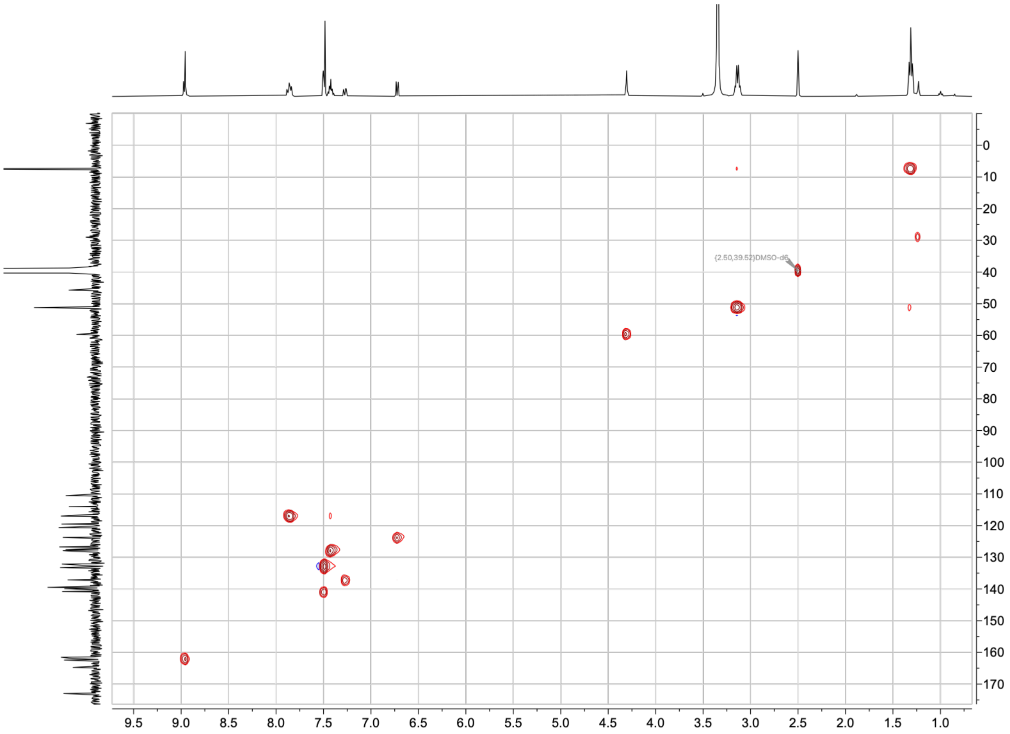


Figure S4. HSQC in DMSO of **Zn2**

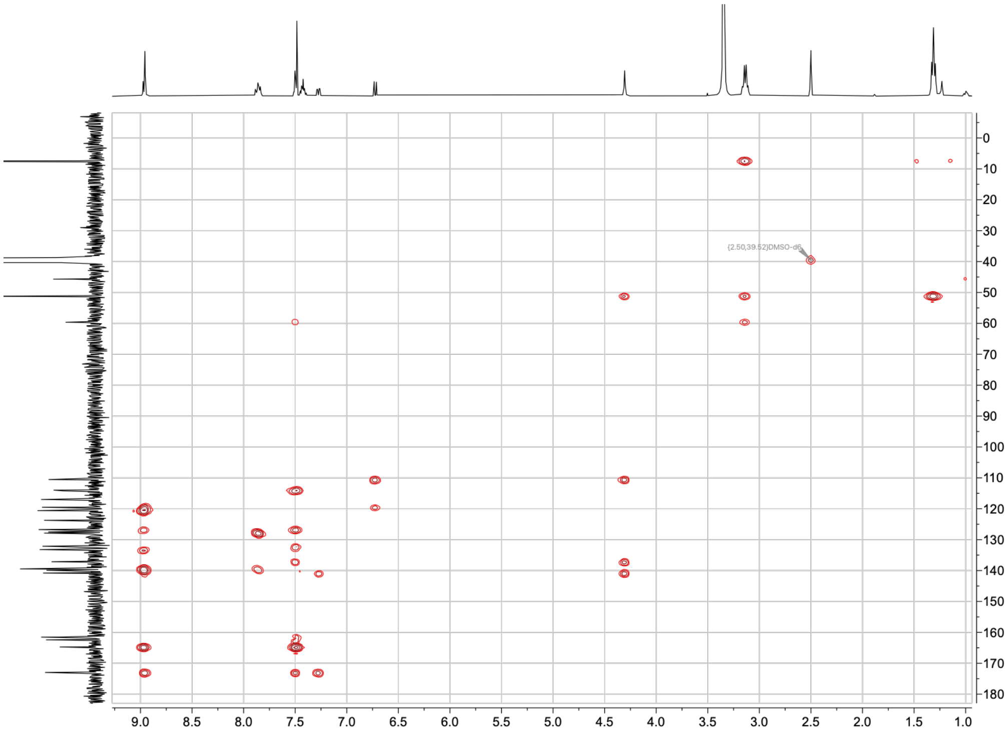


Figure S5. HMBC in DMSO of **Zn2**

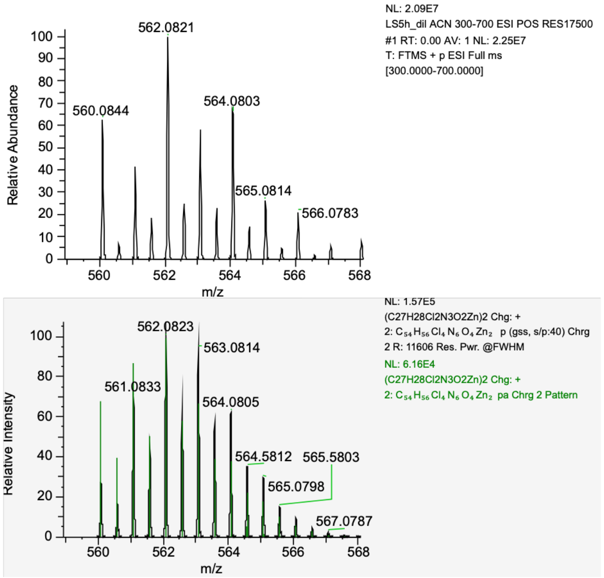


Figure S6. High Resolution mass spectrum of **Zn2** (lower panel includes the calculated spectrum)

X-ray Analysis

X-ray intensity data measured on STOE STADIVARI diffractometer equipped with multilayer monochromator, Mo K/α and Cu K/α micro focus sealed tube and Oxford cooling system. Structure solved by *Direct Methods*. Non-hydrogen atoms were refined with *anisotropic displacement parameters*. Hydrogen atoms of **L1** located on aromatic carbons inserted at calculated positions and refined with riding model, hydrogen atoms of **L2** inserted at calculated positions and refined with riding model. All other hydrogen atoms are refined without AFIX commands because of modelling a possible arrangement. Used Software: X-Area Recipe,[3] *X-Area Pilatus3_SV*,[4] *OLEX2*[5] for cell refinement, data collection, structure solution, refinement, molecular diagrams and graphical user-interface, *Shelxl*[6] for refinement and graphical user-interface *SHELXT-2015*[7] for structure solution, *SHELXL-2015*[8] for refinement*,* *Platon*[9] for symmetry check*.* Experimental data and CCDC-Codes Experimental data (Available online: http://www.ccdc.cam.ac.uk/conts/retrieving.html) can be found in Table 1. Tables 2 and 3 show data related to sample, crystal data, data collection and structure refinement Figure S7 and S10 show asymmetric units. Figures S8, S9, S11 and S12 are related to quality aspects.

Table S1 Experimental parameter and CCDC-Code.

Sample	**Machine**	**Source**	**Temp.**	**Detector Distance**	**Time/ Frame**	**#Frames**	**Frame width**	**CCDC**
			[K]	[mm]	[s]		[°]	
L1	STOE	Mo	100	40	30	2360	0.36	2420748
L2	STOE	Cu	100	40	5	3372	0.5	2420747

L2

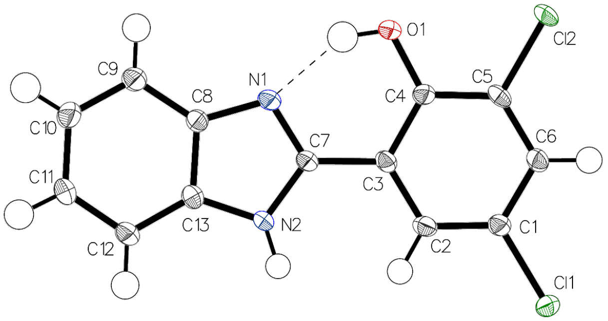


Figure S7. Asymmetric unit drawn with 50% displacement ellipsoid. The bond precision for C-C bonds is 0.0045 Å.

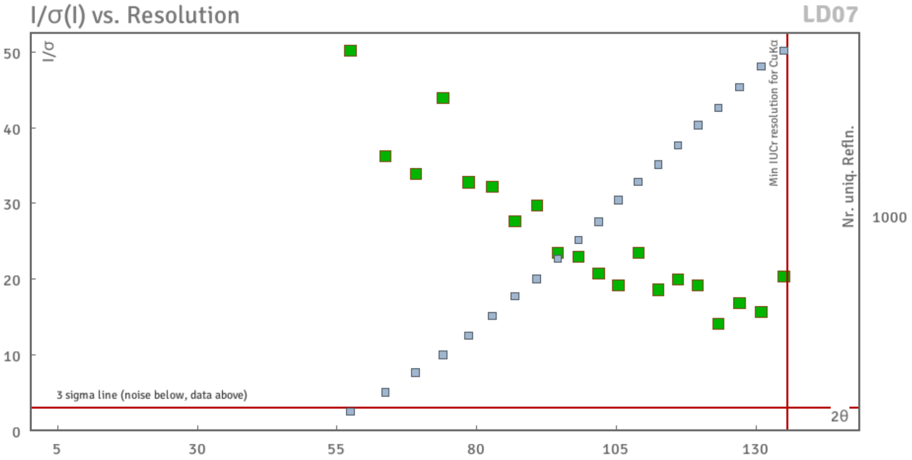


Figure S8. Data quality I 3 sigma line: All data are above the “noise level” line along the min IUCR definition.

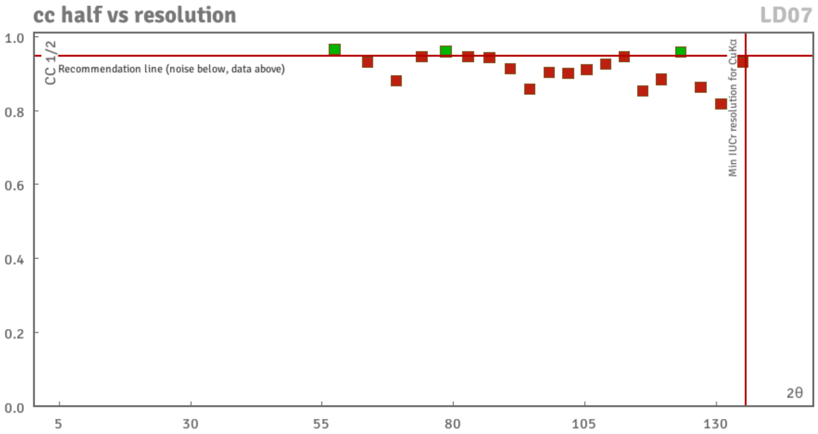


Figure S9 Data quality II CC1/2: Most data are close to the “noise level” line along the min IUCR definition.

Table S2. Sample and crystal data, Data collection and structure refinement

Identification code	**L2**
Empirical formula	$C_{13}H_8Cl_2N_2O$
Formula weight	279.11
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	11.7331(9)
b/Å	7.2792(6)
c/Å	13.7801(11)
α/°	90
β/°	104.101(6)
γ/°	90

Volume/Å^3	1141.46(16)
Z	4
ρ_{calc}g/cm^3	1.624
μ/mm^{-1}	5.013
F(000)	568.0
Crystal size/mm^3	0.31 × 0.147 × 0.01
Radiation	Cu Kα (λ = 1.54186)
2Θ range for data collection/°	53.974 to 137.116
Index ranges	$-10 \le h \le 14$, $-7 \le k \le 8$, $-10 \le l \le 16$
Reflections collected	5166
Independent reflections	1782 [R_{int} = 0.0397, R_{sigma} = 0.0343]
Data/restraints/parameters	1782/0/165
Goodness-of-fit on F^2	1.058
Final R indexes [I>=2σ (I)]	R_1 = 0.0557, wR_2 = 0.1461
Final R indexes [all data]	R_1 = 0.0660, wR_2 = 0.1570
Largest diff. peak/hole / e Å^{-3}	0.52/-0.47

L1

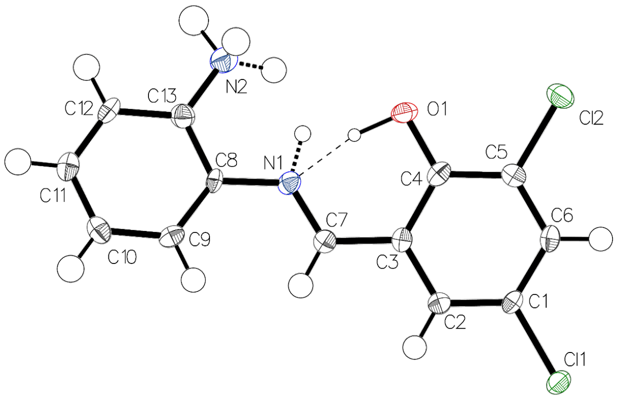


Figure S10 Asymmetric unit drawn with 50% displacement ellipsoid. The bond precision for C-C bonds is 0.0035 Å. Two hydrogen atoms located on N2 are disordered on three positions. One hydrogen atom splits into two positions located on O1 or N1. It seems that at this hydrogen positions (N1,O1) more than one electron could be placed, but we could not explain it.

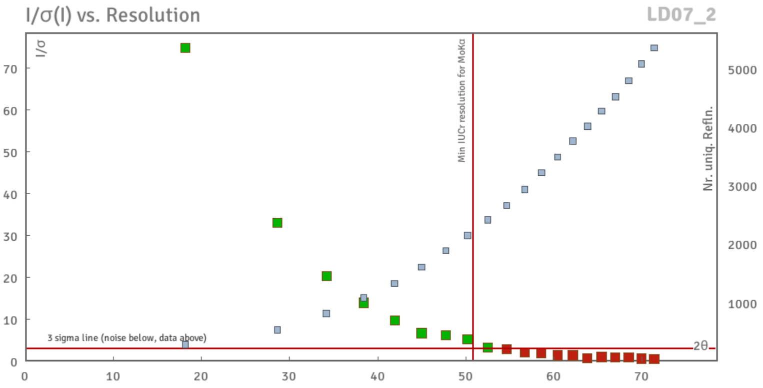


Figure S11. Data quality I 3 sigma line: All data are above the “noise level” line along the min IUCR definition.

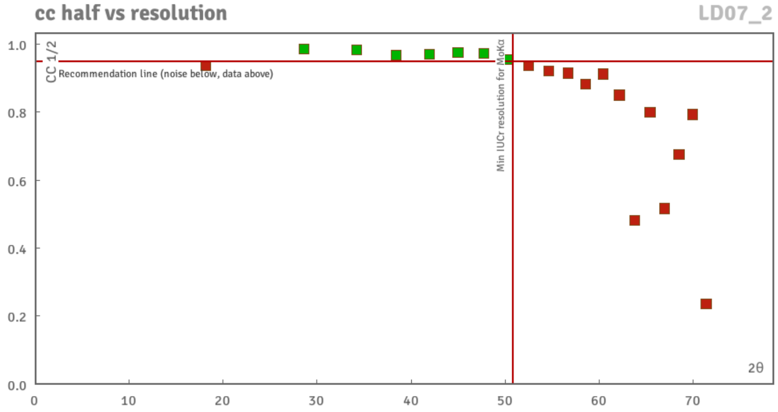


Figure S12. Data quality II CC1/2: Most data are above the “noise level” line along the min IUCR definition.

Table S3. Sample and crystal data, Data collection and structure refinement

Identification code	**L1**
Empirical formula	$C_{13}H_{10}Cl_2N_2O$
Formula weight	281.13
Temperature/K	100(2)
Crystal system	monoclinic
Space group	C2/c
a/Å	28.962(8)
b/Å	3.7880(6)
c/Å	23.455(7)
α/°	90
β/°	112.90(2)
γ/°	90
Volume/Å^3	2370.4(11)

Z	8
ρ_{calc}g/cm^3	1.576
μ/mm^{-1}	0.534
F(000)	1152.0
Crystal size/mm^3	0.07 × 0.047 × 0.02
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.702 to 60.05
Index ranges	$-37 \le h \le 40, -5 \le k \le 5, -32 \le l \le 32$
Reflections collected	14790
Independent reflections	3402 [R_{int} = 0.0421, R_{sigma} = 0.0775]
Data/restraints/parameters	3402/3/180
Goodness-of-fit on F^2	1.031
Final R indexes [I>=2σ (I)]	R_1 = 0.0480, wR_2 = 0.1099
Final R indexes [all data]	R_1 = 0.0926, wR_2 = 0.1214
Largest diff. peak/hole / e Å^{-3}	0.91/-0.57

2. INTERACTION WITH G4 AND B-DNA IN SOLUTION

All absorption and emission spectra were recorded using freshly prepared solutions of the samples. Lyophilized calf thymus DNA (Ct-DNA) was obtained from Sigma-Aldrich and reconstituted in 1.0 mM Tris-HCl (tris-hydroxymethyl-aminomethane) at pH 7.5. The final DNA concentration, in bases, was determined by UV spectrophotometry (Cary 1E double-beam spectrophotometer), measuring absorbance at 260 nm and using a molar absorption coefficient of 6600 M^{-1} cm^{-1}. The oligodeoxynucleotides (ODNs) listed in Table S4 were purchased from Sigma Aldrich and Integrated DNA Technologies (IDT) in HPLC purity grade. Concentrations are reported per strand or per base depending on the experiment, as indicated in the captions. For determining binding constants, concentrations are always expressed per base.

Förster Resonance Energy Transfer (FRET). FRET experiments were performed using an Applied Biosystems™ QuantStudio 6 PCR cycler configured in a 96-well format and equipped with a FAM (6-carboxyfluorescein) filter. Stock solutions of oligodeoxynucleotides (ODNs), labelled with FAM and TAMRA (6-carboxytetramethylrhodamine) probes, were diluted to the requisite concentrations using a 60 mM potassium cacodylate buffer (pH 7.4). The ODNs were then induced to adopt their G-quadruplex (G4) or B-DNA structures by heating the solutions to 80°C for 5 minutes, followed by a gradual cooling process. In the final reaction mixtures, the ODN concentration was standardized to 0.2 µM within a total volume of 30 µL. Metal complexes were initially dissolved in DMSO to create 1 mM stock solutions, and subsequently diluted with the buffer, ensuring that the final DMSO concentration did not exceed 0.1%.

Molecular docking. Molecular docking studies were conducted and evaluated using the Glide module from the Schrödinger Suite. G4 models, we selected the resolved structures of the hybrid conformation of h-Tert (PDB: 2KZD), Bcl2 (PDB: 6ZX7) and Kras (PDB: 6X2G) for docking studies.

Receptor grids were established by designating the original ligands as the center of the grid boxes. Docking was carried out using the Extra Precision (XP) mode as the scoring function, where the generated 3D conformers were docked into the receptor model. During post-docking minimization, each ligand conformer was assessed with a total of 5 poses, and a maximum of 2 docking poses were produced per ligand conformer.

Table S4. 5’-3’ DNA sequences used in this study. In Ds-DNA, Heg linker is (-CH_2-CH_2-O-)$_6$

Name	Sequence
Ds-DNA (FRET)	FAM-TAT AGC TA-Heg-TATA GCT ATA-TAMRA
Bcl2 (FRET)	FAM-GG GGC GGG CGC GGG AGG AAG GGG GCG GGA-TAMRA
Kras (FRET)	FAM-AG GGC GGT GTG GGA AGA GGG AAG AGG GGG AG-TAMRA
h-Tert (FRET)	FAM-AGG GGA GGG GCT GGG AGG GC-TAMRA
Bcl2	AGG GGC GGG CGC GGG AGG AAG GGG GCG GGA
Kras	AG GGC GGT GTG GGA AGA GGG AAG AGG GGG AGG
h-Tert	AGG GGA GGG GCT GGG AGG GC

Table S5. $\Delta T_{1/2}$ values of 0.2 µM ds-DNA and G4s upon interaction with metal complexes at the indicated µM concentration. Uncertainty is ≤ 0.5 for the $\Delta T_{1/2}$ reported. Concentration of DNA is reported in strand.

Zn1	Ds-DNA	Bcl2	Kras	h-Tert
1:5	0.1	0.53	0.28	0.53
1:10	0.2	-0.13	1.09	0
1:20	0.0	0.31	0.04	0.56

Zn2	Ds-DNA	Bcl2	Kras	h-Tert
1:5	0.23	-0.70	0.43	-0.10
1:10	0.20	0.79	2.01	0.14
1:20	0.99	-0.86	1.65	-0.41

Zn3	Ds-DNA	Bcl2	Kras	h-Tert
1:5	0.90	5.57	1.87	7.49
1:10	0.89	8.21	1.91	9.59
1:20	-0.52	11.99	5.14	11.90

Table S6. Docking scores.

	Bcl2	**Kras**	**h-Tert**
Zn1	-3.445	-4.860	-3.088
Zn2	-4.689	-6.572	-2.707
Zn3	-6.045	-7.269	-4.595

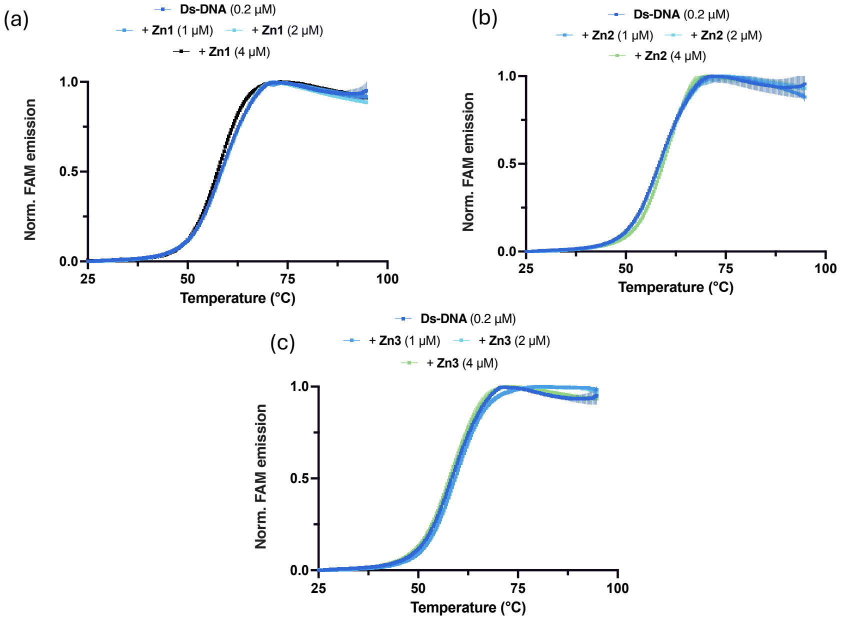


Figure S13. FRET melting profiles of Ds-DNA motif upon interaction with compounds **Zn1**-**Zn3** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

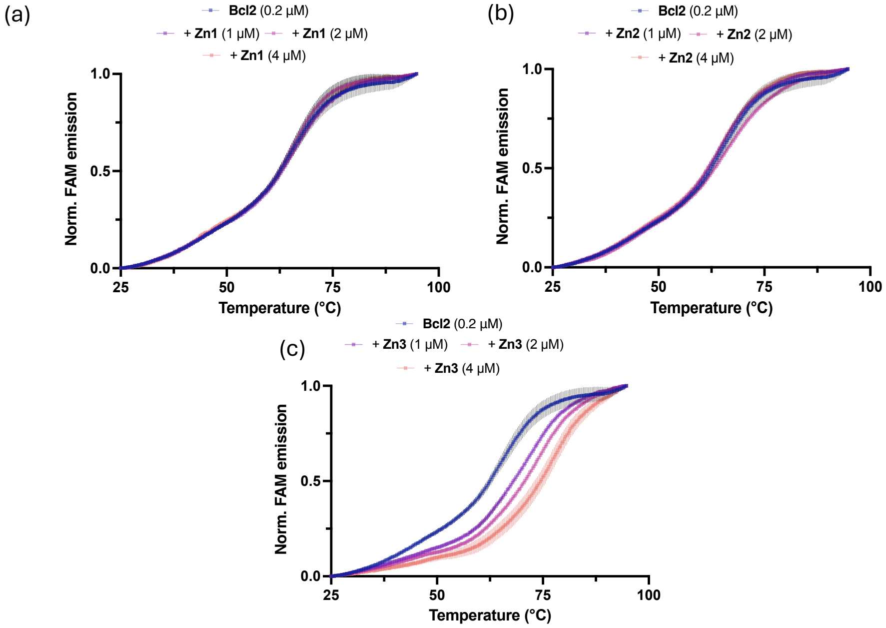


Figure S14. FRET melting profiles of Bcl2 G4 motif upon interaction with compounds **Zn1**-**Zn3** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

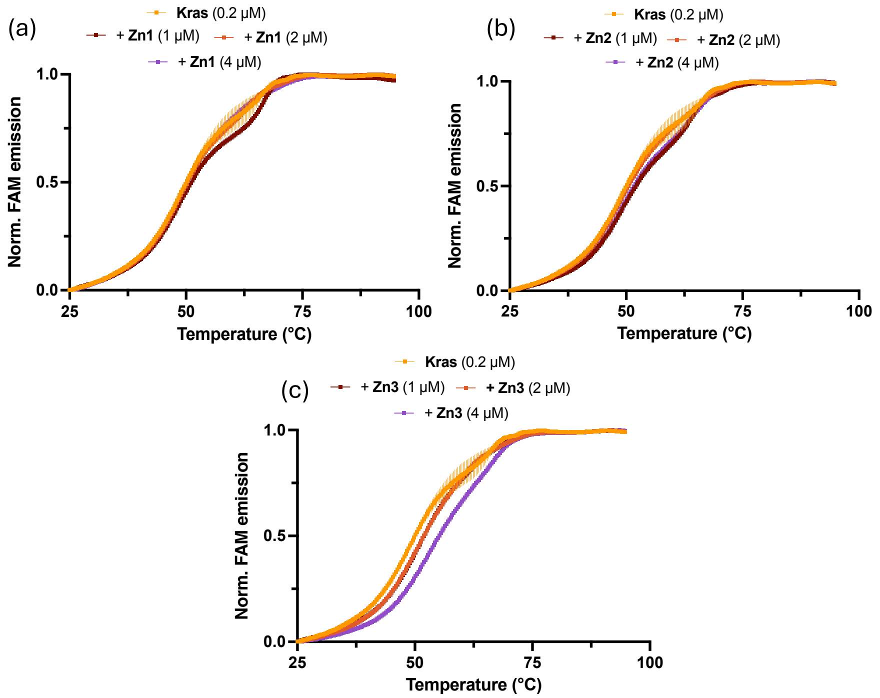


Figure S15. FRET melting profiles of Kras G4 motif upon interaction with compounds **Zn1**-**Zn3** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

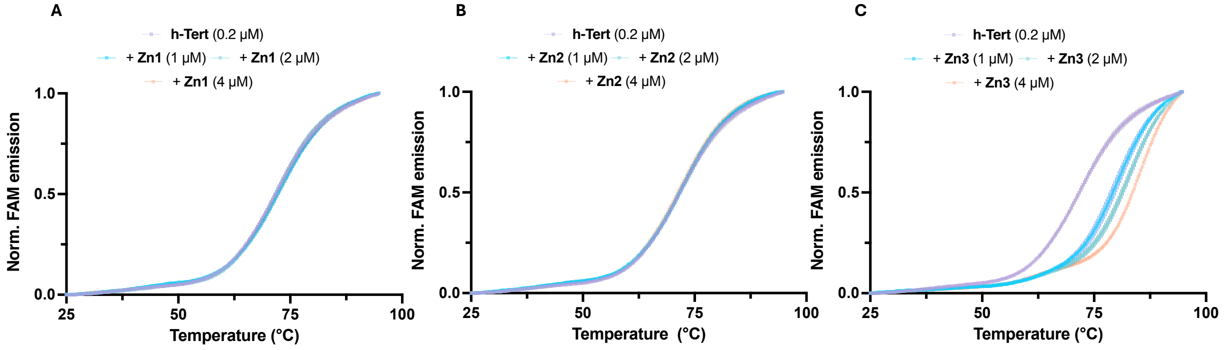
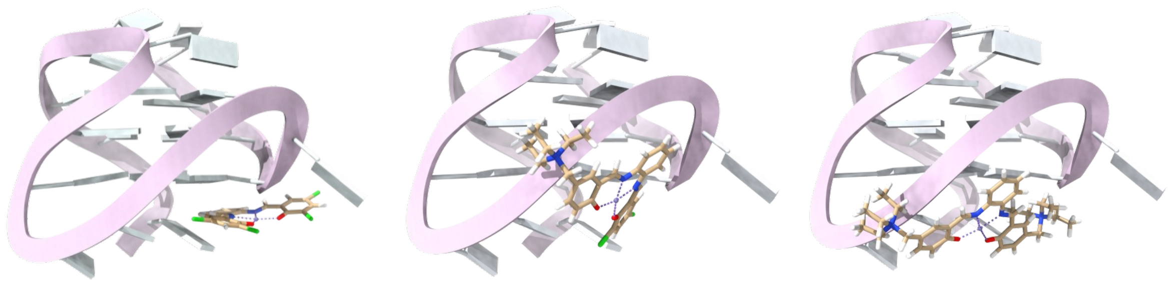
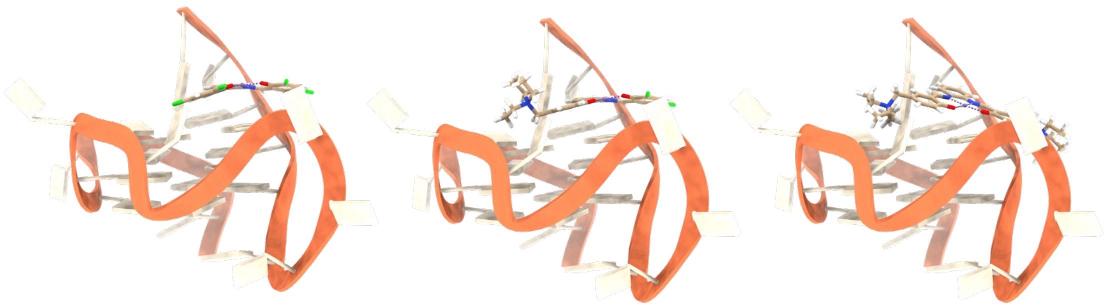


Figure S16. FRET melting profiles of h-Tert G4 motif upon interaction with compounds **Zn1**-**Zn3** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.



Zn1 **Zn2** **Zn3**

Figure S17. Cartoon showing possible binding poses of **Zn1**-**Zn3** with h-Tert G4.



Zn1 **Zn2** **Zn3**

Figure S18. Cartoon showing possible binding poses of **Zn1**-**Zn3** with Kras G4.

Molecular dynamic simulations. To assess for the possibility of membrane internalization we built three model lipid bilayers involving 72 POPC lipids in each leaflet. The systems were solvated with water and 0.15 M physiological salt concentration was enforced and the metal complex was added in the bulk solution. Lipid 24 amber force field was used for the POPC, while water is represented with TIP3P. The force field of the Zn-metal complexes has been parameterized following the gaff procedure and using the MCPB.py utility. All the initial systems have been built using the online charm-gui utility.

MD simulations has been performed with NAMD code in the constant isotherm and isobaric (NPT) system. A time step of 4 fs was used to propagate Newton's equation of motion thanks to the combined use of Rattle and Shake algorithm and Hydrogen Mass Repartitio (HMR). After initial equilibration and thermalization performed by progressively removing constraints on the ligand, a production simulation of about 500 ns has been performed. Showing that the complex remains mainly in the water bulk.

To obtain the free energy profile for the penetration of the ligand inside the membrane we have resorted to enhanced sampling in the form of the combination between metadynamics and extended Adaptive Biased Force (meta-eABF) as implemented in NAMD and COLVAR. To this end we have defined a collective variable consisting in the projection along the normal membrane axis of the distance between the center of mass of the ligand and the center of mass of the P atoms in one of the leaflets. The collective variable was partitioned in 11 consecutive windows, each one having been sampled for 400 ns. This gives a total sampling of 4400 ns for each of the three Zn complex.

MD simulations have been analyzed and visualized using VMD.

3. CELLULAR UPTAKE AND BIOLOGICAL ACTIVITY

Fluorescence. Emission measurements were performed using a JASCO FP-8300 spectrofluorimeter. Full excitation-emission 3D landscape of 15 µM in Tris-HCl 50 mM, KCl 100 mM, pH=7.4, were obtained by setting the parameters as follows: excitation bandwidth 10 nm, emission bandwidth 10 nm, measurement interval 1 nm, and scanning speed 1000 nm/min. Measurements were carried out in a quartz cuvette with an optical path length of 1 cm.

General cell culture. Tumoral pancreatic T3M-4 cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA). T3M-4 cell line was cultured using phenol-free RPMI-1640 culture medium (Sigma-Aldrich), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biowest), 2 mM L-glutamine (Sigma-Aldrich), and 0.1% Penicillin-Streptomycin mixture (Sigma-Aldrich). Cells were maintained as monolayers, in a humidified atmosphere with 5% CO_2, at 37°C. All the experiments were performed with freshly prepared **Zn1-3** compounds, solubilized in DMSO.

IC_{50} determination. T3M-4 cells were seeded in a 96-wells plate (1.3 x10^3 cells/well) and allowed to attach for 24 hours. Cells were treated for 48 hours with increasing concentrations of the considered compound (from 0 to 50 µM). A control condition with the vehicle maximum amount to which cells are exposed (0.2% DMSO) was always included. After 48 hours of treatment, cells were stained with crystal violet, and optical density (OD) recorded at 595 nm. The relative viability of treated cells comparatively to the control was determined using the following equation: (OD_{sample} / $OD_{control}$) x 100. The data were then plotted against the decimal logarithm of the compound concentration log([ZnX]) and the half maximal inhibitory concentration IC_{50} was calculated by non-linear regression using GraphPad Prism 8 software.

Proliferation assay. To study the effect of **Zn1**, **Zn2** and **Zn3** on pancreatic cancer cells proliferation, 8.0 x10^4 T3M-4 cells were seeded per well in a 24-well plate. Twenty four hours post-seeding, cells were treated in triplicate with increasing concentrations of the **Zn1-3** complexes, or with 0.2% DMSO for control. Every 24 hours and for 72 hours, cell density was assessed by a real-time cell imaging system Cytonote (CytonoteScan, Iprasense®) and images were acquired using Horus software (Iprasense®).

Western Blotting. For the study of cell death induction, T3M-4 cells were incubated (1.2 x10^5 cells in a 60 mm culture plate) for 24 hours, prior treatment with 10, 25 or 50 µM of the metal complexes for either 24 or 48 hours. Then, floating and attached cells were harvested using a cell scraper and total proteins extracted incubating cells in Lysis Buffer (10 mM Tris-HCl, 5 mM EDTA, 1% Triton X100, pH 7.4). The total protein concentration was determined by performing Bradford assay. For cleaved PARP detection (89 kDa), 50 µg of total proteins were loaded onto a 10% SDS-PAGE gel and gently transferred to a 0.2 µm PVDF membrane (Bio-Rad®). After a blocking step with a 5% skimmed dry-milk solution, the membrane was incubated overnight with the mouse anti-cPARP primary antibody (BD Biosciences, 552596) at 4°C, before incubation with the appropriate HRP-conjugated secondary antibody for 1 hour at room temperature. Western Blotting chemiluminescence was revealed using the ECL substrate, on a ChemiDoc™ Touch Gel Imaging System (Bio-Rad®). An anti-α-tubulin (55 kDa) antibody (Abcam, Ab52886) was used as loading control. Data were analysed using ImageLab software (Bio-Rad®).

Compounds entrance in cells. To evaluate the entrance of **Zn1**, **Zn2** and **Zn3** complexes in cells, T3M-4 cells were seeded in a 6-well plate (4.0 x10^4 cells/well). After 48 hours, cells were treated with increasing concentrations of the considered metal complex for 24 hours. A 0.2% DMSO condition was always included as control. After a PBS 1X wash, cells were collected by scrapping them in 300 µL PBS 1X. Immediately after harvesting, cells were analysed by flow cytometry on a CytoFLEX flow cytometer (Beckman Coulter, Life Sciences, B53000). Median FITC-A was recorded and quantified using CytExpert software (Beckman Coulter). Histograms were made using the Kaluza 2.1 software. Therefore, to study a possible effect of temperature changes on the compounds entrance into the cells, T3M-4 cells were treated with 25 µM of each compound for the indicated time (from 0 to 2 hours) and incubated either at 37°C or 4°C according to the considered condition. Cells were recovered and analysed using the same procedure as described before.

Statistical analysis. All biological experiments were made with at least three biologically independent replicates. Statistical analyses were performed using the test specified in the legend of each figure, using the GraphPad Prism 8 software. Results with a *p*-value lower to 0.05 were considered as statistically significant.

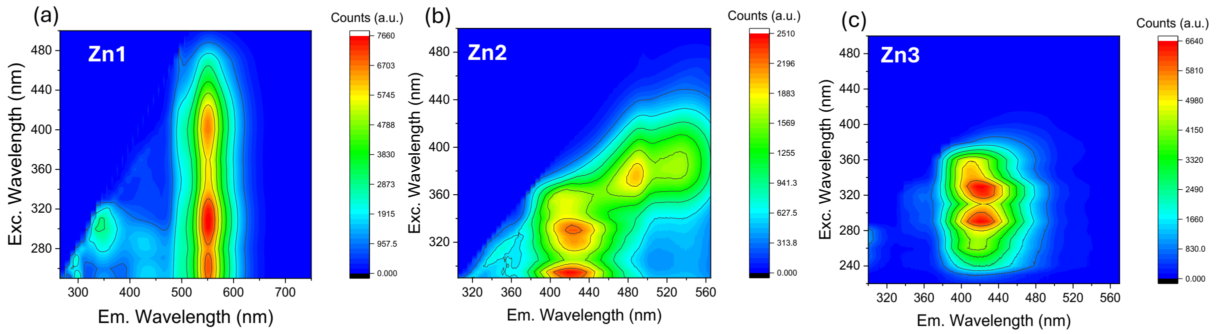


Figure S19. Full excitation–emission profiles of (a) **Zn1** 10 μM, (b) **Zn2** 10 μM and (c) **Zn3** 10 μM in buffer (Tris-HCl 50 mM, KCl 100 mM, pH=7.4).

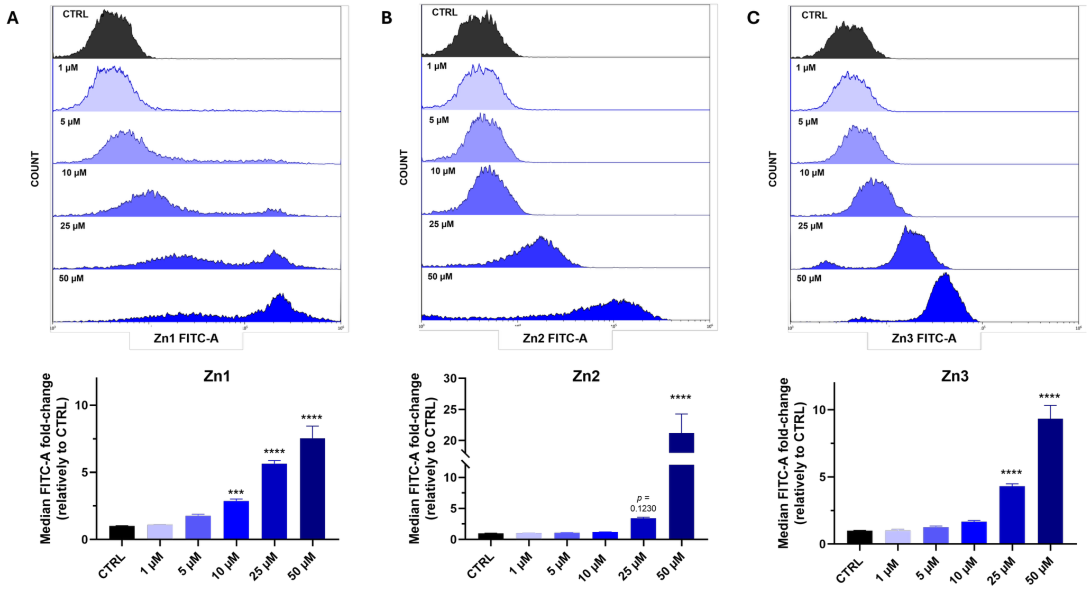


Figure S20. Detection of **Zn1-3** in living cells. T3M-4 cells were treated for 24 hours with increasing concentrations of the **Zn1** (A), **Zn2** (B) or **Zn3** (C) compounds. The median FITC-A fluorescence of the corresponding living cells was measured by flow cytometry. The histograms from the upper panel are representative of three biologically independent replicates. Results are expressed as the median FITC-A fold-change relative to the CTRL condition (*Mean ± SD* (n=3)); ** *p*<0.01 ; *** *p*<0.001 ; **** *p*<0.0001. One-way ANOVA; Holm-Sidak's multiple comparisons test with the CTRL condition.

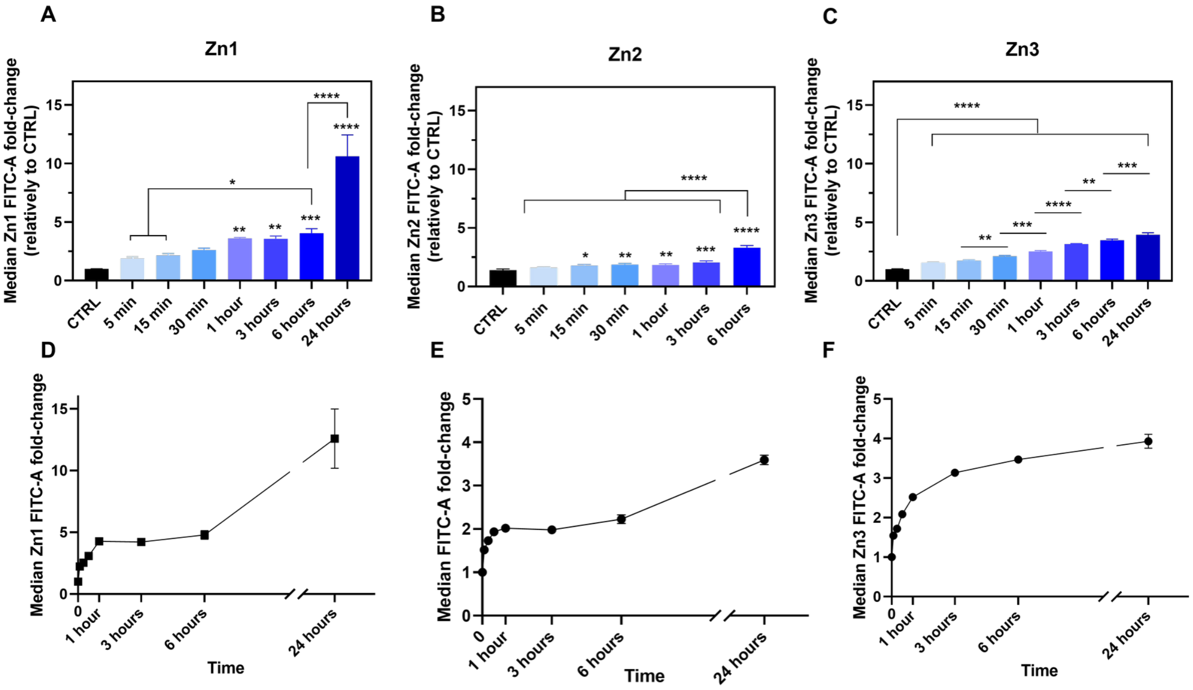


Figure S21. Entrance follow-up of the three **Zn** complexes in living T3M-4 cells over the time. T3M-4 cells were treated with 25 µM of **Zn1** (A,D), **Zn2** (B,E) or **Zn3** (C,F) for different times over 24 hours. The median FITC-A fluorescence of the corresponding living cells was measured by flow cytometry. Data are expressed as the median fluorescence fold-change of treated samples relative to the corresponding timepoint of untreated (CTRL) conditions, presented as Mean ± SD (n=3). For panel (D), y-axis range was adjusted to allow better visualization of the data. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. One-way ANOVA; Tukey's multiple comparisons test with the CTRL condition.<

Zn1

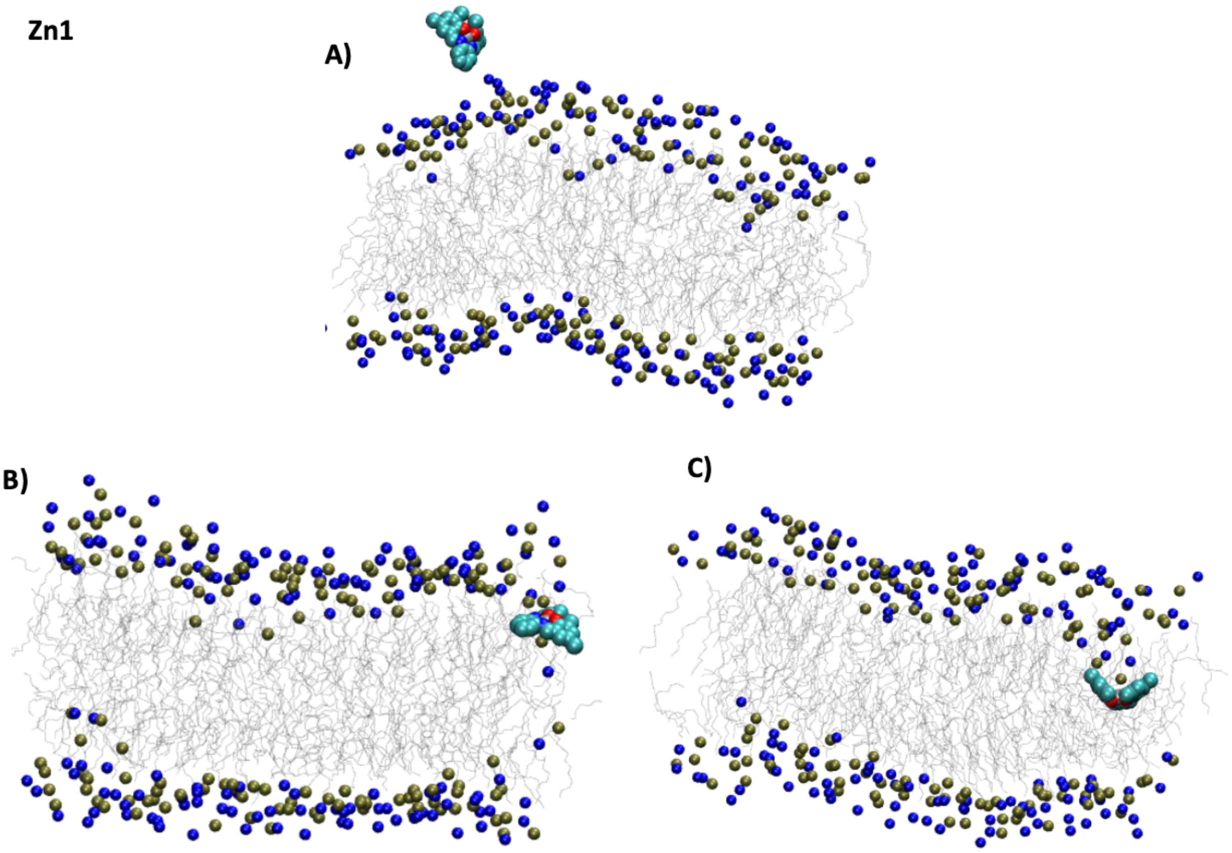


Figure S22. Representative snapshot along the reaction coordinate for the **Zn1** complex namely in bulk water (A), in interaction with the polar heads (B), and at the centre of the membrane (C).

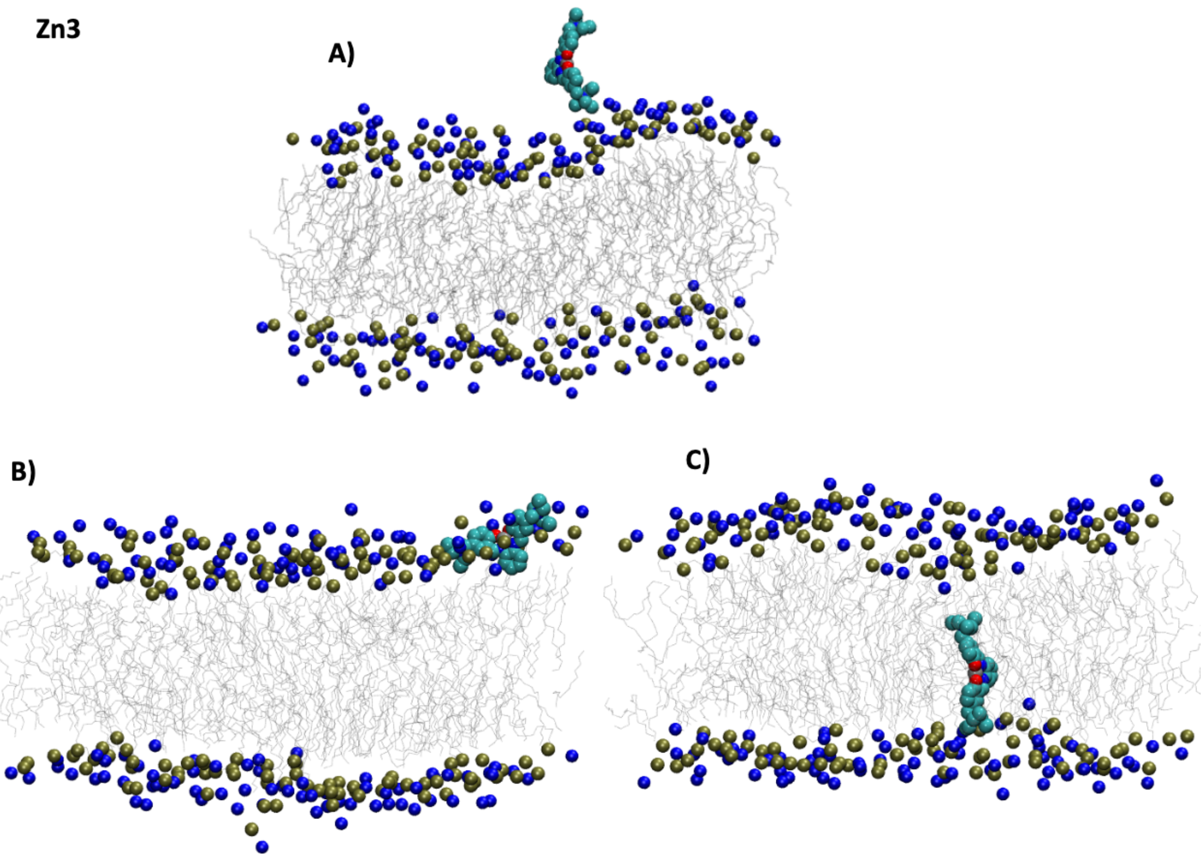


Figure S23. Representative snapshot along the reaction coordinate for the **Zn3** complex namely in bulk water (A), in interaction with the polar heads (B), and at the centre of the membrane (C).

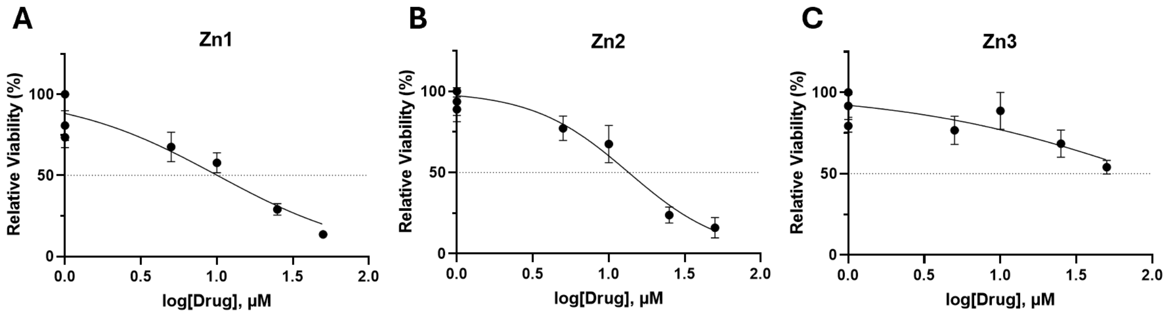
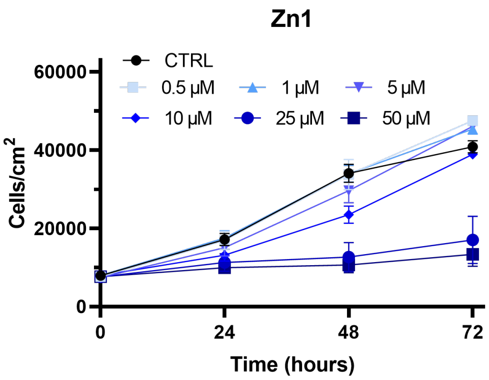


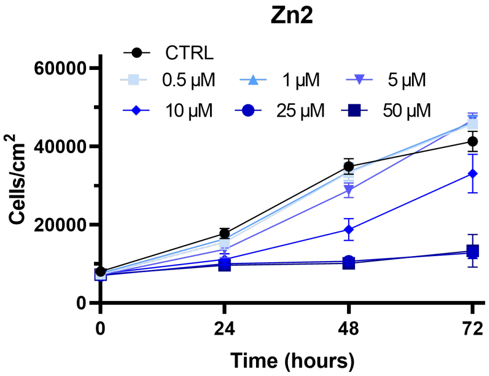
Figure S24. IC_{50} determination of **Zn1-3** complexes on T3M-4 cells. Relative viability (%) of T3M-4 cell line at 48 hours of treatment of the considered Zinc(II) complex, obtained by crystal violet assay. Mean ± SEM (n=4).

Table S7. Half maximal inhibitory concentration IC_{50} of **Zn1**, **Zn2** and **Zn3** on T3M-4 cells at 48 hours of treatment. NA refers to not available data, when $IC_{50} > 50$ µM. *Mean* ± SEM (n=4).

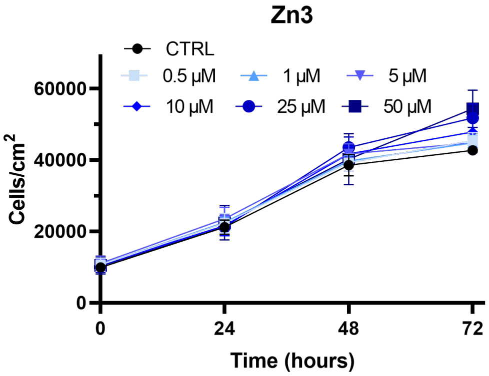
IC_{50} at 48 hours (μM)						
Zn1			**Zn2**			**Zn3**
10.7	±	2.3	13.5	±	3.6	NA



CTRL vs	0 hour	24 hours	48 hours	72 hours
0.5 µM	ns	ns	ns	ns
1 µM	ns	ns	ns	ns
5 µM	ns	ns	ns	ns
10 µM	ns	ns	****	ns
25 µM	ns	*	****	****
50 µM	ns	**	****	****



CTRL vs	0 hour	24 hours	48 hours	72 hours
0.5 µM	ns	ns	ns	ns
1 µM	ns	ns	ns	*
5 µM	ns	ns	**	*
10 µM	ns	***	****	****
25 µM	ns	****	****	****
50 µM	ns	****	****	****



CTRL vs	0 hour	24 hours	48 hours	72 hours
0.5 µM	ns	ns	ns	ns
1 µM	ns	ns	ns	ns
5 µM	ns	ns	ns	ns
10 µM	ns	ns	ns	ns
25 µM	ns	ns	ns	**
50 µM	ns	ns	ns	***

Figure S25. Statistical analysis of T3M-4 cells proliferation study when treated with **Zn1-3**. Proliferation of T3M-4 cells treated with **Zn1**, **Zn2** or **Zn3** was followed every 24 hours for 72 hours by determining the cell density. Statistics are displayed in the table adjacent to the corresponding proliferation graph. Each treated condition was compared to the untreated (CTRL) condition at the same considered time point. Mean ± SD (n=3); * p<0.05 ; ** p<0.01 ; *** p<0.001 ; **** p<0.0001. Two-way ANOVA; Tukey's multiple comparison test.

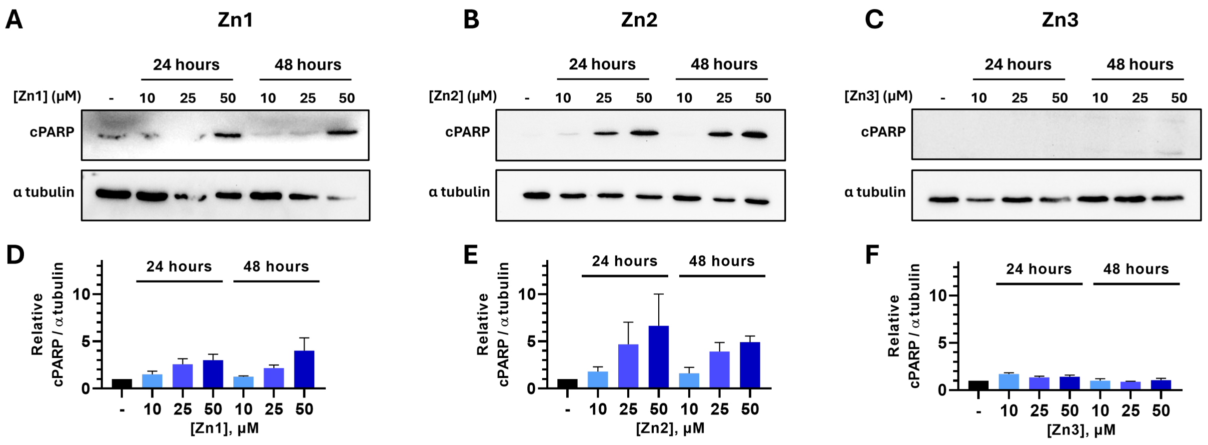


Figure S26. Western Blot experiment was performed to assess the PARP protein cleavage on T3M-4 cells lysates, treated with **Zn1** (A, n=5), **Zn2** (B, n=3) or **Zn3** (C, n=3) at increasing concentrations for 24 or 48 hours. A-tubulin is the loading control. All presented blots are representative of at least three biologically independent experiments. Data are expressed on histograms as Mean ± SEM, relatively to the loading control.

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