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The combination of thioxodihydroquinazolinones and platinum drugs reverses platinum resistance in tumor cells by inducing mitochondrial apoptosis independent of Bax and Bak

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Abstract

The effective management of tumors resistant to platinum drugs-based anticancer therapies is a critical challenge in current clinical practices. The proapoptotic Bcl-2 family proteins Bax and Bak are essential for cisplatin-induced apoptosis. Unfortunately, Bax and its related upstream endogenous apoptotic signaling pathways are often dysregulated in cancer cells. Strategies that are able to bypass Bax- and Bak-dependent apoptotic pathways will thus provide opportunities to overcome platinum drug resistance. We have identified the thioxodihydroquinazolinone mdivi-1 as a member of a novel class of small molecules that are able to induce Bax- and Bak-independent mitochondrial outer membrane permeabilization when combined with cisplatin, thereby efficiently triggering apoptosis in platinum resistant tumor cells. In the present structure activity relationship (SAR) study of a computationally selected library of mdivi-1 related small molecules, we established a pharmacophore model that can lead to the enhancement of platinum drug efficacy and Bax/Bak-independent mitochondrial apoptosis. Specifically, we found that a thiourea function

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Supplementary data

Supplementary data (vendors, biological assay protocols, and complete biological data) associated with this article can be found, in the online version, at [link](#).

The authors have no conflicts of interest.

is necessary but not sufficient for the synergism of this class of thioxodihydroquinazolinones with cisplatin. We were also able to identify more potent mdivi-1 analogs through this SAR study, which will guide future designs with the goal to develop novel combination regimens for the treatment of platinum and multidrug resistant tumors.

Keywords

platinum drug resistance; cisplatin; mdivi-1; thioxodihydroquinazolinone; Bax/Bak-independent apoptosis

Cisplatin (*cis*-diaminedichloroplatinum(II)) and carboplatin (*cis*-diamine (cyclobutane-1,1-dicarboxylate-*O,O'*) platinum(II)) are among the most effective drugs currently in use against a broad spectrum of cancers, including testicular, ovarian, colorectal, bladder, lung, head and neck, and relapsed lymphoma.¹ Formation of DNA-platinum adducts by these drugs is considered to be the major mechanism of action in triggering apoptosis in tumor cells. Despite the high response rate at the initial treatment, the clinical outcome of platinum drugs is often limited by resistance (both acquired and intrinsic) of tumor cells to the drugs, especially in the management of recurrent disease. For instance, in ovarian cancer patients, the initial response rate is up to 70%, however, the disease in 70% of those patients recurs and cisplatin resistance develops in all patients treated for recurrent ovarian cancer.² Furthermore, the development of platinum resistance can also lead to multidrug resistance; for example, the cross-resistance to structurally unrelated sodium arsenite and methotrexate.¹

The mechanism underlying the platinum resistance of tumor cells is extremely complex and multi-factorial. The major factors can be attributed to reduced cellular accumulation of cisplatin, inactivation through covalent reaction with intracellular thiol-containing molecules, enhanced repair activities against cisplatin-DNA adducts, increased tolerance to cisplatin-induced DNA damage, and failure of the apoptotic pathway.³ In addition, cell survival signaling,⁴ epigenetic modification,⁵ extracellular matrix (ECM),⁶ epithelial to mesenchymal transition (EMT),⁷ and microRNA⁸ have also been reported to play certain roles in platinum drug resistance. While extensive efforts have been made to enhance platinum drug sensitivity and overcome platinum resistance, to date, a successful pharmacological intervention in managing platinum drug resistant tumors remains a major challenge.

Cisplatin induces cell death by engaging endogenous apoptotic signaling that activates mitochondrial apoptosis.⁹ Abrogation of the mitochondrial apoptotic pathway is related to acquired multidrug resistance.¹⁰ Bax and Bak are key proapoptotic Bcl-2 family member proteins and required for the antitumor effect of platinum and many other chemotherapeutic drugs. Loss of Bax and Bak leads to complete resistance to cisplatin, due to failure in the induction of mitochondrial outer membrane permeabilization (MOMP) and subsequent apoptotic events.¹¹ Bax is frequently suppressed or mutated in cancers, and its deficiency is related to oncogenic transformation and drug resistance.^{12,13,14,15} Bax expression correlates with the response to chemotherapy and treatment prognosis in cancer patients.^{16,17} In addition, antiapoptotic Bcl-2 family proteins such as Bcl-2 and Mcl-1, which suppress Bax/

Bak-dependent mitochondrial apoptosis, are frequently overexpressed in tumor cells. Conventional targeted strategies currently under preclinical or clinical development for increasing the efficacy of platinum drugs are mostly aimed at enhancing the endogenous apoptotic signaling. For example, inhibitors for PARP, ATR, and Chk1 are known to enhance DNA damage signaling when combined with cisplatin. A direct targeting of antiapoptotic proteins such as Bcl-2 and Bcl-xL is also an attractive strategy; however, the apoptotic effect is dependent on Bax and Bak.^{18,19} A strategy that can stimulate mitochondrial apoptosis by bypassing Bax and Bak is thus a promising therapeutic approach to eradicate cancer cells that are refractory to conventional therapies.^{20,21}

The thioxodihydroquinazolinone mdivi-1 (*mitochondrial division inhibitor*) was first identified as a regulator of apoptosis and mitochondrial fission and fusion in 2005 by a research team from UC Davis (Figure 1).²² By interfering with mitochondrial outer membrane permeabilization, mdivi-1 blocks cytochrome c (Cyt c) release from mitochondria.²³ It has also been reported that inhibition of dynamin-like protein 1/dynamin-related protein 1 (Dlp1/Drp1) by mdivi-1 attenuates mitochondrial defects induced by mutant PTEN-induced putative kinase 1 (PINK1).²⁴ There is growing evidence that mdivi-1's biological profile holds therapeutic value in ischemia/reperfusion injury, myocardial infarction, and neurodegenerative diseases.^{25,26,27,28,29} Furthermore, treatment of tumor cells with mdivi-1 results in a disruption of the cell cycle, genome instability,³⁰ tumor-specific mitotic defects,³¹ and a synergistic apoptotic effect with cisplatin,^{32,33} which provide a basis for using this compound class for cancer treatment.

We have previously identified a combination approach using platinum agents and mdivi-1 to induce apoptosis in multiple types of platinum-resistant tumor cells.³³ Importantly, we identified that a critical mechanism for the synergism of this combination is activating Bax- and Bak-independent mitochondrial apoptosis. We also found that the synergistic combination effect does not follow the SAR on the inhibition of the original molecular target Drp1.³³ Thus, mdivi-1 represents a novel small molecule that can be combined with the standard-of-care platinum anticancer agents to bypass Bax- and Bak-dependent drug resistant pathways.^{32,33} In order to identify the pharmacophore of mdivi-1 that is minimally required for enhancing the efficacy of platinum drugs, in the present work, we have expanded our SAR studies through testing a series of computationally selected mdivi-1 related small molecules. The structural information obtained will provide a basis for developing 2nd generation potent and selective analogs that is critical for the design of a more effective strategy for the management of platinum-resistant cancer.

In the current SAR study, we have selected mdivi-1 related small molecules through substructure and similarity searches³⁴ in the small molecule library collection of the University of Pittsburgh Center for Chemical Methodologies and Library Development (UPCMLD) that currently contains 53,400 small molecules, including 788 thioureas and 2,649 ureas. Mdivi-1 consists of a benzene-fused 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one core with a 2,4-dichloro-5-methoxyphenyl side chain attached to N(3) (Figure 1). Accordingly, an important criterion for the substructure and similarity search was the presence of a pyrimidinedione core fused to either a benzene or a thiophene ring, as well as compounds with an isolated pyrimidinedione. Additionally, we sought to determine the

requirement for a thione group in the core, and therefore we selected specifically compounds with carbonyl replacements. Lastly, we searched for variations in N(1) and N(3) substitutions ranging from aliphatic to aromatic and heteroatom containing side chains. Our searches based on those criteria provided an initial selection of 36 screening samples containing the thioxodihydroquinazolinone or structurally related scaffolds (Figure 2).

Previously, we have shown that the combination of mdivi-1 and cisplatin dramatically enhances the activity of caspase-3/7 compared to single agents treatment in cisplatin-resistant cells.³³ We thus employed the caspase-3/7 activity assay as the primary screening method to evaluate the activity of the first-generation thioxodihydroquinazolinone analogs *in vitro* in a cisplatin-resistant ovarian cancer cell line, A2780cis (Table 1). A microscopic examination of apoptotic morphologic changes was also carried out right before the measurement of caspase-3/7 activity, in order to confirm that the data on the activation of the caspase-3/7 following the treatment is consistent with morphological change thus reflecting the cell death. Thereafter, CellTiter-Blue cell viability assay was further used to validate the activity of hit compounds with varying concentrations of analogs (shown below, Figure 5). After the first round of compound screening, we identified seven active compounds (i.e. **1-4**, **1-6**, **1-8**, **1-17**, **1-19**, **1-21**, and **1-30**) (Figure 2 and Table 1; biological data for all compounds are shown in the SI, Table S1). All active compounds contained the 2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one core. Interestingly, the thioxo group in the pyrimidinone seems to be necessary for bioactivity, since the corresponding quinazoline-2,4(1*H*,3*H*)-diones were inactive. However, the presence of a thiocarbonyl group alone was not sufficient for activity, since all 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-ones and most 2-thioxo-2,3-dihydroquinazolin-4(1*H*)-ones were inactive or only weakly active. Electronic effects of substituents in the active samples ranged from mildly electron-withdrawing (**1-21**) to strongly donating (**1-8**); strongly electron-withdrawing groups seem to be disfavored (**1-16**, **1-22**, **1-36**). Active compounds all have aromatic or alkyl substituents at N(3), whereas N(1) is unsubstituted. However, bulky, basic, or highly functionalized side chains at N(3) are also not tolerated (**1-5**, **1-7**, **1-9**, **1-12**, **1-13**, **1-14**, **1-28**, **1-29**). Surprisingly, all five thioxodihydrothienopyrimidinones were inactive, in spite of the similarity of their core structures to the active thioxodihydroquinazolinones, possibly due to the additional large substituents attached or fused to the thiophene ring (**1-1**, **1-2**, **1-3**, **1-15**, **1-27**).

After identifying the thiourea function as a necessary requirement, we focused on more extensive modifications at the thiourea site and performed a second round of compound selection and screening. We selected another 33 compounds from the same small molecule library collection as used for the first generation compounds, with structural features of an acyclic acyl thiourea core or a 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (thiobarbiturate) moiety (Figure 3).

We then used the same screening method as for the first generation analogs to evaluate compound activity. In this series, only compounds **2-7**, **2-19**, **2-32**, and **2-33** were found to be active (Figure 3 and Table 2; biological data for all compounds are shown in the SI, Table S2). Analogs **2-32** and **2-33** were initially identified through the microscopic observation of cell morphology changes after treatment. The lack of activity of these two

analogues in the caspase-3/7 activity assay while showing a positive response in the CellTiter-Blue cell viability assay (Figure 5) may be due to their interference with the caspase-3/7 activity assay system. In spite of the low hit rate, we gained some additional useful information from this screen. Significantly, the majority of thioureas were inactive, and among the few that showed an effect (**2-19**, **2-32**, and **2-33**), the presence of a nitrogen-containing heterocycle attached to the terminal thiourea nitrogen was noteworthy (but not sufficient, i.e. **2-23** and **2-24**, for example, were closely related inactive analogues). In that regard, the steric and electronic effects of both substituents attached to the nitrogens of the thiourea core appear to be critical for activity (i.e. **2-19** vs **2-2** and **2-23**). The thiobarbiturates were all inactive with the exception of **2-7**. Compounds that are related to **2-7** have been previously reported with an impressive growth inhibition of some human cancer cell lines.³⁵ Overall, while all second-round hits were less potent than the active compounds from the first round, the structural information obtained from these less potent analogues will help to guide future designs of more potent and more selective mdivi-1 analogues. For example, we were able to generate a preliminary pharmacophore model based on the significant SAR information from both analogue series (Figure 4). The heterocyclic core should contain a thiourea moiety, and *N*-substitution by electron-rich aromatic substituents is preferred. The most active compounds also contain a fused benzene ring.

After identifying eleven active compounds through two rounds of screenings, we decided to further evaluate the synergistic capabilities of these compounds with platinum drugs and gain a better understanding of their therapeutic potential. A CellTiter-Blue cell viability assay of the hit compounds with increasing concentrations (10 μ M, 20 μ M, 50 μ M) showed a clear difference in the ability of the eleven hits to act synergistically with cisplatin (Figure 5 and Table S3). Four compounds (**1-6**, **1-8**, **1-17**, and **1-19**) were found to be more potent than the parent mdivi-1 at enhancing the cytotoxicity of cisplatin, as indicated by the larger reduction in cell viability. In order to quantify the combination effect, we calculated the combination index (CI) for the most active analogue, compound **1-6**, based on the method of median-effect principle of Chou and Talalay.^{36,37} As shown in Table 3, the combination of cisplatin and **1-6** produces a synergistic effect ranging from synergism to very strong synergism depending on the concentration of **1-6**. We have further confirmed this synergism for the combination of cisplatin and **1-6** using a fresh sample of high chemical purity (98%). We also found that these four compounds are capable of enhancing the effect of carboplatin, another major and widely used platinum drug (Table 4).

We also sought to identify if the newly identified compounds have the same mechanism of action as mdivi-1 when combined with cisplatin,^{32,33} by using the four most potent lead structures (**1-6**, **1-8**, **1-17**, and **1-19**). The combination of those four analogues with cisplatin enhanced apoptotic cell death same as mdivi-1, when compared to cisplatin alone and the analogues alone (Figure 6). Consistent with the cell viability results (Figure 5), the combination of cisplatin with **1-6**, **1-8**, **1-17**, and **1-19** was also more effective in the activation of caspase-3/7 when compared to mdivi-1 (Figure 6A and Table S4). The cleavage of several major caspases (caspase-8, caspase-9, and caspase-3), as well as poly(ADP-ribose) polymerase (PARP) were also observed after the cells were treated with the combination of cisplatin and all the analogues (Figure 6B). In addition, the upregulation of

a pro-apoptotic Bcl-2 family protein Noxa was also observed only after the combination treatment across all the analogs (Figure 6B).

We then selected compound **1-6** as a representative of the newly identified analogs to further study the details of the mechanism of action. We have shown previously that the combination of mdivi-1 with cisplatin enhances replication stress-mediated DNA damage.³³ Replication protein A (RPA) is a single strand-specific DNA-binding protein, consisting of three subunits, RPA70, RPA32, and RPA14. RPA32 is phosphorylated in response to DNA replication stress.³⁸ The combination of cisplatin and **1-6** enhanced the phosphorylation of RPA32 as early as 4 h post treatment, whereas treatment with cisplatin alone showed an increase in the phosphorylation of RPA32 only after 12 h post treatment (Figure 7A). Both the phosphorylation of RPA32 and upregulation of Noxa occurred earlier than the phosphorylation of H2AX and cleavage of caspase-3, indicating that the replication stress and activation of mitochondrial apoptotic pathway are primary events following the combination treatment. We also observed the uncoupling of mitochondria after combination treatment with cisplatin and compound **1-6** and not after agents alone treatment, as indicated by the lack of response to oligomycin-mediated inhibition of oxygen consumption rate (OCR) following combination treatment (Figure 7B). This uncoupling effect was also observed with mdivi-1, in our previous studies.³³ Importantly, the combination of cisplatin with both mdivi-1 and **1-6** induced Bax/Bak-independent apoptosis, as shown by the cleavage of caspase-8, caspase-9, and caspase-3 in both Bax/Bak wild-type (WT) and double knockout (DKO) MEF cells, while cisplatin alone induced the cleavage of these caspases only in Bax/Bak wild-type cells (Figure 7C). Taken together, we conclude that the mechanism of action of **1-6**, **1-8**, **1-17**, and **1-19** is very likely identical to mdivi-1.

Finally, we evaluated the efficacy of the combination of cisplatin and **1-6** on other types of platinum- or multidrug-resistant cancer cells. As shown in Figure 8A, we observed a potent effect of **1-6** in the combination with cisplatin on ovarian cancer cells (PEO4 and SKOV-3), non-small cell lung carcinoma cells (H1299), triple-negative breast carcinoma cells (MDA-MB-231) and head & neck cancer cells (Cal33), as determined by the cleavage of PARP and caspase-3. We also employed an alternative exposure strategy that more closely mimics in vivo drug administration to test the combination effect. Considering the clearance of cisplatin over the time and the duration of exposure of tumor cells to the effective doses of the drugs in vivo,³⁹ we tested a short-term exposure of cells to the combination of 20 μ M of **1-6** with various doses of cisplatin, followed by cell viability assay after two days. We found that a 3-h exposure of the cells to the combination of cisplatin and **1-6** was sufficient to cause a severe long-term proliferation defect, as compared to the treatment with cisplatin alone, which only had a slight effect on cell viability (Figure 8B).

In conclusion, in a targeted library of 69 analogs of mdivi-1, we identified several potent lead compounds, and we characterized a significant synergistic effect for the combination of cisplatin and compound **1-6**. While cisplatin alone induces apoptosis dependent on the proapoptotic Bcl-2 family proteins Bax and Bak,¹¹ our studies revealed that the combination of certain thioxodihydroquinazolinones and cisplatin induces Bax/Bak-independent apoptosis. This Bax/Bak-independent effect is a critical mechanism underlying the efficient induction of mitochondrial apoptosis by the combination treatment as compared to cisplatin

alone. Accordingly, we were able to establish a preliminary pharmacophore model of this class of thioxodihydroquinazolinones and identify more potent structures, which are essential for guiding future compound optimization for preclinical studies. Given the essential roles of Bax and Bak in mitochondrial apoptotic cell death triggered by many other classes of anticancer drugs in addition to the platinum compounds, such as DNA damaging agents (etoposide,⁴⁰ bleomycin,⁴¹ 5-FU¹¹), microtubule inhibitors,^{42,43,44} EGFR and CDK inhibitors,^{45,46} and the CD20 monoclonal antibody rituximab,⁴⁷ the thioxodihydroquinazolinone / platinum based combination approach can provide a novel drug targeting strategy for a broad spectrum of drug resistant tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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mdivi-1

Figure 1.
Structure of mdivi-1 (3-(2,4-dichloro-5-methoxyphenyl)-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one).

Figure 2.
First-generation mdivi-1 analogs. Active samples are highlighted.

Figure 3.
Second-generation mdivi-1 analogs. Active samples are highlighted.

Figure 4.
Pharmacophore model ($G = O$ or C ; $Q = H$ or C).

Figure 5. Synergistic cytotoxicity of various concentrations of active analogs in combination with cisplatin. A2780cis cells were treated with cisplatin alone, mdivi-1 derivatives alone, or combination at indicated concentrations for 24 h. Cell viability was determined by CellTiter-Blue assay. Data represent the mean±S.D. of triplicates. * P<0.05, ** P<0.01, *** P<0.001.

Figure 6.

The combination of cisplatin and mdivi-1 derivatives induces apoptotic cell death. (A) A2780cis cells were treated with cisplatin alone, mdivi-1 derivatives alone or combination at indicated concentrations for 20 h. Apoptotic cell death was determined by the activation of caspase-3/7. Data represent the mean±S.D. of triplicates. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. (B) A2780cis cells were treated with cisplatin (20 μM) alone, mdivi-1 derivatives (20 μM) alone or the combination for 20 h. The cleavage of caspase-8, 9, 3, PARP and the induction of Noxa were examined by western blot. The human specific anti-caspase-8 antibody recognizes both the full length (FL) and the cleaved fragments.

Figure 7.

The combination of cisplatin and mdivi-1 derivatives enhances DNA damage and mitochondrial dysfunction, leading to hyperactivation of intrinsic mitochondrial apoptotic signaling independent of Bax and Bak. (A) A2780cis cells were treated with cisplatin (20 μ M) alone or with the combination of cisplatin and compound **1-6** (20 μ M) for the indicated time points. The phosphorylation of RPA32 and histone H2AX, the induction of Noxa, and cleavage of caspase-3 were determined by western blot. (B) A2780cis cells were treated with cisplatin (50 μ M) alone, **1-6** (50 μ M) alone, or the combination for 4 h. The oxygen consumption rate (OCR) was measured by a Seahorse XF24 Extracellular Flux Analyzer. Data represent the mean $\pm$ S.D. of triplicates. (C) Bax/Bak wildtype (WT) and double knockout (DKO) MEF cells were treated with cisplatin (10 μ M) alone, or in combination with mdivi-1 (20 μ M) or **1-6** (20 μ M) for 20 h. The cleavage of caspase-8, 9, and 3 was determined by western blot. The mouse specific anti-caspase-8 antibody recognizes only the cleaved fragments as compared to human specific antibody.

Figure 8.

The combination of cisplatin and **1-6** triggers extensive apoptosis in various types of tumor cells and short-term exposure is sufficient to reduce cell survival. (A) Cisplatin-resistant cancer cells PEO4 (ovarian), SKOV-3 (ovarian), H1299 (lung), MDA-MB-231 (breast), Cal33 (head & neck) were treated with DMSO control (D), cisplatin (C) (20 μ M) alone, **1-6** (**6**) (20 μ M) alone, or the combination (C/**6**) for 20 h. The cleavage of PARP and caspase-3 were determined by western blot. (B) A2780cis cells were treated with increasing doses of cisplatin alone or the combination of cisplatin and **1-6** (20 μ M) for 3 h. The compounds were then washed out. Cell survival was determined by CellTiter-Blue assay after 48 h incubation of the cells in drug-free media. Data represents the mean $\pm$ S.D. of quadruplicates.

Table 1

Biological data for active first-generation analogs. Apoptotic cell death was determined by the activity of caspase-3/7 in the cisplatin-resistant ovarian cancer cell line A2780cis, after treatment for 20 h.

entry	compound	caspase-3/7 activity (Lum) ^a	
		compound alone ^b	compound with cisplatin ^c
1	–	13 ± 1	–
2	cisplatin	30 ± 1	–
3	mdivi-1	28 ± 5	102 ± 3
4	1-4	13 ± 1	93 ± 5
5	1-6	12 ± 0	145 ± 6
6	1-8	14 ± 2	121 ± 4
7	1-17	10 ± 2	123 ± 5
8	1-19	12 ± 1	136 ± 12
9	1-21	17 ± 3	90 ± 6
10	1-30	18 ± 2	111 ± 8

^a Data represents the mean ± S.D. of triplicates.

^b Compound tested at 20 μM.

^c Compound tested at 20 μM in the presence of 20 μM cisplatin.

Table 2

Biological data for active second-generation analogs. Apoptotic cell death was determined by the activity of caspase-3/7 in the cisplatin-resistant ovarian cancer cell line A2780cis, after treatment for 20 h.

entry	compound	caspase-3/7 activity (Lum) ^a	
		compound alone ^b	compound with cisplatin ^c
1	–	8 ± 2	–
2	cisplatin	35 ± 4	–
3	mdivi-1	20 ± 3	80 ± 11
4	2-7	25 ± 1	41 ± 3
5	2-19	9 ± 1	50 ± 8
6	2-32 ^d	0 ± 1	4 ± 1
7	2-33 ^d	11 ± 2	23 ± 3

^aData represent the mean ± S.D. of triplicates.

^bCompound tested at 20 μM.

^cCompound tested at 20 μM with 20 μM of cisplatin.

^dThe lack of activity is probably due to the interference of the analogs with the caspase-3/7 activity assay system.

Table 3

Combination index (CI) for the combination of the most active analog, compound **1-6**, with cisplatin (20 μ M). CI was calculated using CompuSyn software.

compound	1-6		
	10 μ M	20 μ M	50 μ M
combination Index	0.67	0.24	0.01
grading	synergism	strong synergism	very strong synergism

Table 4

Synergistic cytotoxicity of various concentrations of active analogs in combination with carboplatin. Cell viability was determined by a CellTiter-Blue assay in the cisplatin-resistant ovarian cancer cell line A2780cis, after treatment for 24 h.

entry	compound	survival (%) ^a of cells with compound and carboplatin (200 μ M)		
		10 μ M	20 μ M	50 μ M
1	mdivi-1	92.6 $\pm$ 3.5	75.1 $\pm$ 3.9	40.6 $\pm$ 3.0
2	1-6	64.5 $\pm$ 2.7	48.0 $\pm$ 3.0	2.5 $\pm$ 0.4
3	1-8	89.5 $\pm$ 2.1	65.8 $\pm$ 3.5	42.5 $\pm$ 2.3
4	1-17	90.7 $\pm$ 0.9	79.3 $\pm$ 3.9	69.7 $\pm$ 4.1
5	1-19	103.5 $\pm$ 1.6	70.0 $\pm$ 2.9	31.6 $\pm$ 4.2

^aData represent the mean $\pm$ S.D. of triplicates. Concentrations refer to the tested compound. Untreated cells are at 100% survival and cells treated with carboplatin alone (200 μ M) are at 99.6% survival.