Electronic Supporting Information

A metal-based tumour necrosis factor-alpha converting enzyme inhibitor

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1. General experiment

Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received.

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d_6: ^{1}H 2.05, ^{13}C δ 29.8; CD_3Cl: ^{1}H δ 7.26, ^{13}C δ 76.8). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for 1H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

2. Synthesis of iridium(III) complexes

The following complexes were prepared according to (modified) literature methods. All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

The precursor iridium(III) complex dimer $[M_2(C\hat{}N)_4Cl_2]$ was prepared according to a reported method.[1] Then, a suspension of $[M_2(C\hat{}N)_4Cl_2]$ (0.2 mmol) and corresponding N^N ligands (0.44 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was then allowed to cool to room temperature, and filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaoration until precipitation of the crude product occurred. The precipiate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound.

Complex **1**. Yield: 52%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.18 (d, J = 8.0 Hz, 2H), 8.48 (d, J = 8.0 Hz, 2H), 8.22 – 8.27 (m, 4H), 8.17 (d, J = 4.0 Hz, 2H), 7.98 (d, J = 4.0 Hz, 3H), 7.86-7.81 (m, 3H), 7.56 (d, J = 8.0 Hz, 2H), 7.46-7.43 (m, 5H), 7.20 (t, J = 4.0 Hz, 3H), 6.45 (d, J = 4.0 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 157.2, 153.3, 149.4, 148.9, 148.7, 147.1, 145.2, 140.8, 137.5, 134.4, 133.8, 132.5, 130.3, 129.9, 129.8, 129.5, 129.0, 127.2, 126.7, 124.1, 122.4, 120.6. HRMS: Calcd. for $C_{45}H_{28}IrN_4[M–PF_6]^+$: 845.2037, found: 845.2005.; Anal. Calcd for ($C_{45}H_{28}IrN_4PF_6$): C, 53.62; H, 3.00; N, 8.34., found: C, 53.6; H, 2.89; N, 8.20.

Complex **2**. (Reported)[2]

Complex **3**. (Reported)[3]

Complex **4** ^{1}H NMR (400 MHz, CD_3CN) δ 8.36-8.34 (d, J = 8.0 Hz, 4H), 7.96-7.94 (d, J = 8.0 Hz, 2H), 7.47-7.44 (d, J = 8.0 Hz, 2H), 7.33-7.32 (d, J = 4.0 Hz, 2H), 7.07-7.03 (t, J = 8.0 Hz, 2H), 6.95 (s, 2H), 6.88-6.84 (d, J = 8.0 Hz, 2H), 6.57-6.56 (t, J = 4.0 Hz, 2H), 6.28-6.26 (d, J = 8.0 Hz, 2H), 2.83-2.79 (t, J = 8.0 Hz, 4H), 1.77-1.69 (m, 4H), 1.35-1.27 (m, 24H), 0.88-0.85 (t, J = 8.0 Hz, 6H); ^{13}C NMR (400 MHz, CD3CN) δ 157.7, 157.6, 151.7, 144.6, 139.7, 134.5, 134.0, 129.2, 129.1, 127.9, 125.7, 124.5, 113.2, 109.5, 36.3, 33.0, 31.3, 30.5, 30.4, 30.37, 30.2, 23.8, 14.8; HRMS: Calcd. for $C_{46}H_{58}IrN_6[M–PF_6]^+$: 887.4350, found: 887.4325.; Anal. Calcd for ($C_{46}H_{58}IrN_6PF_6$): C, 53.53; H, 5.66; N, 8.14., found: C, 53.69; H, 5.63; N, 8.09.

Complex **5**. Yield: 55%. ^{1}H NMR (400 MHz, CD_3CN) δ 9.08 (s, 2H), 8.48 (d, J = 8.0 Hz, 2H), 8.25 (s, 1H), 8.20 (s, 1H), 8.10 (d, J = 8.0 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H), 7.93-7.86 (m, 4H), 7.72-7.65 (m, 4H), 7.39 (s, 2H), 7.30 (t, J = 8.0 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 6.45 (d, J = 8.0 Hz, 2H), 5.47 (s, 1H), 5.51 (s, 1H); ^{13}C NMR (400 MHz, CD_3CN) δ 167.2, 167.0, 158.4, 158.3, 154.1, 148.6, 139.5, 136.7, 133.6, 131.8, 129.9, 129.1, 129.0, 128.0, 127.0, 125.7, 123.7, 122.2, 121.1, 117.0, 115.4. Calcd. for $C_{45}H_{28}RhON_6[M–PF_6]^+$: 771.1380, found: 771.1394; Anal. Calcd for ($C_{45}H_{28}RhON_6PF_6$+2H_2O): C, 56.73; H, 3.39; N, 8.82.; found C, 56.75; H, 3.59; N, 9.01.

3. Photophysical measurement

Emission spectra and lifetime measurements for complexes were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (±1 nm); τ (±10%); φ (±10%). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw.

Luminescence quantum yields were determined using the method of Demas and Crosby[4] $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

4. Computational methodology

DFT calculations were performed on the cations in complexes **1** and **5** using the ORCA software package (version 3.0.2).[5] Their electronic ground states were optimized using the BP86 functional[6] accompanied with the zero-order regular approximation (ZORA)[5] to account for relativistic effects. The def2-SVP basis sets were used for the N, C and H atoms, while the def2-TZVP(-f) basis sets were used for the Ir and Rh atoms.[7] Auxiliary basis sets, used to expand the electron density in the calculations, were chosen to match the orbital basis sets. The resolution of the identity algorithm (RI) was used to accelerate all calculations. Tight SCF convergence criteria (1×10^{-8} Eh in energy, 1×10^{-7} Eh in the density charge, and 1×10^{-7} in the maximum element of the DIIS error vector) were used throughout.

5. Cells and reagents

Human monocytic leukemia THP-1 cells were cultivated in RPMI-1640 medium with high glucose and L-glutamine and supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 units/ml)/streptomycin (100 μg/ml). Cells were maintained at a cell

density of 1–2 × 10^6 cells/mL. SensoLyte® 520 TACE (α-Secretase) Activity Assay Kit *Fluorimetric* was purchased from AnaSpec (San Jose, CA). Human TNF-α Elisa kit was purchased from NeoBioscience (Shenzhen, China). TACE, Recombinant Protein was purchased from Merck Millipore (Guyancourt, France). Phorbol myristate acetate (PMA) and lipopolysaccharides (LPS) were obtained from Sigma-Aldrich (St Louis, MO). All the compounds were dissolved in dimethyl sulfoxide (DMSO).

6. TACE enzymatic assay

The TACE enzymatic assay was performed using a SensoLyte® 520 TACE (α-Secretase) activity assay kit following the instructions from the manufacturer. 5-FAM fluorescent substrate Abz-LAQAVRSSSR-Dpa was used to measure the TACE activity. In the primary screening, the human purified TACE protein (1 μg/mL) was pre-treated with the compounds at the final concentration of 1 μM for 10 min at room temperature in a black 384-well plate. The reaction was initiated by the addition of fluorescent substrate at a final concentration of 100 nM to the mixture. The plate was shaken gently for 30 sec and the reaction was incubated for 1 h at room temperature. The active TACE protein cleaves the FRET substrate into two separate fragments, resulting in an increase of 5-FAM fluorescence. The reaction was quenched by the addition of stop solution and the fluorescence intensity was monitored at an excitation of 490 nm and emission of 520 nm in a SpectraMax M5 microplate reader (Molecular Devices). For the dose-response analysis, TACE protein was pre-treated with compounds at various concentrations for 10 min at room temperature and the assay was conducted as before.

7. TACE kinetic assay

The TACE kinetic study was carried out by using SensoLyte® 520 TACE (α-Secretase) activity assay kit. Briefly, the human purified TACE protein (1 μg/mL) was pretreated with different concentrations of the Ir(III) complex ranging from 6.25 μM to 100 μM for 10 min at room temperature in a black 384-well plate. Different concentrations of the 5-FAM fluorescence substrate was added to each well. After shaking for 30 sec, the

reaction was incubated for 30 min. The fluorescence of 5-FAM was quenched by the addition of stop solution and the fluorescence of the wells was measured in a SpectraMax M5 microplate reader with excitation and emission of 490 nm and 520 nm.

8. TNF-α immunoassay

THP-1 cells were seeded with PMA (100 ng/mL) in the growth medium at a density of 5 $\times 10^5$ cells/mL in a 96-well plate for three days. After differentiation, cells were cultivated in the growth medium for overnight. PMA-differentiated THP-1 cells were seeded in the growth medium at a density of 5 $\times 10^5$ cells/mL in a 96-well plate overnight. After 24 h, the cells were co-treated with different concentration of complex **1** and LPS (1 ug/mL) for another 24 h at 37 ℃. The quantity of the LPS-induced secretion of TNF-α in the culture supernatant from PMA-differentiated THP-1 cells was determined using Human TNF-α Elisa kit according to the manufacturer's instructions. 100 μL of supernatant samples were added to each well immobilized with human TNF-α monoclonal antibody and incubated at 37 ℃ for 1.5 h. Each well was then washed by adding 300 μL of 1 × wash solution for four times, and incubated with 100 μL of yellow antibody solution at 37 ℃ for 1 h. Washed as before, the well was incubated with 100 μL of substrate solution at room temperature for 30 min. After incubation, 100 μL of stop solution was added to each well, and the optical density of each well was read at 450 nm using a SpectraMax M5 microplate reader (Molecular Devices).

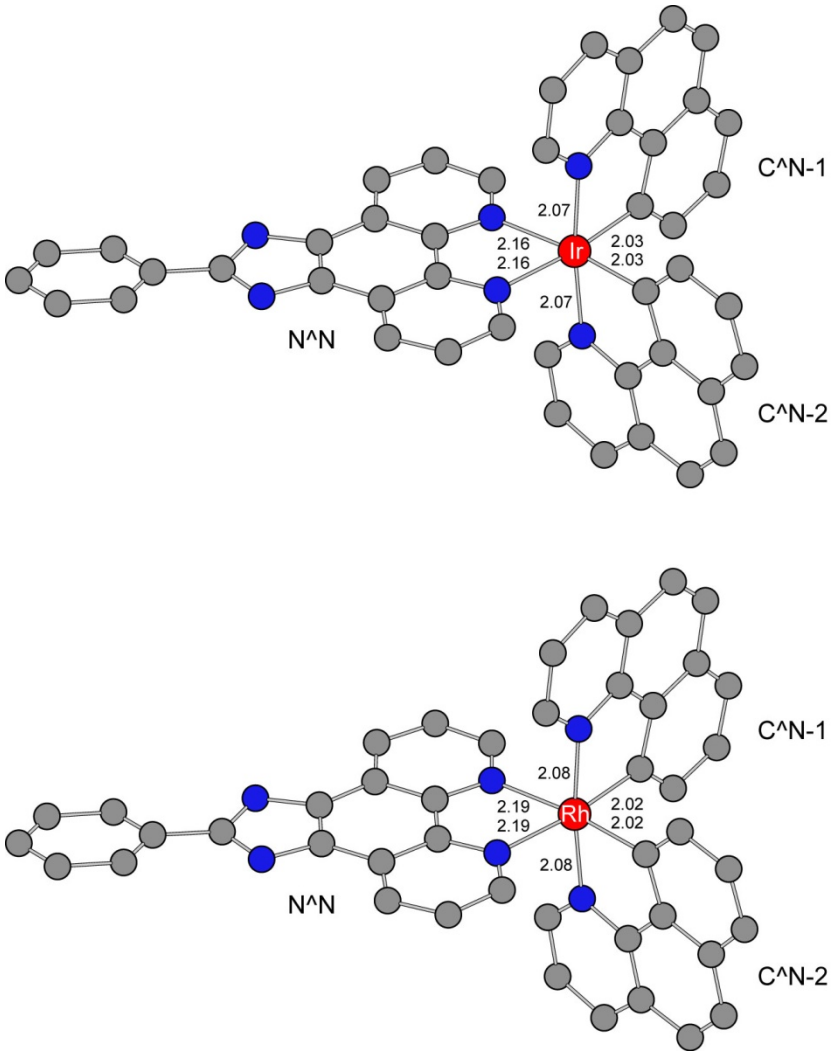
9. Western immunoblotting

PMA-differentiated THP-1 cells were seeded in a 6-well plate at a density of 5 × 10^5 cell/mL in the complete medium overnight. Cells were then treated with complex **1** (in 0.05% DMSO) or vehicle control and LPS (1 μg/mL) in low FBS medium for additional 24 h. After the treatment, cells were lysed and the concentration of protein samples was quantified using the BCA protein method. 45 μg of cell lysates were subjected to electrophoresis on 12% SDS-PAGE. After electrophoresis, protein samples were transferred to a PVDF membrane, and then incubated in blocking buffer at room

temperature for 1 h. Immunoblotting was performed by treating the membrane with primary antibody at 4 °C overnight and probing with secondary antibody for 1 h at room temperature. Signals of protein bands were detected using enhanced chemiluminescent Plus reagents (GE Healthcare) and analyzed by Image Lab. The antibodies used for western blotting were phospho-p38 MAPK (Thr180/Tyr182) antibody (CST, #9211) and p38 MAPK antibody (CST, #9212), β-Actin antibody (CST, #4967) and anti-rabbit IgG, HRP-linked antibody (CST, #7074).

Table S1. Photophysical properties of complexes **1–5**.

Complex	λ_{em} / nm	UV/vis absorption λ_{abs} / nm (ε / $dm^3mol^{-1}cm^{-1}$)
1	585	413 (7520), 281 (58600)
2	598	272 (4.5×10^5), 382 (1.2×10^4), 438 (9.6×10^3)
3	611	265 (2.11×10^4), 303 (1.06×10^4), 355 (1.9×10^3), 396 (2.46×10^3)
4	536	292 (7.8×10^4), 317 (4.2×10^3)
5	535	394 (12716), 301 (71720, sh), 279 (88940)



Löwdin Atomic Charges

Ir	-1.67
C^N-1	0.77
C^N-2	0.77
N^N	1.13

Löwdin Atomic Charges

Rh	-0.43
C^N-1	0.36
C^N-2	0.36
N^N	0.71

Fig. S1 Molecular structures for the cations in **1** (top) and **5** (bottom) optimized at DFT level. Hydrogen atoms are omitted for clarity. Important bond distances around the metal centers (unit in Å), together with the Löwdin atomic charge distribution within different moieties, are depicted.

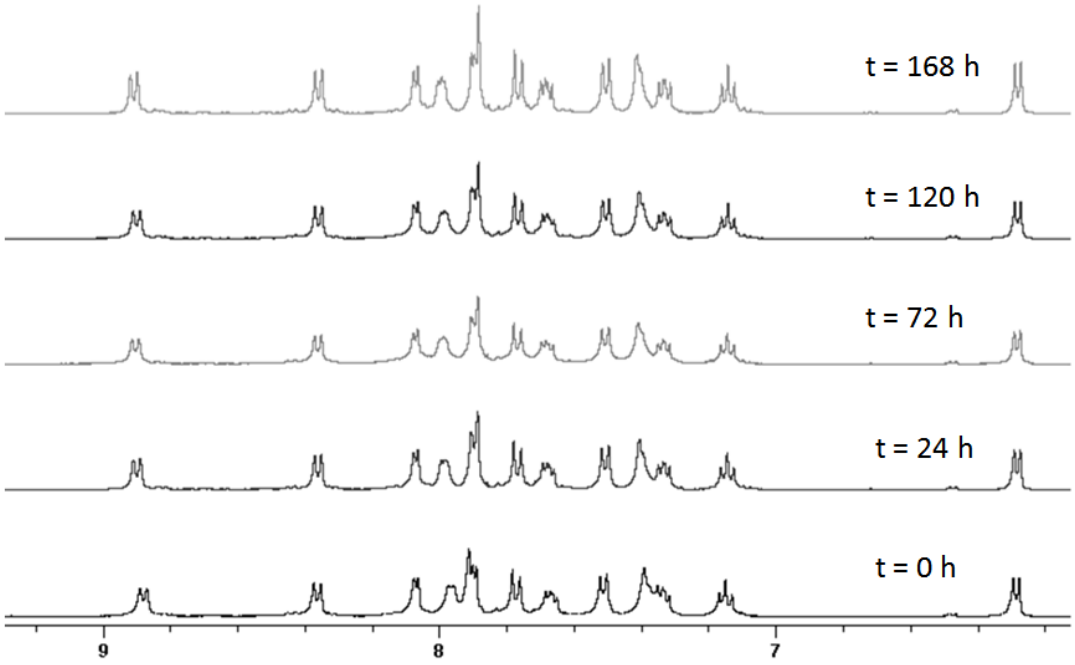


Fig. S2 Aromatic region of the ^{1}H NMR spectra of complex **1** at a concentration of 5 mM in 65% [D_6]DMSO/35% D_2O at t = 0 h and after incubation for 24, 72, 120 and 168 h at 310 K.

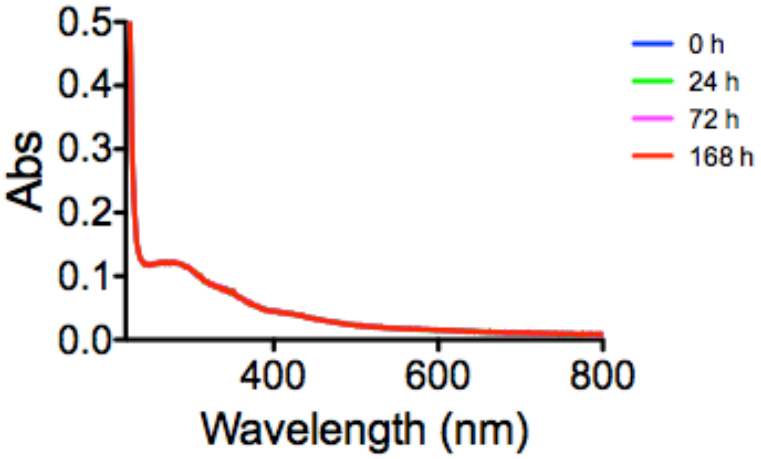


Fig. S3 UV/Vis absorption of complex 1 at a concentration of 50 μM in 65% DMSO/35% H_2O at t = 0 h and after incubation for 24, 72, 120 and 168 h at 310 K.

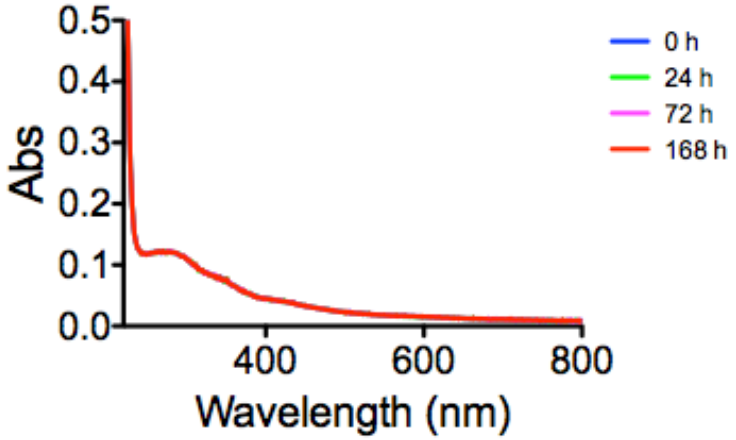


Fig. S4 UV/Vis absorption of complex 1 at a concentration of 50 μM in 65% DMSO/35% H_2O with dilutied serum media at t = 0 h and after incubation for 24, 72, 120 and 168 h at 310 K.

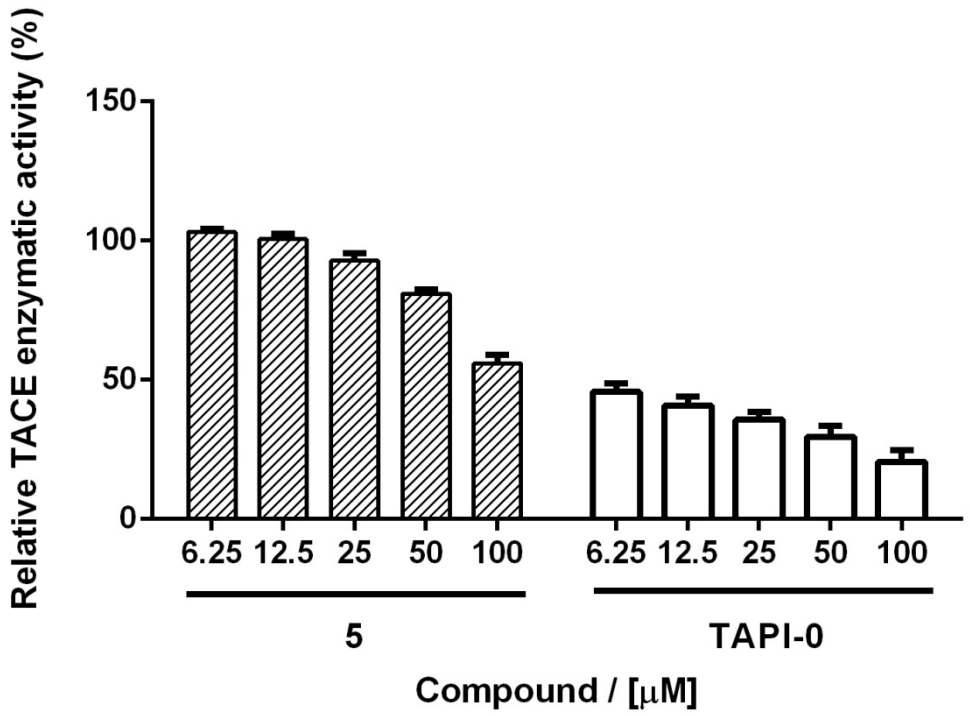


Fig. S5 Complex **5** and TAPI-0 inhibits TACE enzymatic activity as determined by a fluorimetric assay. Human purified TACE protein (1 μg/mL) was pre-incubated with the compounds at the indicated concentrations for 10 min, and the reaction was initiated by the addition of 5-FAM fluorescent substrate (100 nM). After 30 min, the reaction was stopped and the fluorescence intensity of the wells was monitored at an excitation of 490 nm and an emission of 520 nm using a microplate reader. IC_{50} value of complex **5**: >100 μM. IC_{50} value of TAPI-0: < 6.25 μM. Error bars represent the standard deviations of the results from three independent experiments.

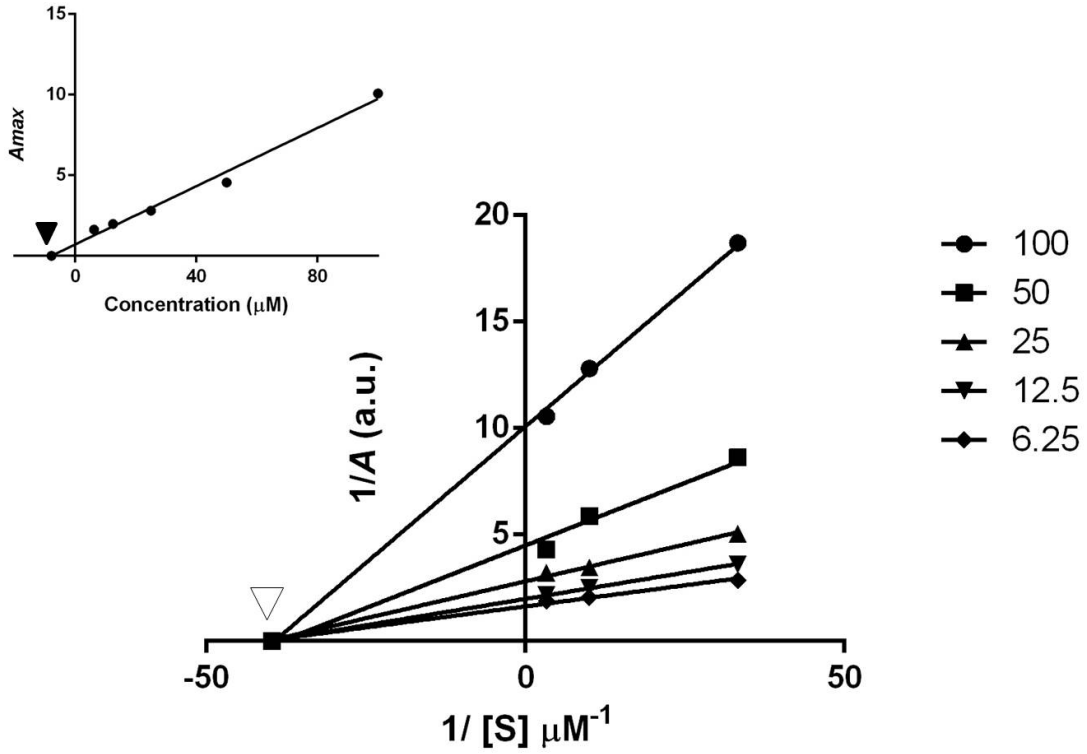


Fig. S6 Lineweaver-Burke analysis of complex **1** on TACE activity as determined by a fluorimetric assay. After preincubating the human purified TACE protein (1 μg/mL) with complex **1** at the indicated concentrations, the reaction was initiated by the addition of different concentrations of the 5-FAM fluorescent substrate. The increase of 5-FAM fluorescence intensity was monitored at an excitation of 490 nm and emission of 520 nm. ▽, $-1/K_{d\ app.}$ ▼, $-K_{i\ app}$.

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