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**Oleanolic acid derivative methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate inhibits ABCC1/MRP1 protein function and reduces its level in acute promyelocytic leukemia cells**

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**Background:** The aim of our research work was to find modulator of ABCC1/MRP1 protein which is related to the multidrug resistance of cancer cells. Thus, concerning the commonly known biological activity of oleanolic acid, we decided to test a group of its semisynthetic derivatives.
**Materials and Methods:** We tested four oleanolic acid derivatives which have been chemically modified comparing to the parental compound base structure at C-3, C-11 and C-28 positions: methyl 3,11-dioxoolean-12-en-28-oate, methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (Fig. 1), 12α-bromo-3-hydroxyiminoolean-28->13-olide and methyl 3,12-dioxo-12a-aza-C-homoolean-28-oate. As an experimental model two cell lines were used: HL-60 (acute promyelocytic leukemia cells) and its multidrug resistant subline HL-60/AR overexpressing *ABCC1* gene. MTT test was performed to assess the potency of compounds in reduction of leukemic cells viability. The influence of the compounds on the MRP1 function was checked using fluorescent MRP1 substrate − calcein accumulation and retention assay with cytometric detection method and changes in MRP1 protein level were measured using SDS-PAGE electrophoresis and Western Blot technique.
**Results**: The studied oleanolic acid derivatives showed high activity revealing significant reduction of leukemic cells viability (MTT assay). All of tested compounds were more effective than parental compound. Methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (HIMOXOL) possessing = NOH group in C-3 position showed even higher activity against multidrug resistant cells than against wild type after 72 h of treatment ($IC_{50}$: 3.17±0.48 μM, 4.68±0.28 μM, respectively). Test assessing the ABCC1 function modulating activity (calcein retention assay) gave the information that HIMOXOL used in 10 μM almost completely blocked calcein efflux from HL-60/AR cells within three hours after the loading of the cells with calcein-AM. Calcein accumulation test confirmed effectiveness of the compound. Moreover 24 h treatment of HL-60/AR cells with 5, 10 and 20 μM HIMOXOL yielded dose dependent reduction of MRP1 protein level.
**Conclusion:** Obtained results prove that oleanolic acid derivative − methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate is efficient ABCC1/MRP1 modulator able to decrease level of MRP1 protein and inhibit transport of its substrate out of the cell.

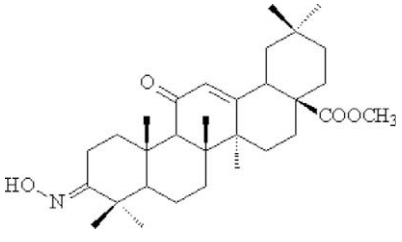


Figure 1. Structure of 3-hydroxyimino-11-oxoolean-12-en-28-oate.

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**Iron chelators that overcome drug resistance**

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**Introduction:** Previously, our laboratory demonstrated that the di-2-pyridylketone thiosemicarbazone (DpT) and 2-benzoylpyridine thiosemicarbazone (BpT) series of iron chelators possess highly potent and selective anti-cancer activity *in vitro* and *in vivo*. Interestingly, one of our leading compounds, Dp44mT, was more cytotoxic to drug resistant KBV1 cells which over-express P-glycoprotein (P-gp), a classical drug export pump, than the parent cells, KB31 which do not possess P-gp (Whitnall *et al.* 2006).
We investigated if this observation was indeed dependent on P-gp function and assessed the cytotoxicity using specific inhibitor of P-gp, PSC833. We also determined whether increased toxicity was due to reduced efflux of the chelator, Dp44mT.
**Materials and Methods:** KB31 and KBV1 (P-gp over-expressing) cells have been derived from the HeLa cell line. To maintain drug resistance, KBV1 was cultured in the presence of vinblastine (1 μg/mL). The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay was used to assess the effect chelators have on cellular proliferation. $^{14}$C-labeled Dp44mT was used to study the uptake and efflux of drug in the cells.
**Results:** We have demonstrated using the MTT cell proliferation assay that both Bp4eT and Dp44mT were more cytotoxic in P-gp over-expressing KBV1 cells compared to the parent KB31 cells which do not express P-gp. This increased cytotoxicity in P-gp expressing cells was reversed in presence of the P-gp inhibitor, PSC833 (1 μM), making the resistant cells far less sensitive to our chelators ($IC_{50}$ increased 13.5-fold for Bp4eT and 3.4-fold for Dp44mT relative to control). In contrast, PSC833 had no significant effect on the sensitivity of non resistant cells to Dp44mT or Bp4eT (see Table).
We have also examined whether this increased cytotoxicity in P-gp cells was due to reduced efflux of the chelator. We used $^{14}$C-labeled Dp44mT to compare the efflux of drug over various time points at 37ºC. It was shown over a 2 h re-incubation period that $^{14}$C-Dp44mT was consistently effluxed more in KBV1 compared to KB31 cells. However, this was unlikely to be due to P-gp as PSC833 had no effect on the efflux of Dp44mT in KBV1 cells.
**Conclusion:** Further studies are underway to elucidate the mechanism of increased cytotoxicity of Dp44mT and Bp4eT in P-gp over-expressing cells. This study has shown that these iron chelators are more effective against P-gp over-expressing cells than those without P-gp. Further understanding of the mechanism of how these chelators overcome drug resistance will be crucial in developing chemotherapeutic drugs that are able to combat multi-drug resistance in cancer patients.

| | $IC_{50}$ (μM) ±SD | | | |
|---|---|---|---|---|
| Cell line | Bp4eT | Bp4eT + PSC833 | Dp44mT | Dp44mT + PSC833 |
| KB31 | 0.022±0.002 | 0.018±0.001 | 0.017±0.001 | 0.013±0.001 |
| KBV1 | 0.013±0.005 | 0.176±0.024 | 0.007±0.001 | 0.024±0.006 |

**References**

Whitnall M *et al* (2006) Proc Natl Acad Sci USA, 103, 14901−6.

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**Targeting the MAPK pathway to increase sensitivity of ovarian carcinoma cells to platinum compounds**

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Aberrant activation of the MAPK pathway may be relevant in reduced sensitivity of cancer cells to conventional and target specific agents. We previously showed that ovarian carcinoma cells selected from the IGROV-1 cell line for resistance to oxaliplatin (IGROV-1/OHP) exhibited decreased sensitivity to gefitinib, due to increased phospho-Extracellular signal Regulated Kinase (ERK)1/2 levels, suggesting that the signaling occurring through EGFR-mediated activation of downstream events may contribute to resistance of ovarian carcinoma cells. The present study was designed to investigate whether targeting the MAPK cascade may be a promising approach to improve cell sensitivity to platinum compounds and to explore the molecular mechanisms leading to activation of survival pathways in these cell systems. Since MEK1/2 regulates ERK activation, we examined whether the pharmacological inhibition of the MEK1/2 kinase may increase sensitivity to platinum compounds in the platinum-sensitive and -resistant cells. When IGROV-1 cells were exposed to cisplatin/oxaliplatin in combination with the MEK1/2 inhibitor CI1040, a synergistic effect was observed, as evaluated by the combination index method. In the IGROV-1/OHP subline, a supra-additive interaction was found with the cisplatin/CI1040 combination. In IGROV-1 cells, the drug combinations resulted in increased caspase 3 cleavage as compared to single drug treatment, thereby supporting an increased apoptotic response. Because ERKs are known to be dephosphorylated by Dual Specificity Phosphatases (DUSPs), we used a genome-wide approach to examine the expression levels of DUSPs in the studied cell lines. We found that the mRNA level of different members of the DUSP family (e.g., DUSP5, DUSP6, DUSP23) were significantly reduced in IGROV-1/OHP and in other Pt drug-resistant ovarian carcinoma cell lines as compared to parental cells. In silico analysis of transcription factor binding sites indicated that p53 may bind DUSP5 but not DUSP6 or DUSP23 promoters, thus suggesting that p53 mutation of IGROV-1/OHP cells may lead to DUSP5 down-regulation. Overall, our results support that targeting the MAPK pathway may increase sensitivity of ovarian cancer cells to platinum compounds. However, the efficacy of