

# **Biological evaluation in resistant cancer cells and study of mechanism of action of Arylvinyl-1,2,4-Trioxanes**

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## Supplementary Materials

<b>Table S1. Additional cell lines data.....</b>	<b>4</b>
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<b>Cytotoxicity Assays .....</b>	<b>5</b>
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<i>Figure S1 Cytotoxicity assay against A549T cell lines .....</i>	<i>5</i>
<i>Figure S2 Cytotoxicity assay against A2780/WT cell lines .....</i>	<i>6</i>
<i>Figure S3 Cytotoxicity assay against A2780/CDDP cell lines .....</i>	<i>7</i>
<i>Figure S4. Cytotoxicity assay against HCT-8/WT cell lines .....</i>	<i>8</i>
<i>Figure S5. Cytotoxicity assay against HCT-8 TR cell lines.....</i>	<i>9</i>
<i>Figure S6. Cytotoxicity assay against MCF-7 WT cell lines .....</i>	<i>10</i>
<i>Figure S7. Cytotoxicity assay against MCF-7/ADR cell lines.....</i>	<i>11</i>
<i>Figure S8. Cytotoxicity assay against SGC7901 WT cell lines.....</i>	<i>12</i>
<i>Figure S9. Cytotoxicity assay against SGC7901/CDDPR cell lines.....</i>	<i>13</i>
<i>Figure S10. Cytotoxicity assay against LO2 and CCD19Lu normal cell lines.....</i>	<i>13</i>

<b>Rhodamine 123 Exclusion Assays.....</b>	<b>14</b>
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<i>Figure S11. Compound 1.....</i>	<i>14</i>
<i>Figure S12. Compound 2.....</i>	<i>14</i>
<i>Figure S13. Compound 3.....</i>	<i>15</i>
<i>Figure S14. Compound 4.....</i>	<i>15</i>
<i>Figure S15. Compound 5.....</i>	<i>16</i>
<i>Figure S16. Compound 6.....</i>	<i>16</i>
<i>Figure S17. Compound 9.....</i>	<i>16</i>
<i>Figure S18. Compound 10.....</i>	<i>16</i>

<b>UV-Vis analysis .....</b>	<b>19</b>
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<i>Figure S19. UV-Vis analysis of oxidation of reduced riboflavin (RFH2) by trioxane 8.....</i>	<i>Error! Bookmark not defined.</i>
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<b>In silico studies .....</b>	<b>20</b>
--------------------------------	-----------

<i>Figure S20. Validation of docking reliability by using the known crystallize X-ray structure of target protein P-gp complexed with Zosuquidar.....</i>	<i>20</i>
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<i>Table S2. Docking scores of synthesized and standard compound 7 and 8 against P-gp protein (PDB ID: 6FN1)</i>	20
<i>Table S3. MM-GBSA result for 7, 8 and Artesunic acid</i>	21
<i>References</i>	21

## Cytotoxicity studies

**Table S1. Additional data of cell lines**

Cytotoxicity of compounds **1-10** against normal, wild-type cancer and resistant cancer cell lines with selectivity index (S.I), compared to paclitaxel (**PTX**) and Cisplatin (**CDDP**).

Selectivity index (S.I.) = IC<sub>50</sub> for wild type cells / IC<sub>50</sub> for resistant cancer cells

HCT-8/TR: Taxol-resistant human colorectal cancer cell line

SGC7901/CDDP: Cisplatin-resistant human gastric cancer cell line

Compounds	LO2 cells IC <sub>50</sub> [μM] (SD)	CCD-19Lu IC <sub>50</sub> [μM] (SD)	HCT-8/WT cells IC <sub>50</sub> [μM] (SD)	HCT-8/TR cells IC <sub>50</sub> [μM] (SD)	S.I.	SGC7901 cells IC <sub>50</sub> [μM] (SD)	SGC7901/CD DP cells IC <sub>50</sub> [μM] (SD)	S.I.
<b>1</b>	-	-	41.052±2.1	55.16±4.4	0.74	>100	>100	---
<b>2</b>	-	-	52.28±3.2	58.43±5.3	0.89	>100	>100	---
<b>3</b>	-	-	51.48±2.77	39.05±3.2	1.32	>100	>100	---
<b>4</b>	-	-	17.44±5.2	19.20±4.2	0.9	68.39±2.2	28.73±0.1	2.38
<b>5</b>	-	-	50±3.9	38.16±3.60	1.31	>100	>100	---
<b>6</b>	-	-	>100	>100	---	>100	>100	---
<b>7</b>	5.27±0.03	2.36±0.20	12.20±1.22	3.51±0.2	3.47	7.82±1.2	7.44±1.1	1.05
<b>8</b>	4.31±0.05	0.56±0.01	27.75±3.08	7.64±0.77	3.63	20.10±2.1	18.69±1.02	1.07
<b>9</b>	-	-	>100	28.76±1.08	3.48	58.43±0.1	NA	NA
<b>10</b>	-	-	>100	>100	---	>100	NA	NA
<b>PTX</b>	-	-	58.29±6.2	>100	0.58	0.17±0.1 <sup>b</sup>	NA	NA
<b>CDDP</b>	-	-	>100	49.35±2.2	2.04	10.19±0.2	13.54±1.2	0.75

<sup>a</sup>Data retrieved from [1]; NA = Not applicable.

IC<sub>50</sub> values for BEAS-2B were available in our previous publication [2].

## Cytotoxicity Assays

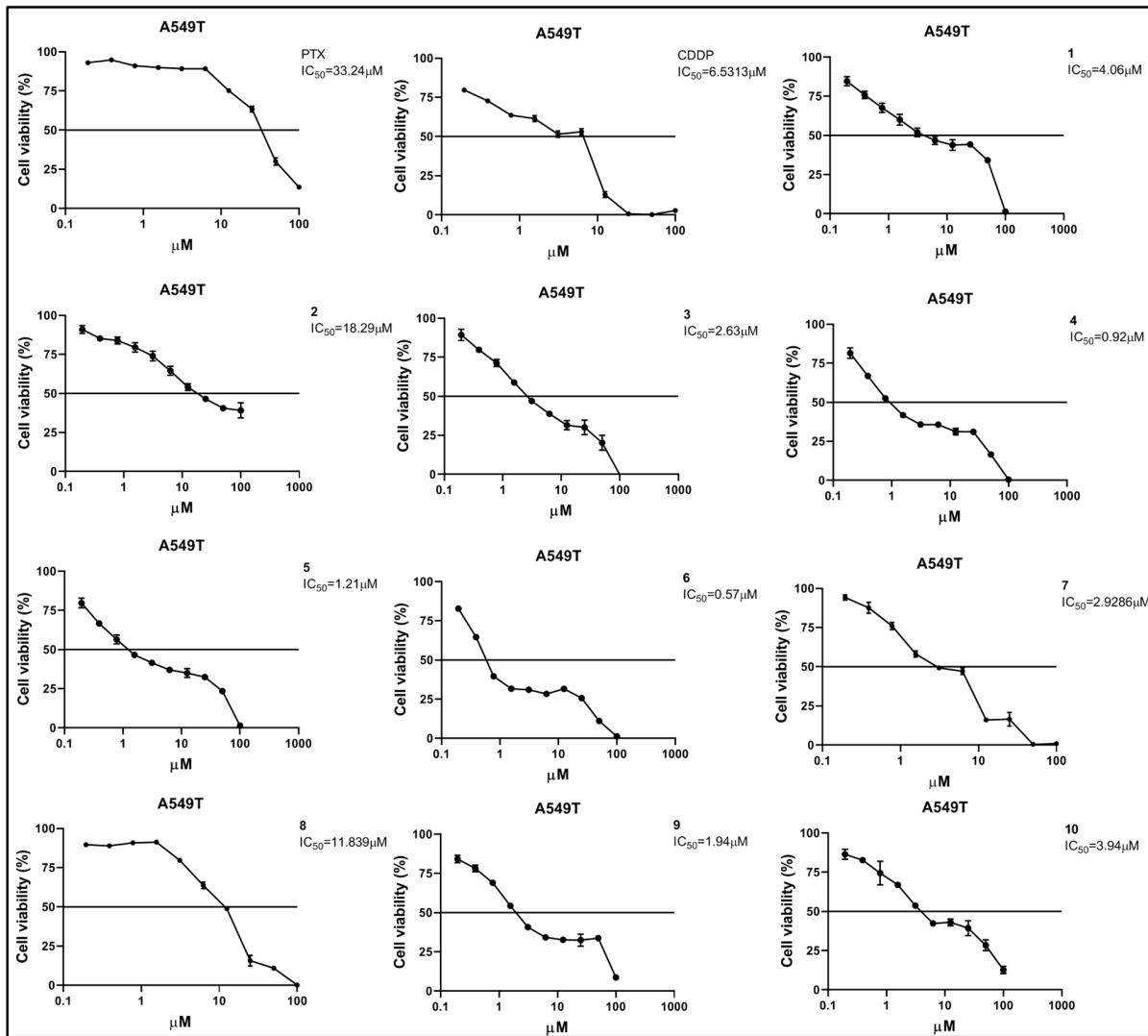


Figure S1 Cytotoxicity assay against A549T cell lines

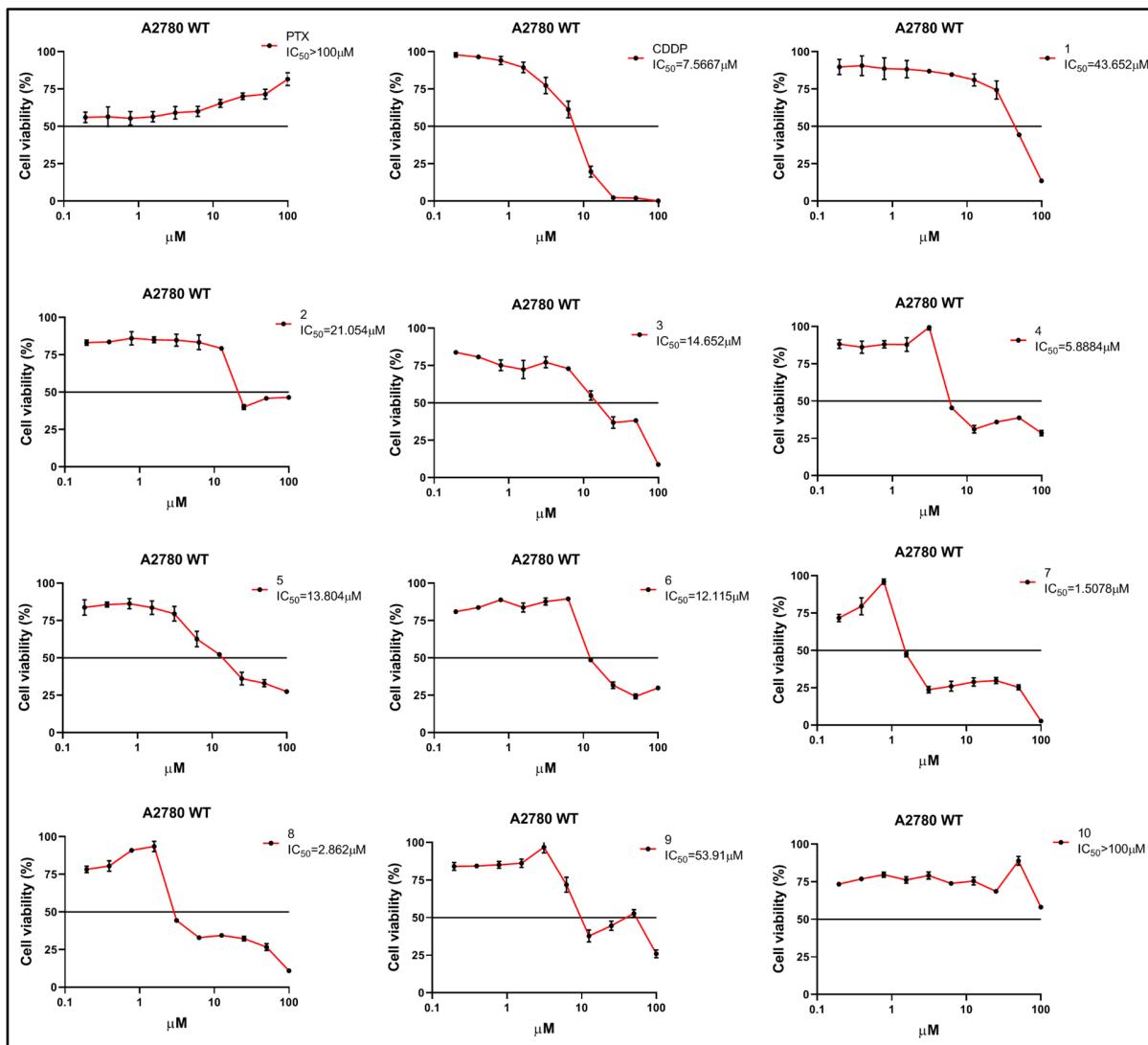


Figure S2 Cytotoxicity assay against A2780/WT cell lines

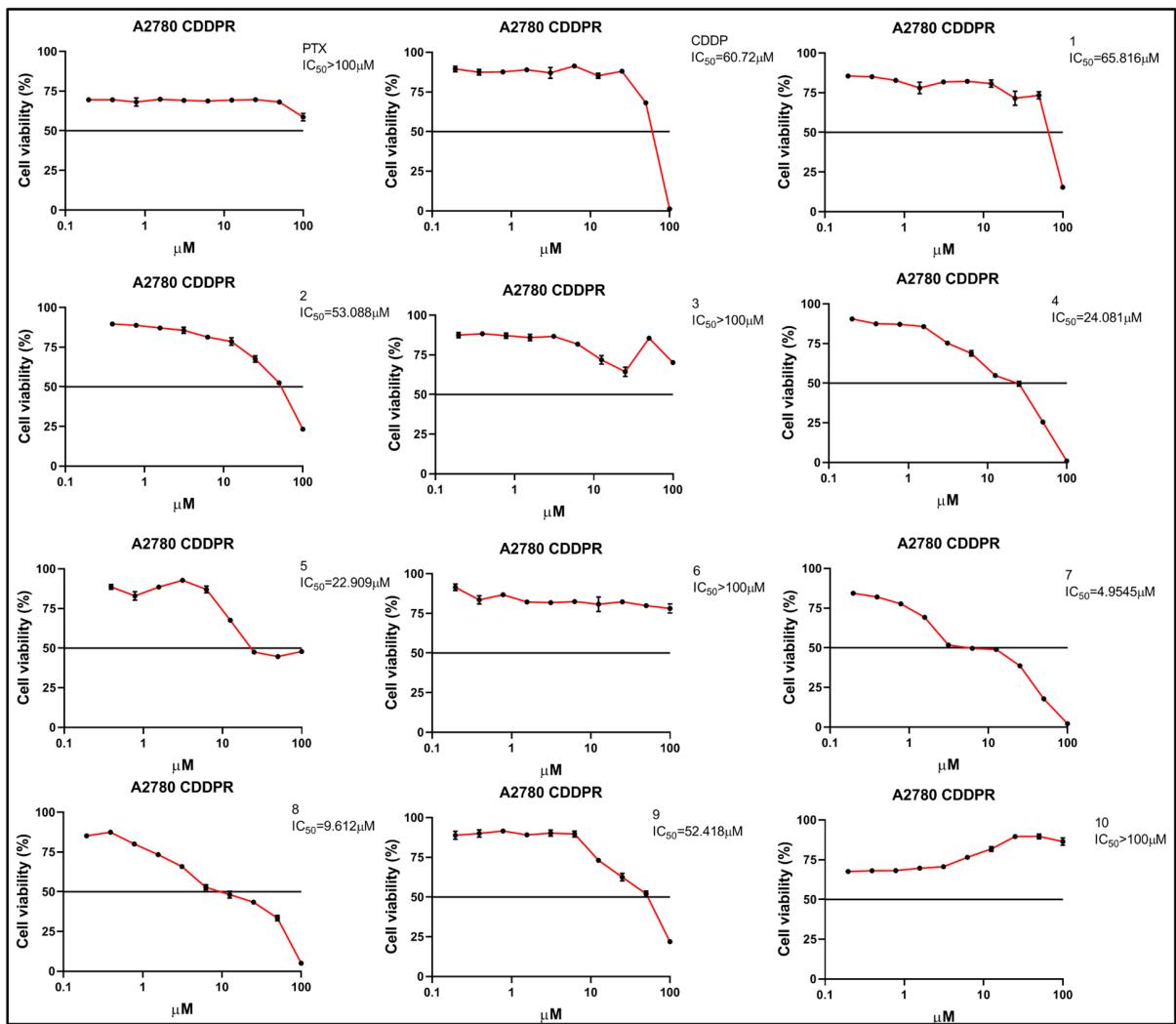


Figure S3 Cytotoxicity assay against A2780/CDDP cell lines

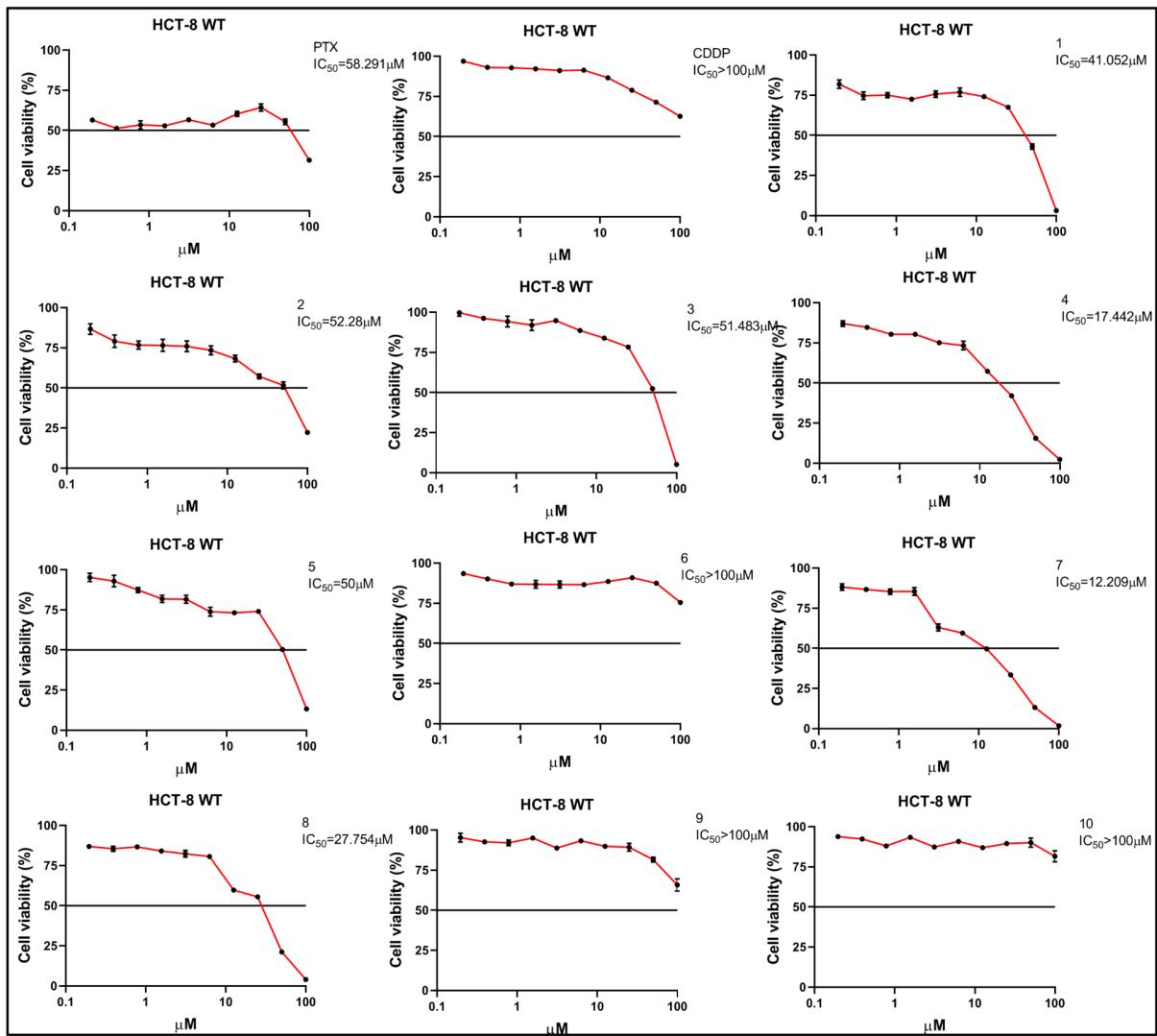


Figure S4. Cytotoxicity assay against HCT-8/WT cell lines

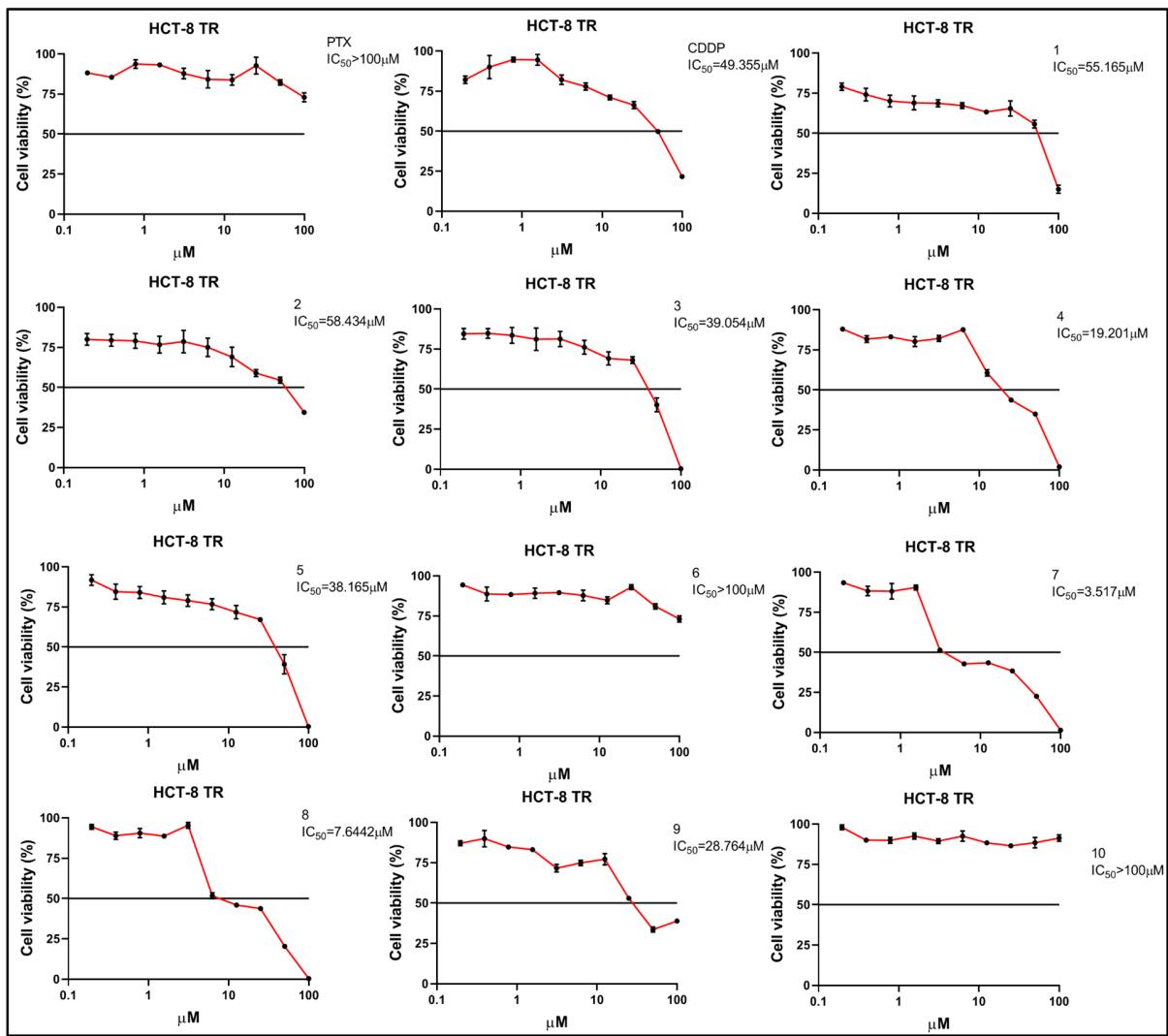


Figure S5. Cytotoxicity assay against HCT-8/TR cell lines

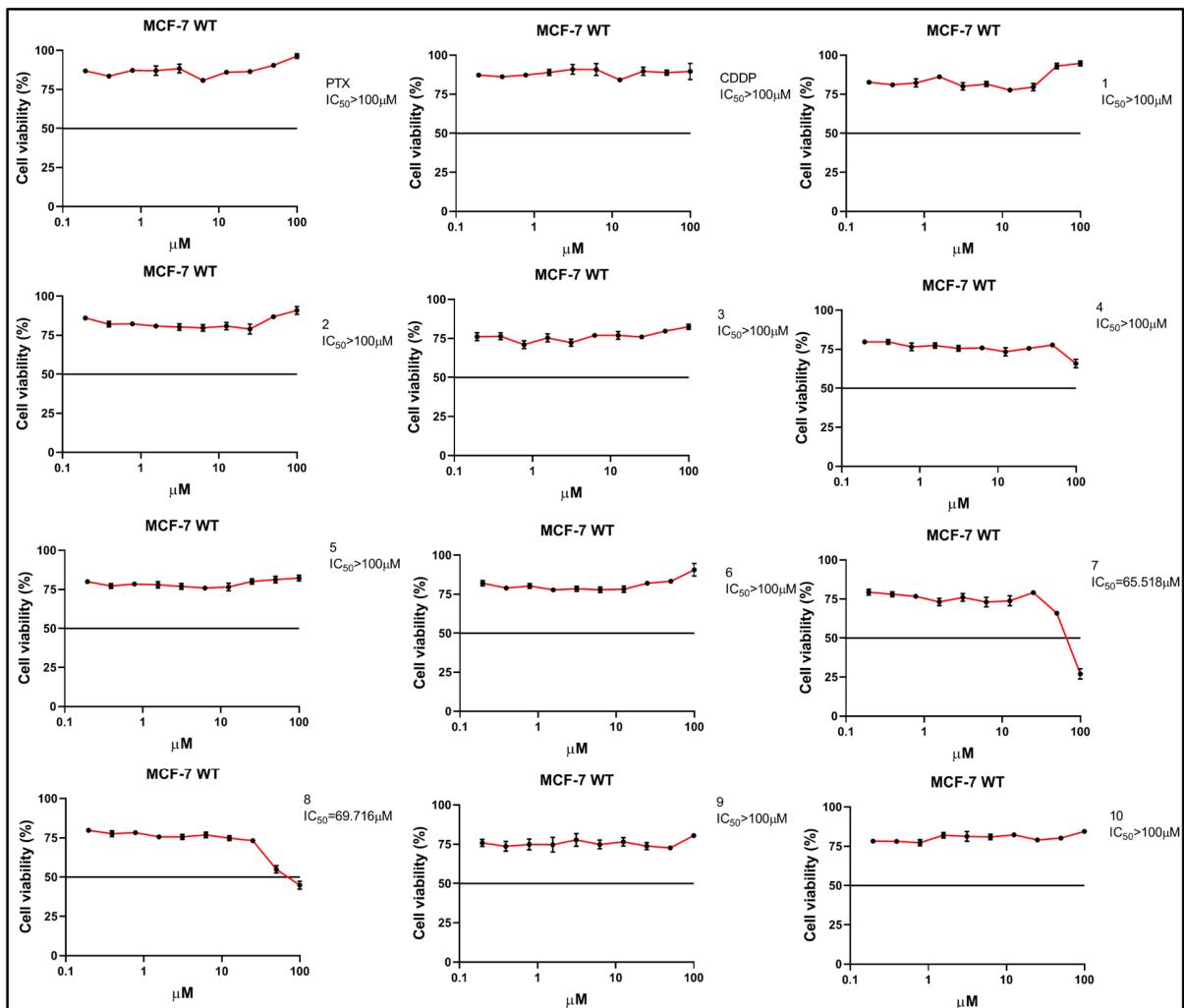


Figure S6. Cytotoxicity assay against MCF-7/WT cell lines

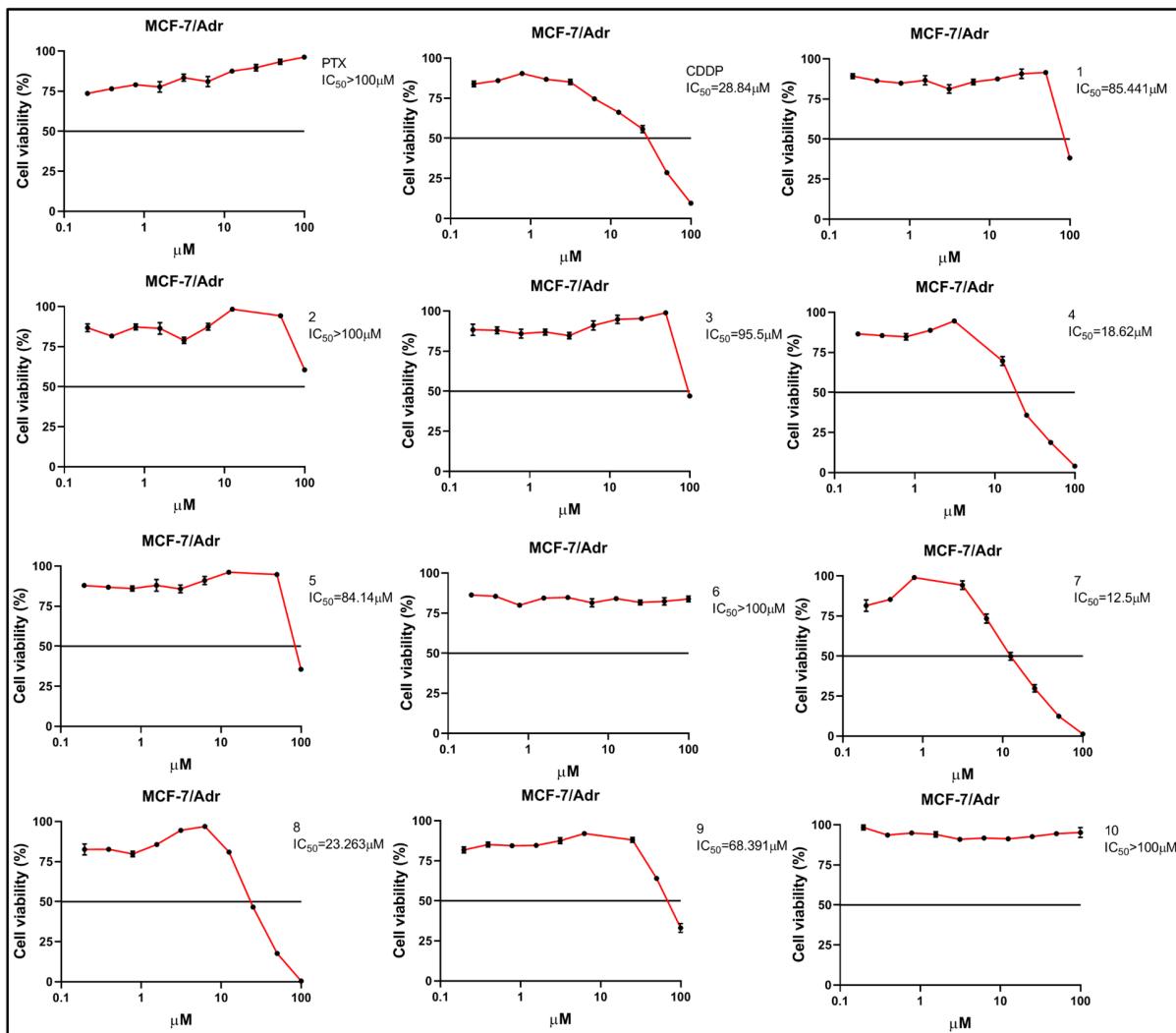


Figure S7. Cytotoxicity assay against MCF-7/ADR cell lines

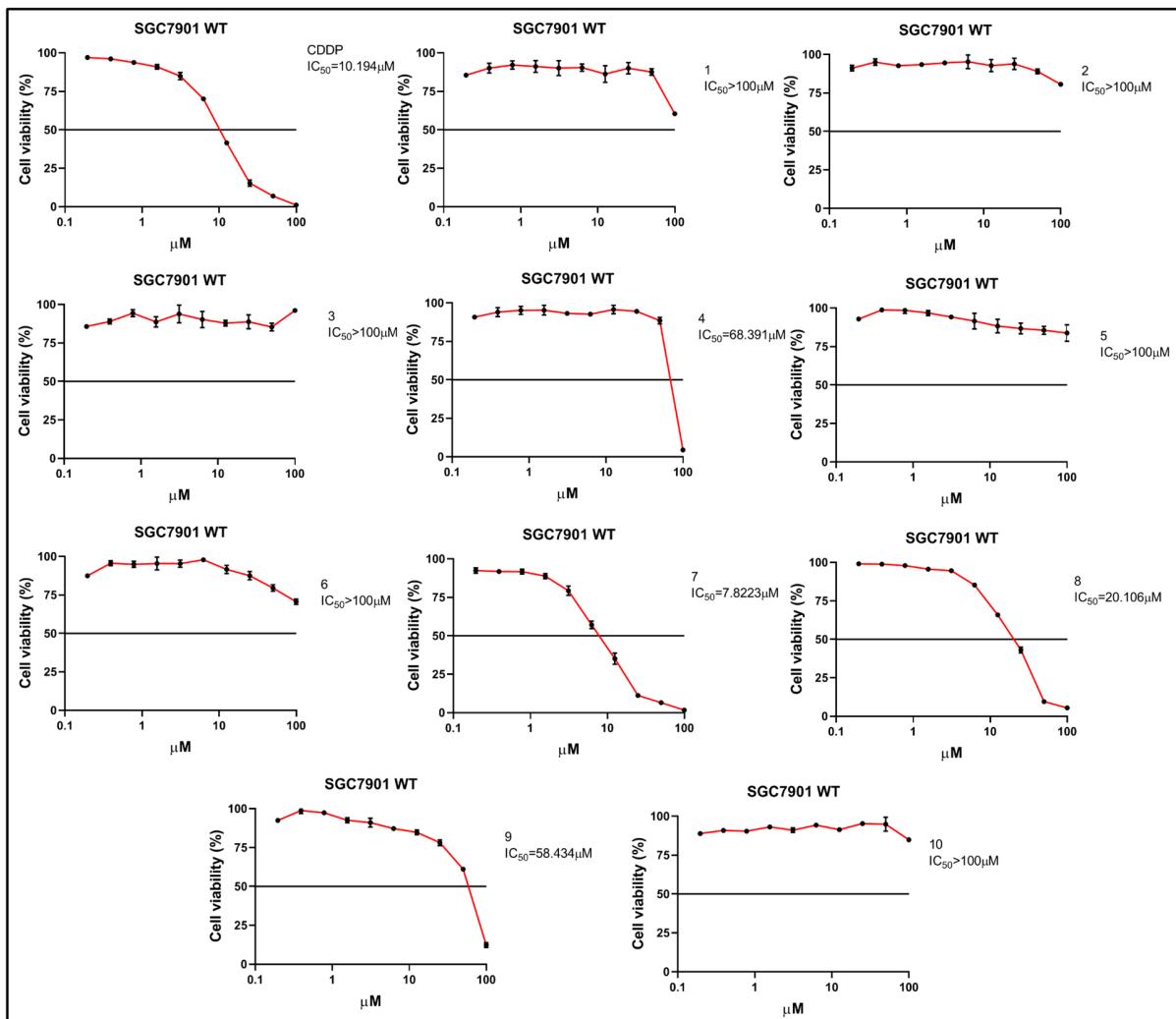


Figure S8. Cytotoxicity assay against SGC7901/WT cell lines

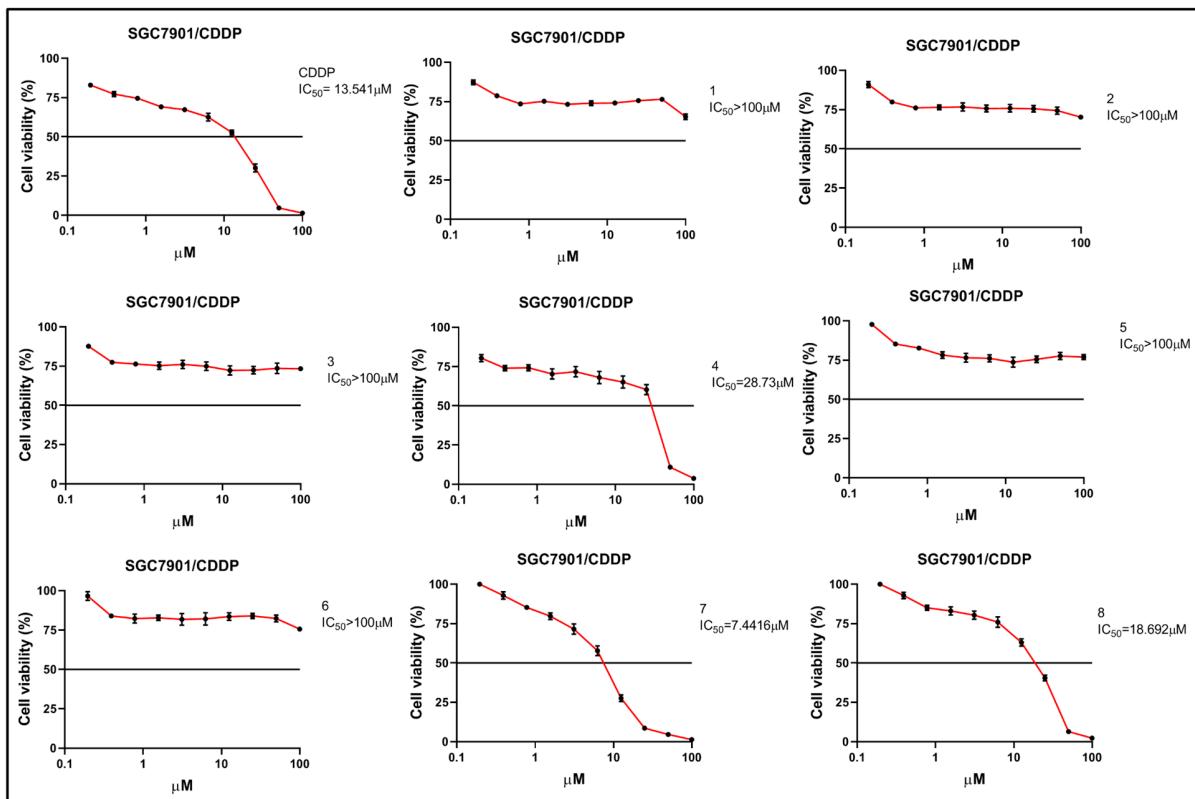


Figure S9. Cytotoxicity assay against SGC7901/CDDP cell lines

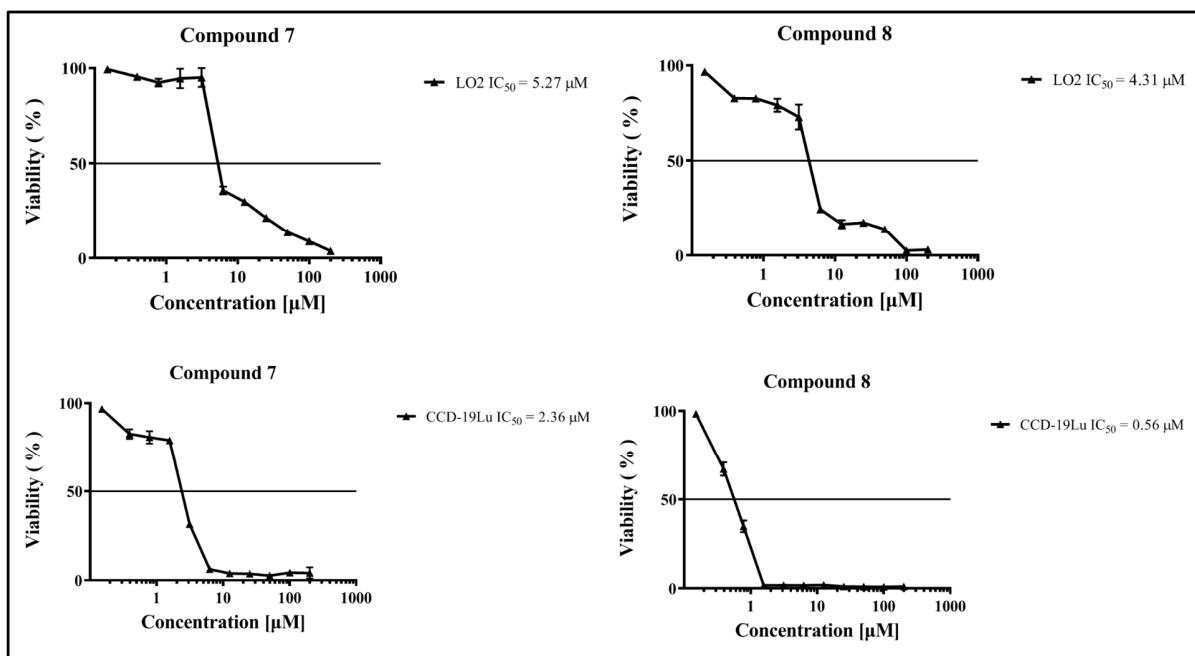


Figure S10. Cytotoxicity assay against LO2 and CCD19Lu normal cell lines

## Rhodamine 123 Exclusion Assays

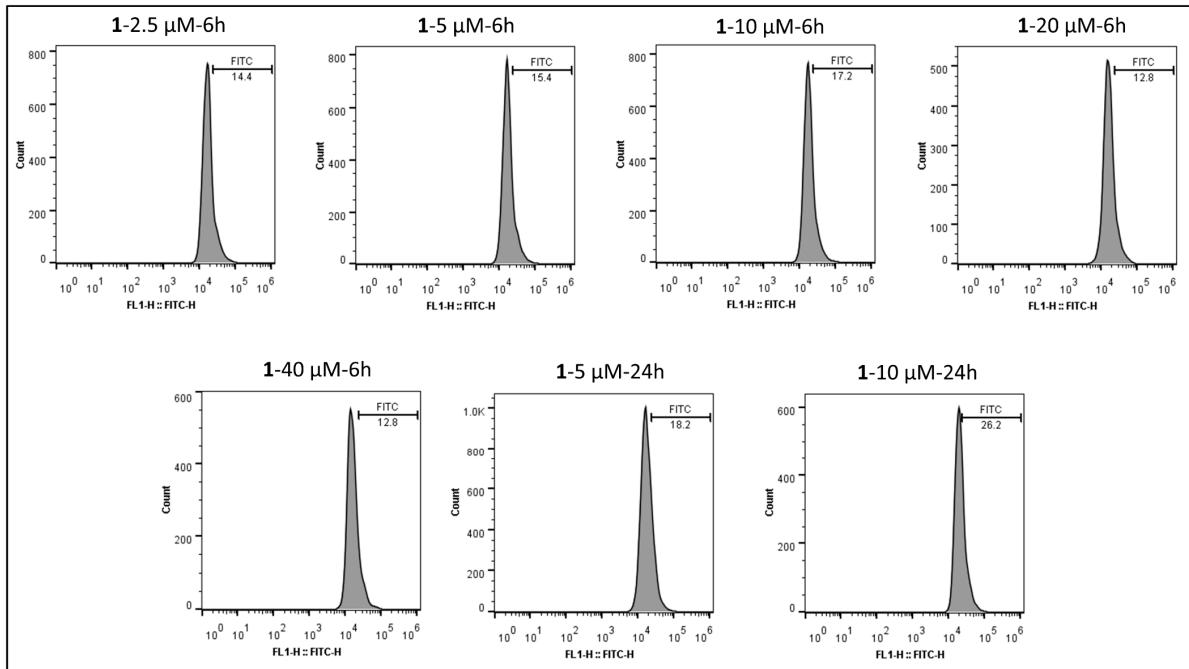


Figure S11. Compound 1

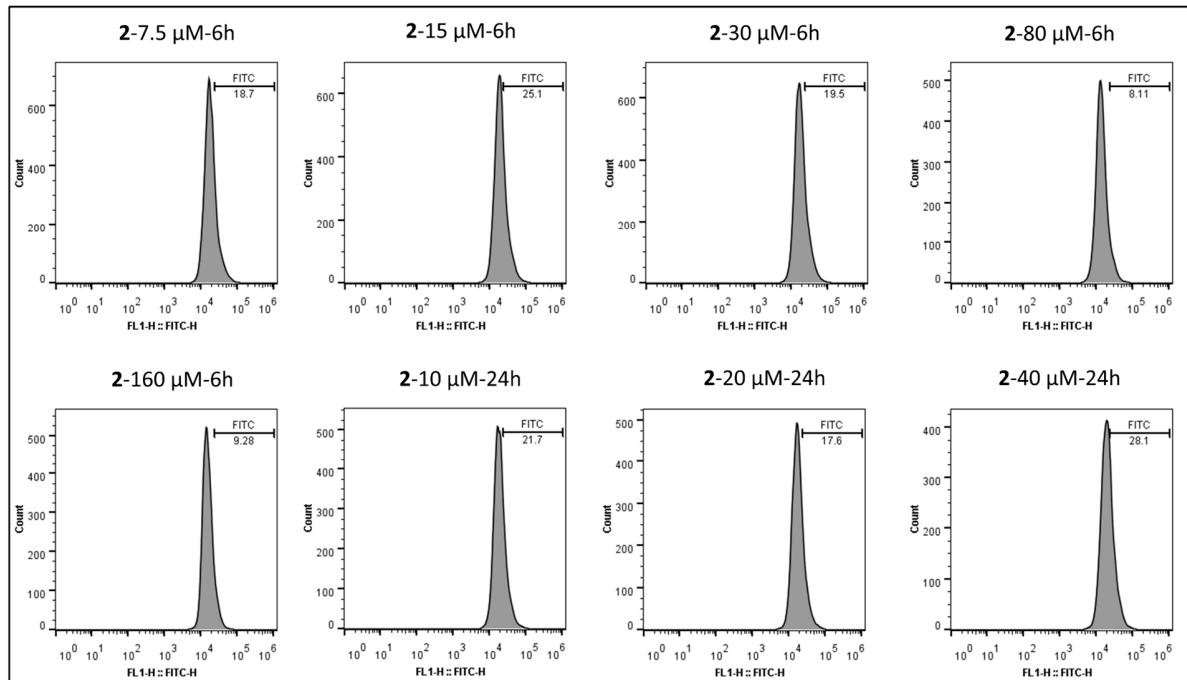
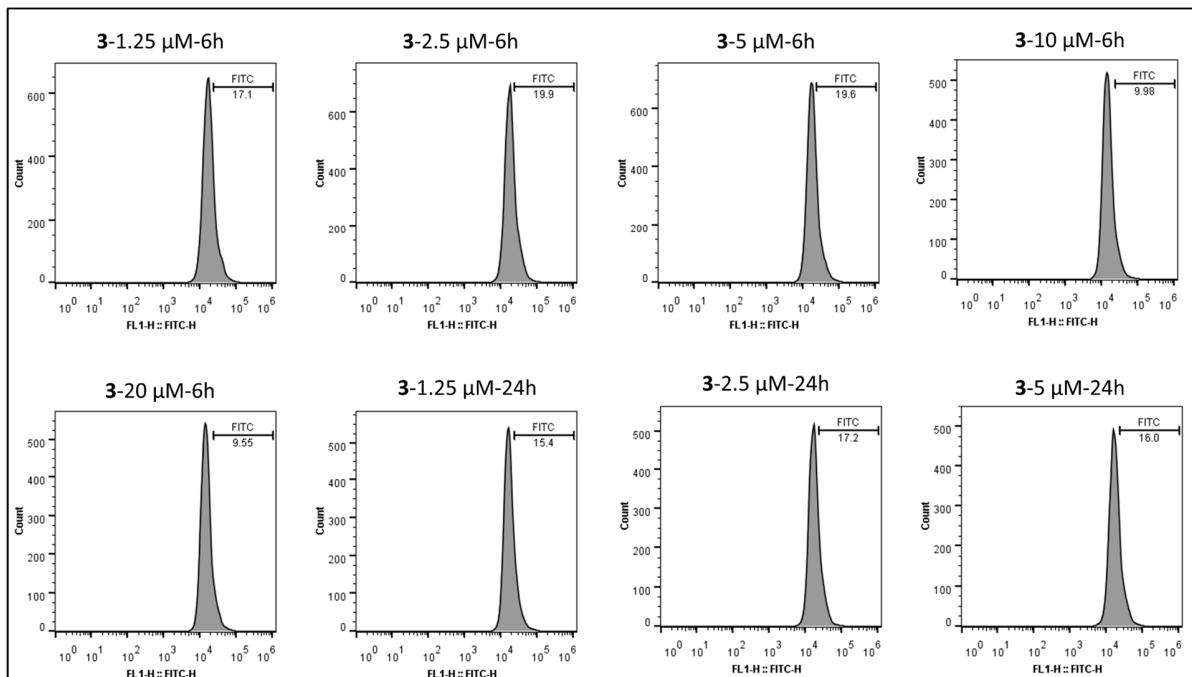
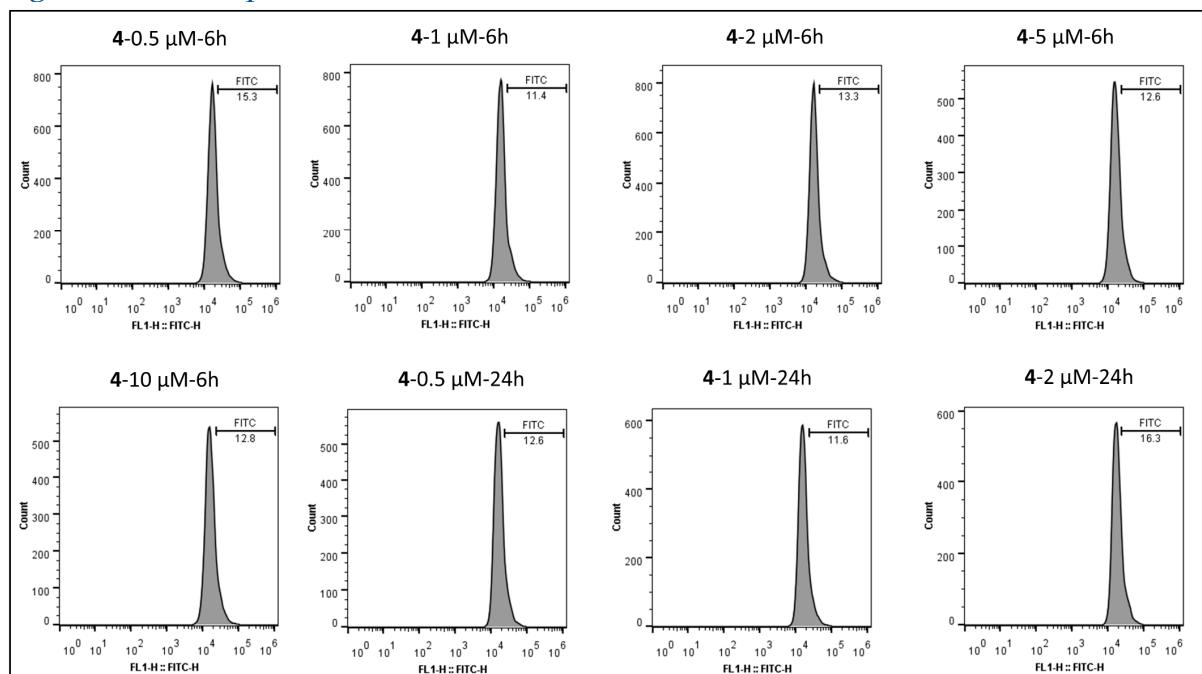


Figure S12. Compound 2



**Figure S13. Compound 3**



**Figure S14. Compound 4**

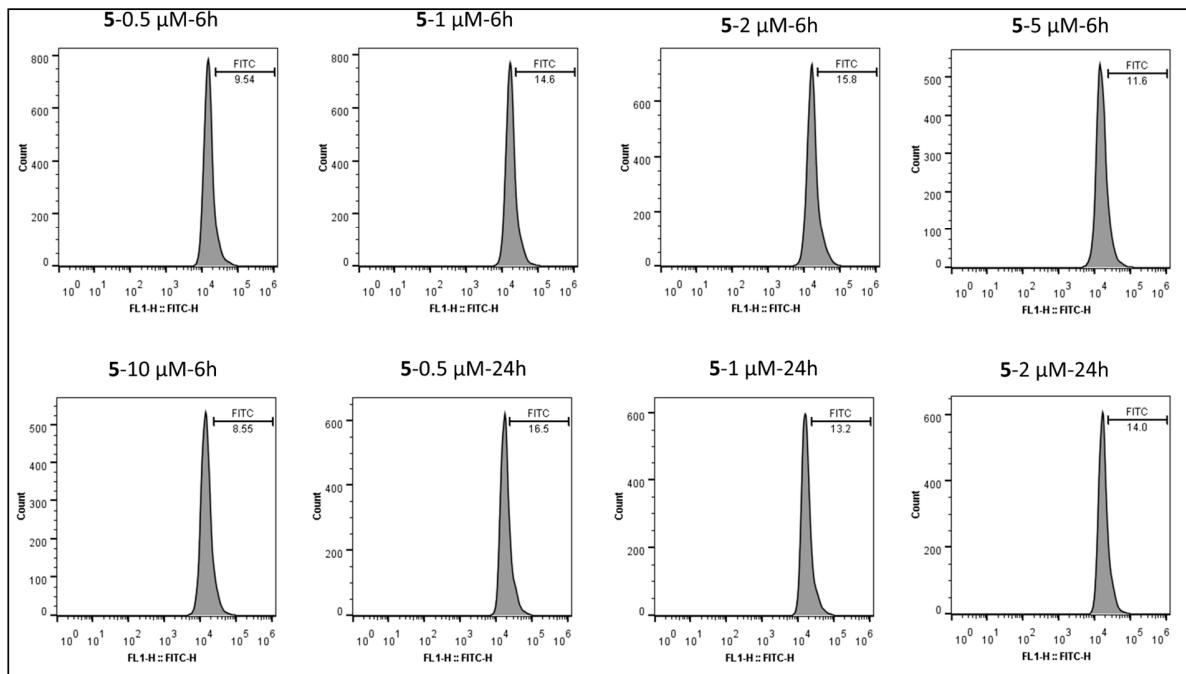


Figure S15. Compound 5

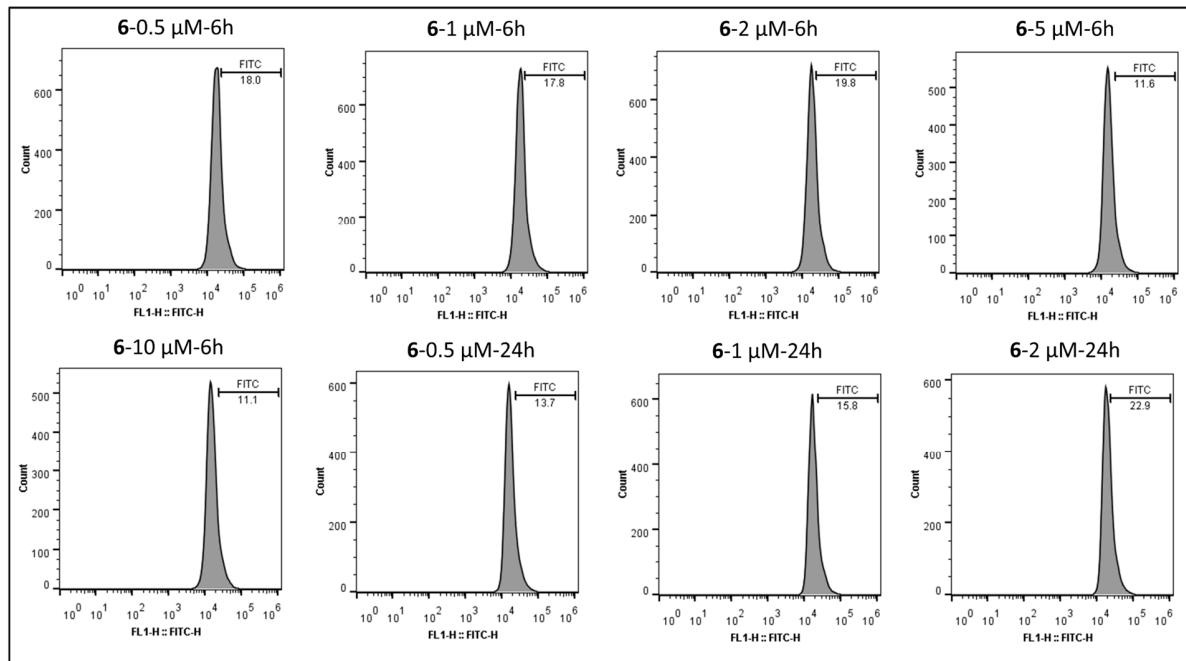


Figure S16. Compound 6

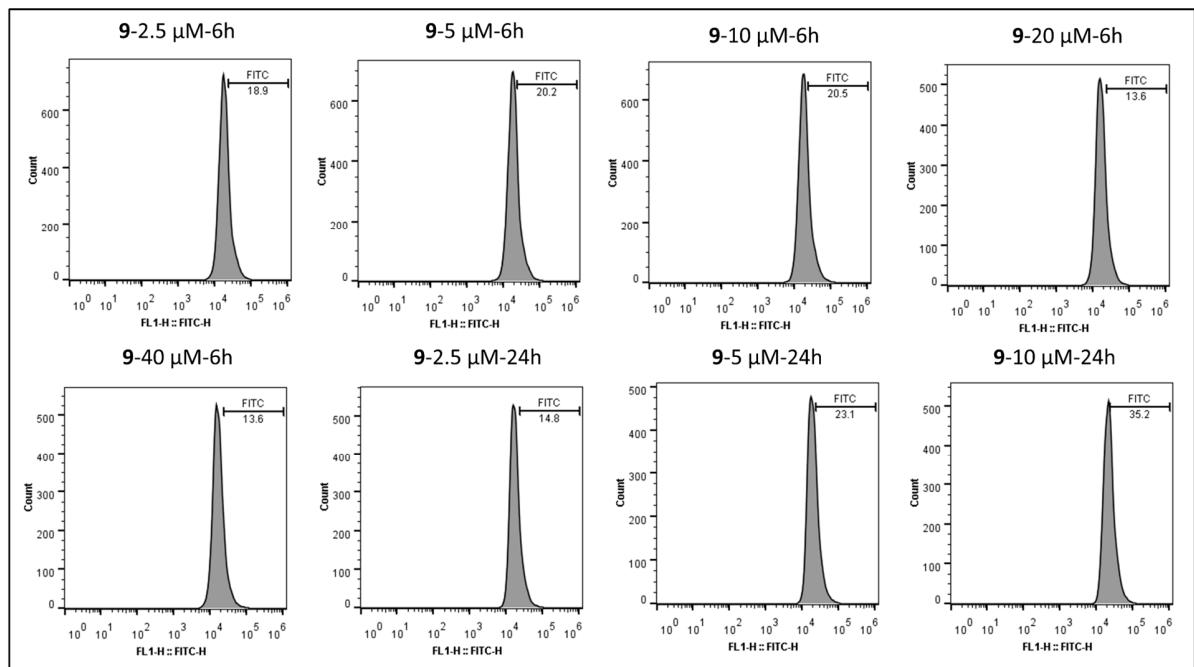


Figure S17. Compound 9

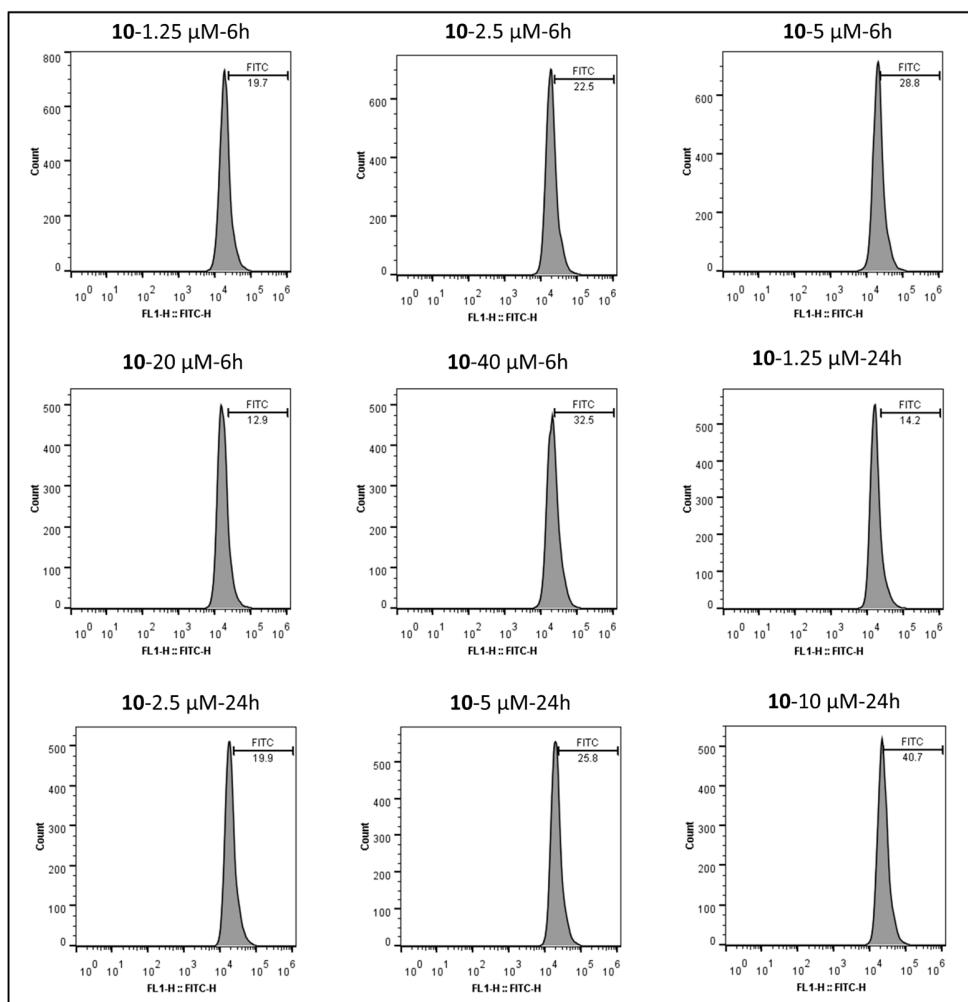
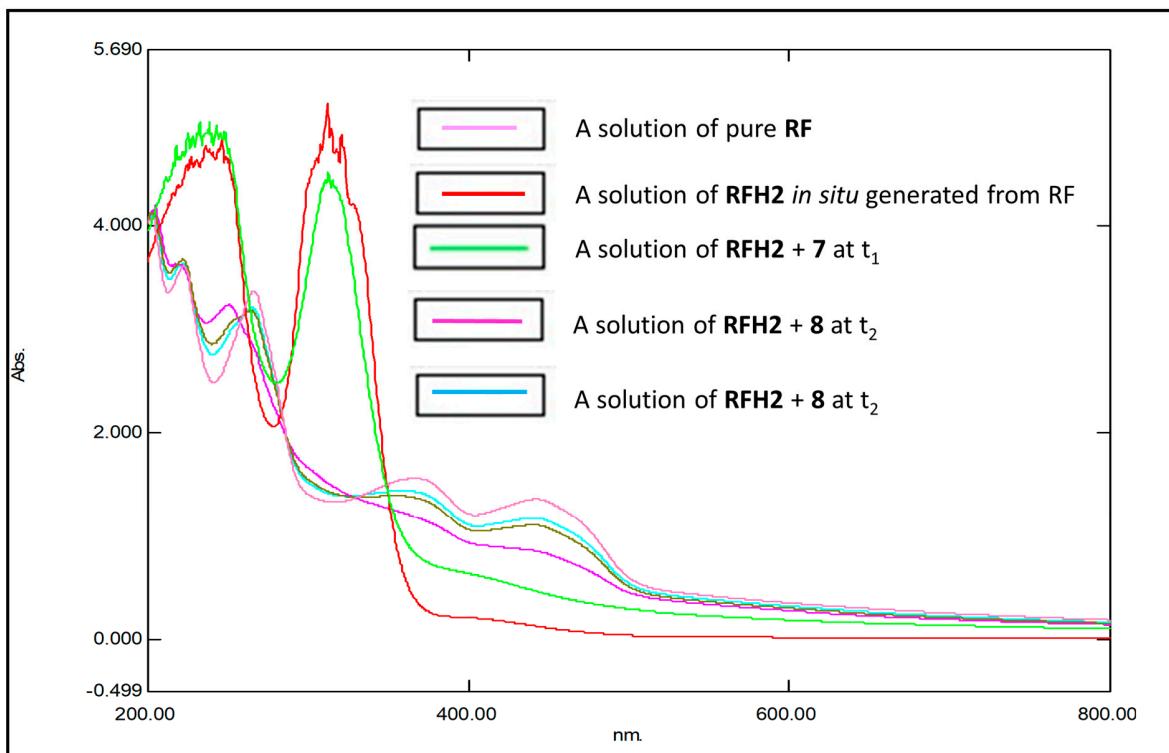


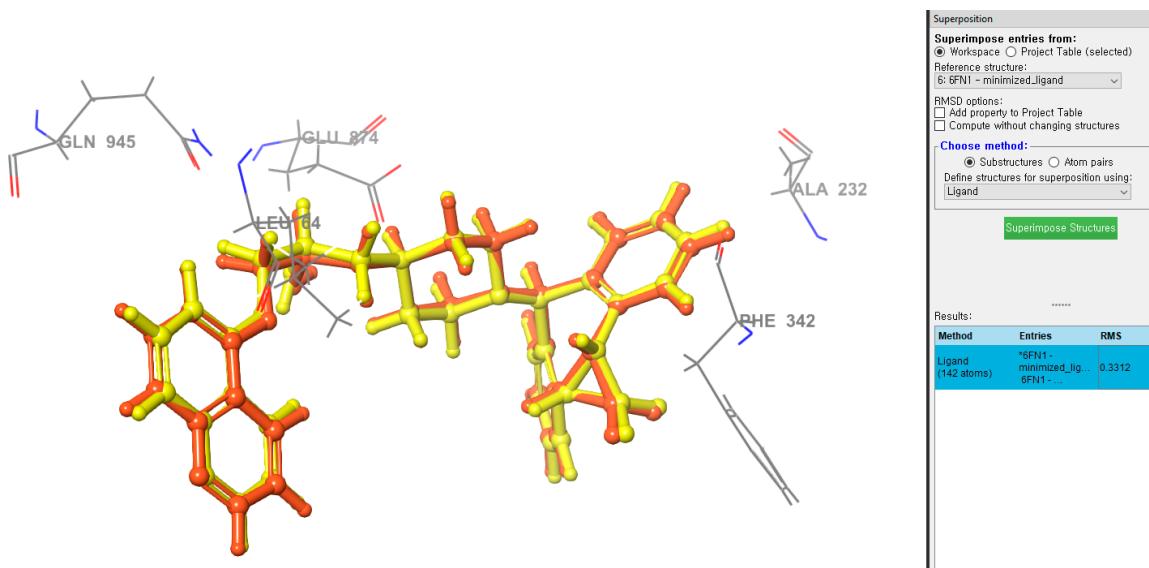
Figure S18. Compound 10

## UV-Vis analysis



**Figure S19** UV-Vis analysis of oxidation of reduced riboflavin (RFH2) by trioxane 8. RF ( $6.6 \times 10^{-4}$  mmol) was completely converted to RFH2 (disappearance of absorption bands at 370 and 445 nm) by the treatment with sodium dithionite ( $1.4 \times 10^{-4}$  mmol) under argon at 37 °C. Trioxane 8 ( $4 \times 10^{-4}$  mmol) in MeCN was added, and the absorptions of the mixture at 370 and 445 nm were monitored at an interval ( $t_1-t_3$ ) of 20 s until complete oxidation of RFH2 back to RF (within 10 min).

## In silico studies



**Figure S20.** Validation of docking reliability by using the known crystallize X-ray structure of target protein P-gp complexed with Zosuquidar.

The docking reliability was validated by using the known X-ray crystal structure of target protein P-gp complexed with Zosuquidar. The co-crystallized Zosuquidar inhibitor was redocked into the binding site, and the docked conformation with the highest score of -7.479 was selected as the most probable binding conformation. The low root mean-square deviation (RMSD) of 0.3312 Å between the docked and the crystal conformations indicates the high reliability of Glide software in reproducing the experimentally observed binding mode for this inhibitor. As shown in Figure S20, redocked molecules were almost in the same position with co-crystallized at the active site of Zosuquidar inhibitor.

**Table S2.** Docking scores of synthesized and standard compound **7** and **8** against P-gp protein (PDB ID: 6FN1)

Entry	Compound	Docking score (kcal/mol)	Glide XP Energy (kcal/mol)	Glide XP Emodel (kcal/mol)
1	<b>7</b>	-7.089	-7.089	-42.051
2	<b>8</b>	-8.196	-8.196	-36.935
3	Artesunic acid	-6.252	-6.253	-36.817

Table S3:MM-GBSA result for **7**, **8** and Artesunic acid

Entry	dG (Bind)	dG (Coulomb)	dG (Covalent)	dG (Hbond)	dG (Lipo)	dG (Solv_GB)	dG (vdW)
<b>7</b>	-96.714	-5.118	0.032	-0.010	-53.757	10.733	-45.193
<b>8</b>	-93.841	-3.696	0.766	-0.10	-54.440	10.042	-46.006
Artesunic acid	-70.435	-13.595	1.111	-0.027	-39.027	25.087	-43.984

## References

1. Shang, C.; Sun, L.; Zhang, J.; Zhao, B.; Chen, X.; Xu, H.; Huang, B. Silence of cancer susceptibility candidate 9 inhibits gastric cancer and reverses chemoresistance. *Oncotarget.* **2017**, *8*, 15393-15398, doi: 10.18632/oncotarget.14871.
2. Tiwari, M. K.; Coghi, P.; Agrawal, P.; Yadav, D. K.; Yang, L. J.; Congling, Q.; Sahal, D.; Wong, V. K. W.; Chaudhary, S. Novel Halogenated Arylvinyl-1,2,4 Trioxanes as Potent Antiplasmodial as well as Anticancer Agents: Synthesis, Bioevaluation, Structure-Activity Relationship and In-silico Studies. *Eur. J. Med. Chem.* **2021**, *224*, 113685.