Supporting Information

Quenching the Firefly Bioluminescence by Various Ions

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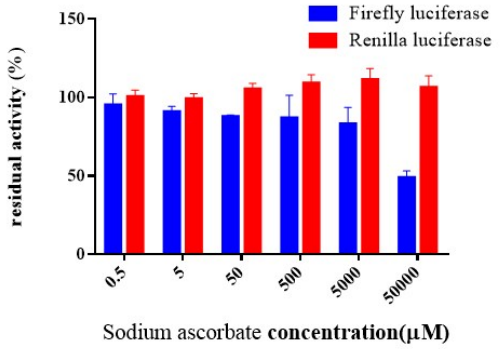

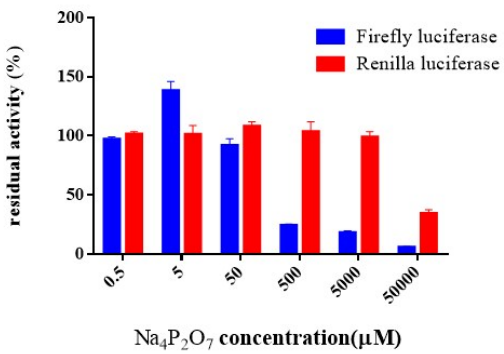

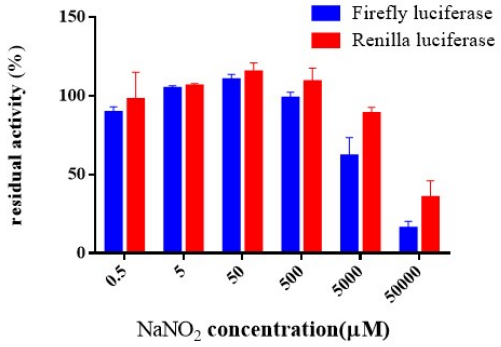

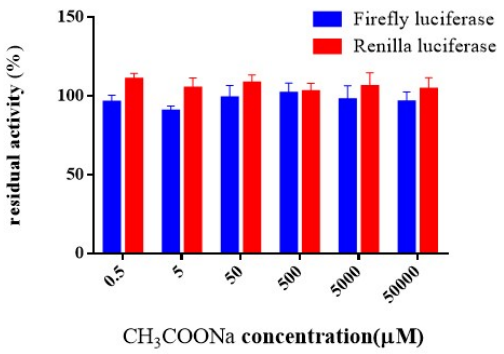

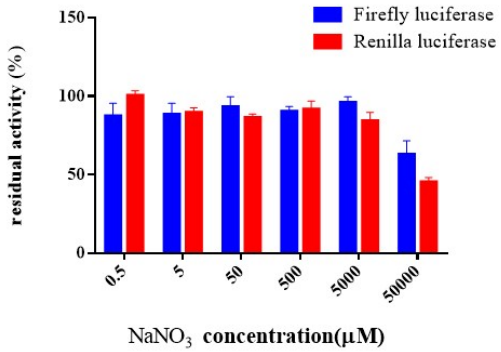

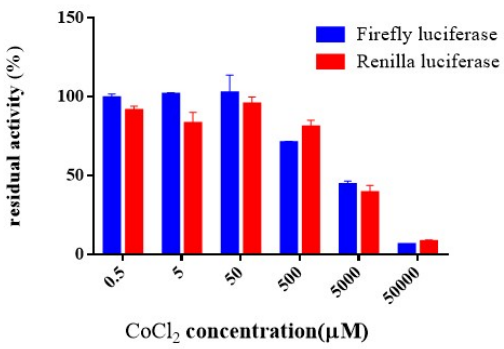

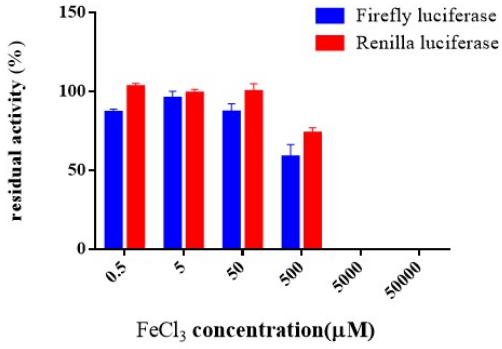

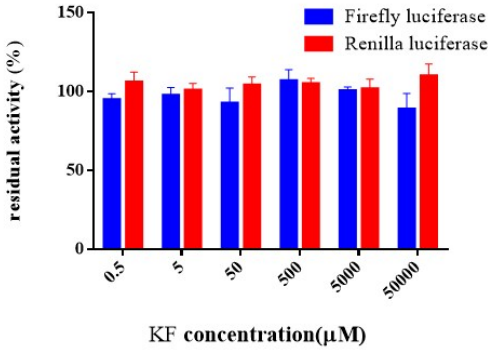

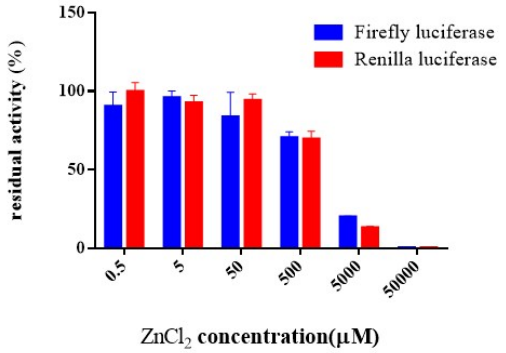
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50
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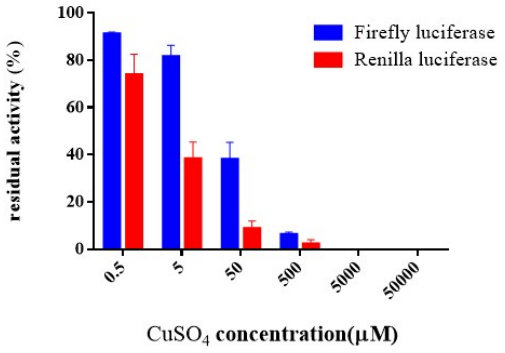
Firefly luciferase
Renilla luciferase
0.5
5
50
500
5000
50000
ZnCl2 concentration(μM)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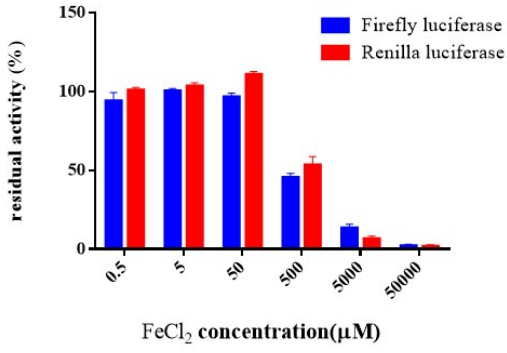
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Firefly luciferase
Renilla luciferase
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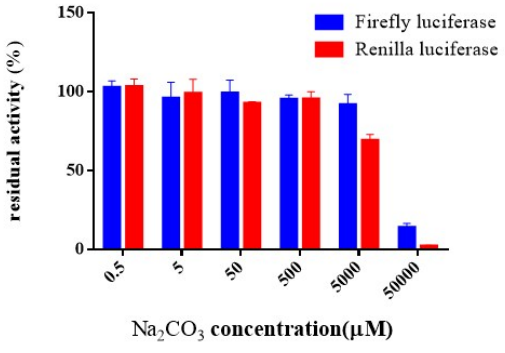
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Firefly luciferase
Renilla luciferase
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5
50
500
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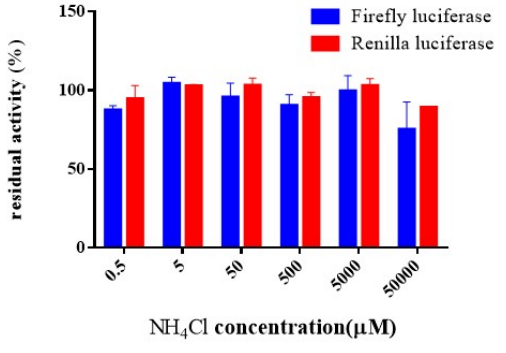
Na2CO3 concentration(μM)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Firefly luciferase
Renilla luciferase
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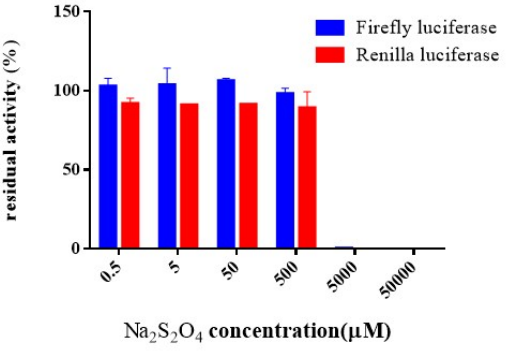
NH4Cl concentration(μM)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Firefly luciferase
Renilla luciferase
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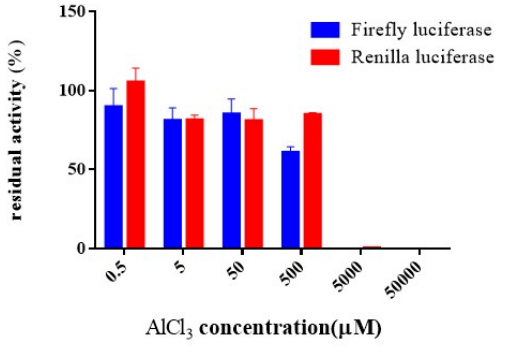
Na2S2O4 concentration(μM)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Renilla luciferase
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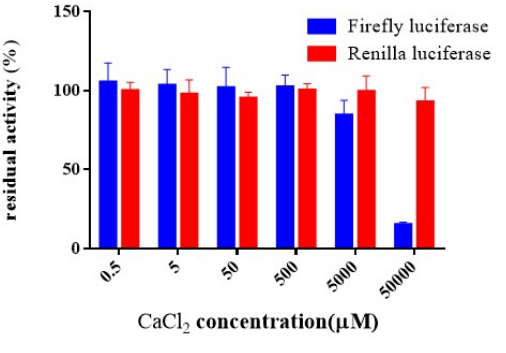
AlCl3 concentration(μM)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Renilla luciferase
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CaCl2 concentration(μM)

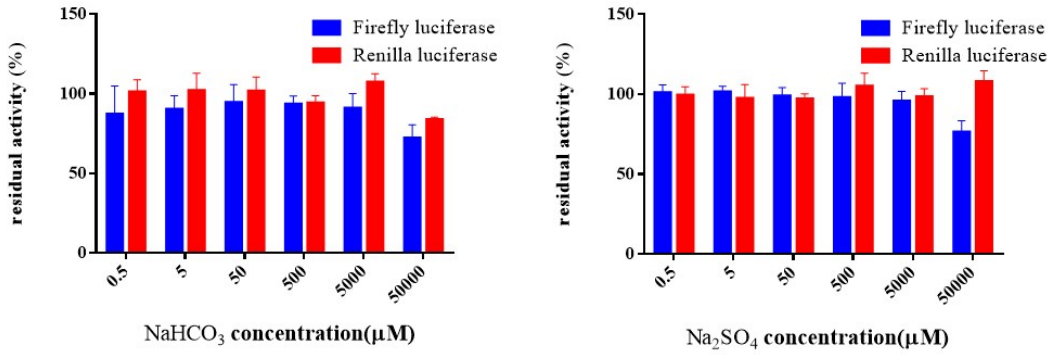

Figure S1. The results of dual luciferase reporter gene simulated assay using other's ionic compounds *in vitro.*

2. Materials and instruments

All salts available from commercial sources were analytical grade. Ultra-pure water was used to prepare all aqueous solutions. Measurements for bioluminescent assays were performed in 50 mM Tris buffer, pH 7.46 with 10 mM $MgSO_4$ at 37°C. The recombinant firefly luciferase was purchased from Promega (E1702, America). Recombinant *Renilla reniformis* luciferase was purchased from RayBiotech. ATP and Tris base were purchased from Aladdin. Coelenterazine was purchased from Chemedir. Luminescence produced by the luciferase was measured with Omega microplate reader (POLARstar Omega, Germany). Bioluminescence imaging was determined with an IVIS Kinetic (Caliper Life Sciences, USA) equipped with a cooled charge-coupled device (CCD) camera. Circular regions of interest (ROI) were drawn and quantified using Living Image software. The intensity of luminescence was reported as total photon flux within an ROI in photons per second.

3. Dual luciferase reporter gene simulated assay *in vitro*

All salts were dissolved in 1M in ultra-pure water, and they were diluted to various concentrations (0 μM, 1 μM, 10 μM, 100 μM, 1 mM, 100 mM) in Tris buffer, containing 5 μM of coelenterazine. The Fluc was diluted to 20 μg/mL and Recombinant *Renilla reniformis* luciferase was diluted to 1 μg/mL in the Tris buffer. Firefly luciferase substrate solution is also prepared in this Tris buffer, containing 40 μM of amino-luciferin and 2 mM of ATP.

To a 96-well plates (WHB, black) each well containing 25 μL of the Fluc solution and 25 μL of the renilla luciferase solution, Tris buffer was added instead of two luciferases as blank groups. After that 50 μL of the firefly luciferase substrate solution was added. Then 100 μL of various concentrations (0 μM, 1 μM, 10 μM, 100 μM, 1 mM, 100 mM) of ionic compounds solutions were added immediately. Tris buffer only containing 5 μM of coelenterazine was set as a control group. Luminescence produced by the luciferase-catalyzed reactions was measured with Omega microplate reader (POLARstar Omega, Germany). The data were analyzed with the GraphPad Prism software.

4. ES-2-FLuc cell and ES-2-Rluc cell bioluminescence inhibition assays *in cellulo*

ES-2 cells (human ovarian cancers cell line) were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences. ES-2 cells expressing Fluc (ES-2-FLuc cells) were supplied by Cellcyto and ES-2 cells expressing Rluc (ES-2-Rluc cells) were purchased from BioDiagnosis. ES-2-FLuc cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and ES-2-Rluc cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). They were also cultured at 37°C in a humidified atmosphere with 5% CO_2 incubator.

In ES-2-FLuc cell bioluminescence inhibition assays, 100 μL cell suspension were added into 96-well plates (Corning, 3603) to make the number of cells in each well at about 4×10^4. After incubated for 16 hours, 100 μL ionic compounds of different concentrations (1 μM, 10 μM, 100 μM, 1 mM, 100 mM) dissolved by RPMI 1640 medium without FBS were added as three triplicates. And RPMI 1640 medium without FBS was added directly as a negative control. After incubation for 1.5 hours, the medium was removed, and 100 μL amino-luciferin solution (100 μM, dissolved in Tris buffer of pH 7.46) was added. Also blank group (without cells) containing only 100 μL amino-luciferin solution were set. After the amino-luciferin solution was added, the bioluminescence intensity was measured immediately with IVIS Kinetic (Caliper Life Sciences, USA) instrument equipped with a cooled charge coupled device (CCD) camera for bioluminescent imaging. The exposure time was one second. Circular regions of interest (ROI) were drawn and quantified using Living Image software. The data were reported as total photon flux within an ROI in photons per second and were analyzed using the GraphPad Prism software.

In ES-2-Rluc cell bioluminescence inhibition assays, the experimental procedure was preformed as described previously except using DMEM medium instead of RPMI 1640 medium and coelenterazine solution (10 μM, dissolved in saline). The exposure time was ten seconds. As showed in Fig S1, NaN_3 showed selectively inhibit Fluc while having little effect on Rluc *in cellulo*.

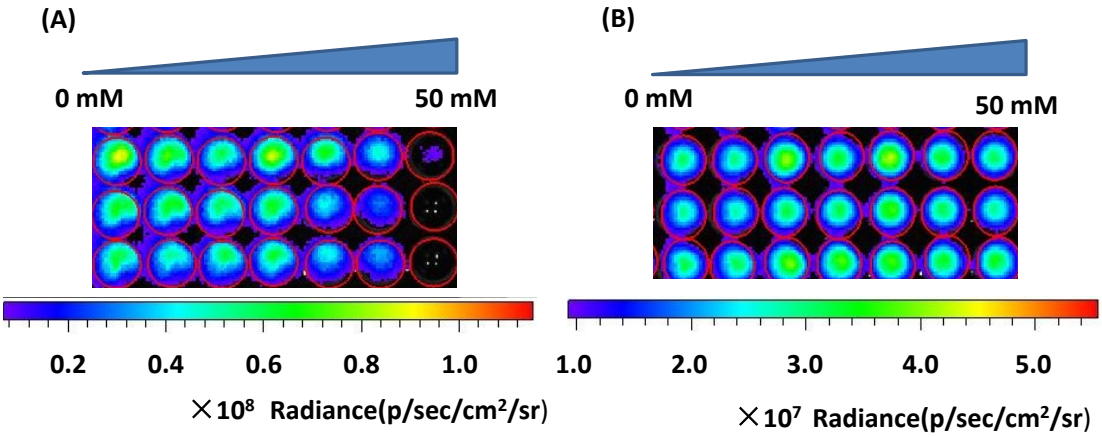

Figure S2. Bioluminescence imaging of Fluc inhibition (A) and Rluc inhibition (B) by NaN_3 *in cellulo*.

5. ES-2-FLuc cell bioluminescence inhibition assays for different incubation time

100 μL cell suspension were added into 96-well plates (Corning, 3603) to make the number of cells in each well at about 4×10^4. After incubated for 16 hours, 100 μL ionic compounds of different concentrations (1 μM, 10 μM, 100 μM, 1 mM, 10mM, 100 mM) dissolved by RPMI

1640 medium without FBS were added. And RPMI 1640 medium without FBS was added directly as a negative control. After incubation for 0.3, 10, 30 min in the incubator, the medium removed, and 100 μL amino-luciferin solution (100 μM, dissolved in Tris buffer of pH 7.46) was added. The bioluminescence intensity was measured immediately with IVIS Kinetic (Caliper Life Sciences, USA) instrument equipped with a cooled charge coupled device (CCD) camera for bioluminescent imaging. The data were reported as total photon flux within an ROI in photons per second and were analyzed using the GraphPad Prism software. These results demonstrated that some ionic solution under high concentration were able to rapidly inhibit the firefly luciferase activity in few seconds *in cellulo,* which might be used in improved dual luciferase reporter gene assay.

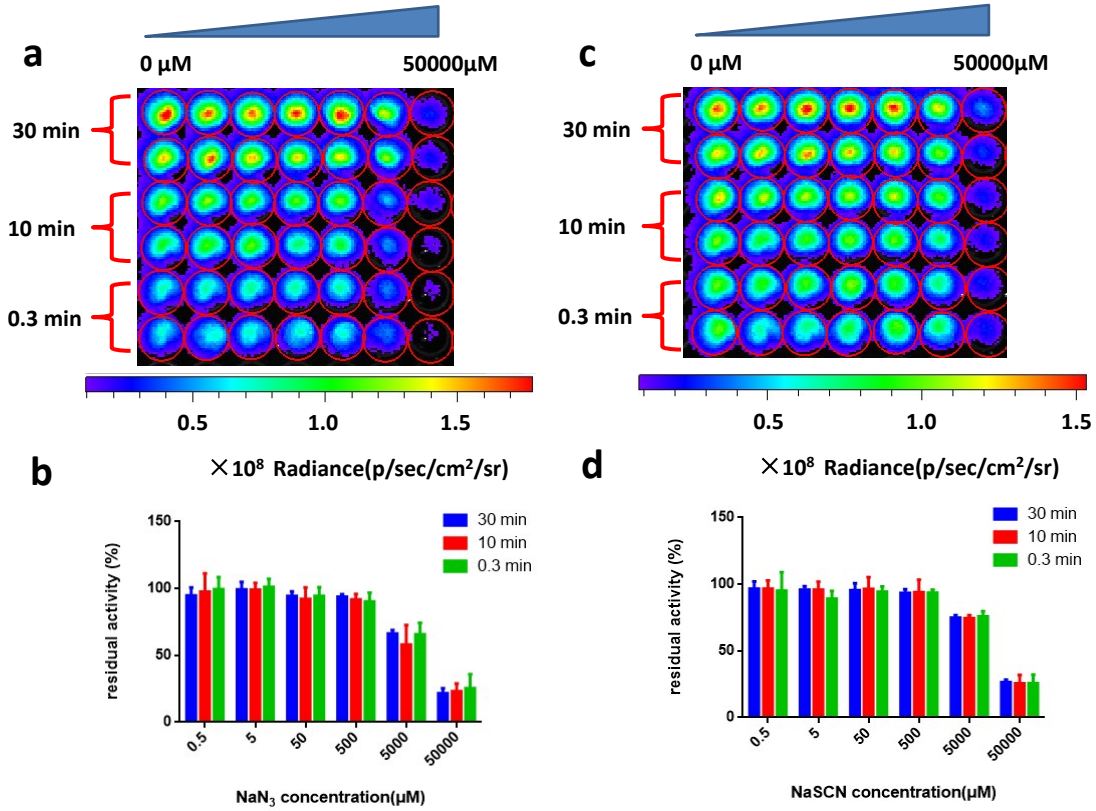

Figure S3. Bioluminescence imaging of ES-2-FLuc cell incubated with NaN_3 (a) and NaSCN (c) for different times; (b) quantification of residual luciferase activity of (a); (d) quantification of residual luciferase activity of (c).

6. The effect of absorption spectra of amino-luciferin and emission spectra of Fluc by ions

For the recording changes of absorption spectra of amino-luciferin，2 mL of amino-luciferin solution (40 μM, containing ionic compounds of different concentrations or not in Tris buffer of pH 7.46) was added into a quartz cell and the mixture was measured with a TU-1901 UV-VIS Spectrophotometer. The results indicated that some strong salt solution (5 mM, 50 mM) didn't change absorption spectra of aminoluciferin (Figure S4).

For the recording changes of bioluminescence emission spectra, 0.5 mL of Fluc substrate solution (containing ionic compounds of different concentrations or not Tris buffer of pH 7.46) was added into 0.5 mL of the Fluc solution (20 μg/mL) in a quartz cell

and the mixture was immediately measured with a F-2500 FL Spectrophotometer in luminescence mode with the lamp off at a scan rate of 3000nm/min. The result showed that some strong salt solution (5 mM, 50 mM) didn't change firefly luciferase bioluminescence maximum emission wavelength. (Figure S4)

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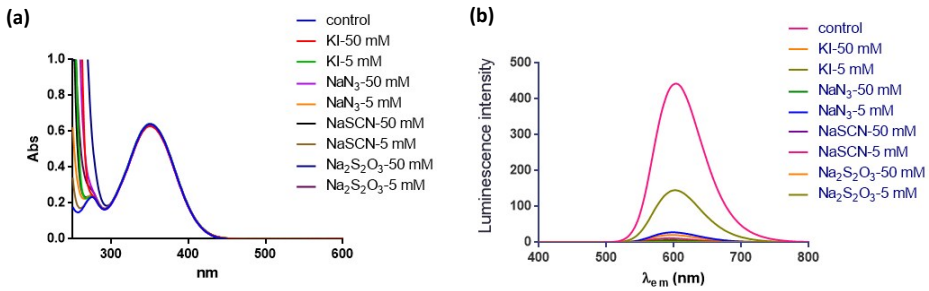

Figure S4. The effect of absorption spectra of amino-luciferin (a) and emission spectra of Fluc bioluminescence (b) by ions