differential BAX/BAK regulation despite equivalent R8BIDBH3 uptake kinetics. Subcellular fractionation confirmed constitutive BAX localization to the mitochondrial membrane in H460 cells in the absence of cytochrome/SMAC release, suggesting priming for death, and enhanced susceptibility to R8BIDBH3. In summary, machine vision based modelling of BAX/BAK dependent mitochondrial permeabilization is a useful tool for quantitative study of differential apoptosis dynamics in living cancer cells.

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Growth prevention of cancer cells by naphtoquinone derivatives

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The mitogen activated protein kinases (MAPKs) family consists of serine/threonine kinases. Extracellular signal regulated protein kinases (ERK1/2), c-Jun amino-terminal kinases (JNKs) and p38 MAPKs are the major members in this family. ERK1/2 are generally associated with cell proliferation and survival, whereas JNKs and p38 are usually related to apoptotic response. Activated MAPKs pathways have been detected in carcinoma of the colon. In this research, we focused on the EGFR-induced signal transduction in HT29 cells, which are colorectal adenocarcinoma cells.

The purpose of this research is to examine the effects of naphtoquinones derivatives (NQs) on cell growth, apoptosis and cell signaling. Furthermore, we intend to clarify the mechanism by which the NQs prevent growth of HT-29 cancer cells.

In order to examine the effect of the NQs on biological activities we preformed the following assays:

1. XTT assay – to measure cell viability.
2. DNA fragmentation and FACS analysis – to determine cell apoptosis.
3. Western blot – to examine cell signaling, using specific antibodies against phosphorylated and thus activated ERK1/2 and p38 MAPKs.

Among the various NQs that we screened, TW69 was the first that had a significant effect on cell proliferation. Using DNA fragmentation assay, we demonstrated that extended incubation with TW69 (25 μM for 48h) leads to apoptotic cell death. In addition, FACS analysis of cells treated with TW69 (25 μM for 48h), showed a high percent of apoptotic cells (33.97%). These preliminary results have led to synthesis of a new derivative, TW96, which is based on TW69 structure. Cell viability assays revealed that TW96 induces cell death with IC_{50} values of 0.3 μM. Furthermore, FACS analysis showed a high percent of apoptotic cells (78.75%) after treatment with 5 μM of TW96. In addition, cell signaling analysis demonstrates an increased activation of ERK1/2 and p38 in response to TW96 in a time dependent manner. Recent reports indicate that NQs cause an increase in reactive oxygen species (ROS) formation. It is well known that high levels of ROS lead to apoptosis. Experiments with N-acetyl-L-cysteine, (NAC), which is a ROS scavenger, showed inhibition of both ERK1/2 and p38 activation in response to TW96, suggesting that TW96-induced p38 activation is ROS-dependent. Further research is underway in order to determine whether ROS formation is involved in TW96-induced cell death.

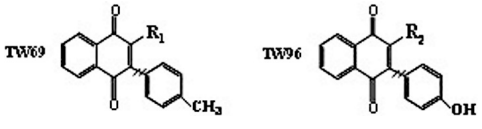

Fig. 1. Chemical structure of naphthoquinone derivatives.

Our study reveals newly synthesized compounds which affect cell viability and mediate apoptosis of HT29 cells. We suggest that these NQs may serve as lead compounds for the development of potential anti-cancer drugs.

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The inhibition of PI3K/Akt pathway as a major molecular determinant of bortezomib-induced apoptosis in hepatocellular carcinoma cells

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Background: Hepatocellular carcinoma (HCC) is one of the most common and aggressive human malignancies. Bortezomib, a proteasome inhibitor, has been shown good clinical responses in hematological malignancies and has been approved by FDA in refractory multiple myeloma in 2003. In this study, we examined the efficacy of bortezomib in HCC cells and identified the molecular change responsible for the resistance of bortezomib.
Methods: Five human HCC cells were used, including HuH7, HepG2, HepG2.2.15, Hep3B and PLC/PRF/5. Cells were exposed at various concentrations of bortezomib for 72 hours and cell viability was assessed by using the MTT assay. Cell cycle assay and apoptosis assay were done by flow cytometry. Protein expressions were assessed by western blot assay.
Results: Our data indicated bortezomib has biphasic effects in HCC cells. Bortezomib induced cell cycle arrest in G2/M phase starting at the lower concentration in all types of HCC cells. At the higher concentrations, bortezomib would induce massive apoptosis in HCC cells within 24 hours except PLC/PRF/5 cells. Bortezomib was unable to induced apoptosis at the clinical relevant concentrations (below 1000 nM) in PLC/PRF/5 cells. Compared with the molecular change between PLC/PRF/5 and other cells, we found PI3K/Akt pathway played a very important role in mediating the resistance of bortezomib-induced apoptosis. Our first evidence showed that bortezomib downregulated phospho-Akt in a dose- and time-dependent manner in sensitive HCC cells. However, in PLC/PRF/5 cell, bortezomib could not inhibit the activity of Akt even at the concentration of 1000 nM. Secondly, we applied a well-known PI3K inhibitor, LY294002, in our study and found that the combination of bortezomib and LY294002 induced apoptosis in PLC/PRF/5 cells in a dose- and time-dependent manner, indicating the importance of PI3K/Akt pathway in bortezomib-induced apoptosis in HCC cells.
Conclusions: Bortezomib could induce apoptosis at the clinical relevant concentration in most of HCC cells except PLC/PRF/5. Bortezomib downregulated phospho-akt in sensitive HCC cells but not in PLC/PRF/5 cells. Combination of bortezomib and PI3K inhibitor, LY294002 abrogated the resistance of bortezomib in PLC/PRF/5 cells. The inhibition of PI3K/Akt signaling pathway might improve the efficacy of bortezomib and overcome the drug resistance in the treatment of HCC.
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Imidazoacridinone derivative C-1311 (SymadexTM) induces apoptosis, mitotic catastrophe or senescence in human colon carcinoma HCT116 cells depending on p53 status

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Antitumor imidazoacridinone C-1311 (Symadex^{TM}), active especially against colon carcinomas, has recently entered phase II clinical trials. C-1311 has been shown to inhibit catalytic activity of DNA topoisomerase II and to induce interstrand DNA crosslinking after metabolic activation. Here, we studied the cellular response of colon carcinoma HCT116 cells to C-1311 treatment in relation to p53 function.

HCT116 $p53^{-/-}$ and HCT116 $p53^{+/+}$ cells were treated with C-1311 at EC_{80} concentration for different time periods varying from 24 to 144 h. Cell cycle distribution was analysed by flow cytometry. Phosphorylation status of Tyr15 on cdc2 was studied to monitor mitotic progression. DAPI staining was used to identify micronucleated cells. Annexin V/PI, TUNEL and caspase-3 activity assays were used for studies on apoptosis. Analysis of cellular morphology and expression of SA-β-galactosidase were performed to identify cells with senescence-like phenotype.

HCT116 $p53^{-/-}$ cells underwent a short-term G2M arrest after 48 h of C-1311 treatment. The arrest however, could not be sustained and we observed the reduction in G2M arrested cells which was associated with gradual increase in cells with a subG1 and a slight increase in cells with >4n DNA content. Tyr15-phospho-Cdc2 was initially up-regulated but decreased after 48 h, which indicated that G2 arrested cells progressed into mitosis. Enlarged cells with multiple micronuclei, typical for mitotic catastrophe, appeared after 48 h. Early apoptotic cells occurred after 48 h of C-1311 exposure, as demonstrated by Annexin V/PI assay. Prolonged incubation with C-1311 led to increase in apoptotic population (50% of cells were TUNEL-positive and had active caspase-3 after 144 h of treatment). In contrast, HCT116 $p53^{+/+}$ cells showed an apparent cell growth arrest at G1 and G2M phases. This growth arrest was accompanied by apoptosis of only 20% of treated cells at the 144 h time point. Surviving HCT116 $p53^{+/+}$ cells, starting from 96 h, developed features of drug-induced senescence with flattened, enlarged morphology and increasing degree of SA-β-galactosidase staining. Such effect was not observed in p53-null cells.

The overall results suggest that in HCT116 $p53^{-/-}$ cells, C-1311 induced transient G2M arrest followed by mitotic catastrophe and p53-independent apoptosis. In HCT116 $p53^{+}/^{+}$ senescence-like arrest appears to be a major p53-induced cellular response to C-1311 treatment.