Electronic supplementary material

Characterization of platinum(II) complexes exhibiting inhibitory activity against 20S proteasome

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Off-target activity against other proteases

Inhibition of cathepsin B

Cathepsin B (50 ng) was incubated in 100 L assay buffer (100 mM sodium actetate, 5 mM EDTA, 5mM DTT; pH 5.5) with different concentrations of platinum complexes and 20 M of the fluorogenic peptide substrate Z-RR-MCA for 1 h at 37°C. After incubation, the production of hydrolyzed AMC groups was measured at 450 nm (λex = 365 nm).

Inhibition of -chymotrypsin

****In 100 L assay buffer (25 mM HEPES and 0.5 mM EDTA, pH 7.8), -chymotrypsin (50 ng) was incubated with different concentrations of platinum complexes and 25 M of the fluorogenic peptide substrate Suc-LLVA-MCA for 1 h at 37°C. After incubation, the production of hydrolyzed AMC groups was measured at 450 nm (λex = 365 nm).

**Figure S1**. Residual activity of cathepsin B (■), -chymotrypsin (▲) and 20S proteasome (●) in the presence of complex **1**(a), complex **2** (b) and complex **3** (c).

**Inhibitory activity of N-9-anthracenylmethyl-1,2-ethanediamine and (ethylenediamine)bispyridine platinum(II) chloride against the purified 20S proteasome**

For the proteasome inhibition assays, the peptide-AMC substrates (25 M Suc-LLVY-AMC) and inhibitors were added to assay solutions. The assay buffer had the following composition: 25 mM HEPES, 0.5 mM EDTA, 0.03% SDS (pH 8.0). Human erythrocyte 20S proteasome (100 ng) was added to the assay buffer containing substrates and inhibitors at a final volume of 100 L. After incubation at 37°C for 1 h, the fluorescence emission spectrum at 450 nm (λex, 365 nm) was measured by using a fluorescence microplate reader.

**Figure S2**. Inhibitory rate of (ethylenediamine)bispyridine platinum (II) chloride (gray) and N-9-anthracenylmethyl-1,2-ethanediamine (black) against ChTL activity of the purified 20S proteasome.

**Inhibitory activity of N-9-anthracenylmethyl-1,2-ethanediamine against the 20S proteasome in the whole cell lysate**

For the determination of inhibitory activity in the whole cell lysate, peptide-AMC substrates (50 M Suc-LLVY-AMC), inhibitors, and the whole cell lysate (15 g) were added to the assay solutions. The following assay buffer was used: 25 mM HEPES, 0.5 mM EDTA, 0.03% SDS (pH 8.0). After incubation at 37 °C for 4 h, the fluorescence emission was measured at 450 nm (λex = 365 nm) by using a fluorescence microplate reader.

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**Figure S3**. Inhibitory rate of N-9-anthracenylmethyl-1,2-ethanediamine against ChTL activity of the 20S proteasome in the whole cell lysate.



**Figure S4.** Theplausible structure of 1:1 adduct ofcomplex **2** with N-acetylcysteine.