Table: *In vitro* chemosensitivity data for temozolomide and example compounds from two new classes of imidazotetrazine

Compound	A2780 MGMT$^+$/MMR$^+$	A2780 + PaTrin2 MGMT$^-$/MMR$^+$	A2780-cp70 MGMT$^+$/MMR$^-$	A2780-cp70 + PaTrin2 MGMT$^-$/MMR$^-$
Temozolomide	>250	8.58 (0.32)	>250	231(10)
DP86	54(11)	34(3.1)	62(10)	94(3.3)
DP68	0.7(0.23)	1.3(0.07)	6.5(1.0)	7.0(0.1)

All data are IC_{50}/mean(SD) μM.

439 POSTER

Rapid effects of Irvalec on tumor cell integrity associated with changes in the ionic membrane conductance

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Irvalec® (PM02734, Elisidepsin) is a marine-derived cytotoxic depsipeptide that is currently undergoing phase II clinical studies in non-small cell lung cancer. In vitro treatment of tumor cells with Irvalec® induces necrotic cell death, a process associated with rapid loss of membrane integrity and subsequent cell permeabilization. In dose-response experiments, very similar IC50 values were obtained after short (30 min) and long (72 h) exposure times to the drug, suggesting that the compound exerts its cytotoxic effect immediately after drug treatment. Treated cells underwent rapid and dramatic morphological changes, including cell blebbing, severe swelling, plasma membrane permeabilization and cell lysis. Apart from the numerous small blebs, membranes from damaged cells also re-organized to form enormous bubbles surrounded by cell membrane. Using a fluorescent derivative of Irvalec®, it was demonstrated that the compound mostly localized in the plasma membrane of treated cells. Using electrophysiological techniques, it was shown that Irvalec® induced an important increase in membrane conductance. The compound permeabilized the plasma membrane to ions, even when the cells were not pulsed, causing important changes in the holding current. It has been described that zinc attenuates the drastic effects of some membrane disrupting agents. Hence, to test if zinc exerted some protective effect against the cytotoxicity of Irvalec®, A549 cells were treated with this drug in the presence or absence of zinc salts and its cytotoxicity evaluated by both propidium iodide uptake, using plate fluorimetry, and by electrophysiology, measuring the variations in the ion currents induced by the drug. Interestingly, in the presence of zinc, a 90% decrease in the cytotoxicity of Irvalec® was observed, that was accompanied by a decrease in the conductive properties of the cell membrane. Altogether, these results suggest that Irvalec® rapidly alters the ionic membrane conductance, inducing a hydroelectrolytic disbalance that leads to necrotic tumor cell death.

440 POSTER

The dietary phytochemical fisetin triggers apoptosis in breast cancer cell lines: Basis for a therapeutic modality?

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Background: Breast cancer remains the most commonly diagnosed cancer in Canadian women. The lifetime probability of diagnosis is approximately 1 in 9. Although the current management strategies for breast cancer are effective, there is still significant morbidity and mortality associated with the disease and its treatments. This study explores the flavonoid fisetin (present in strawberries and other fruits and vegetables) as a possible novel therapeutic modality for breast cancer.
Methods: Breast cancer cells used in this study consisted of adenocarcinoma cell lines MCF-7, MDA-MB-231, and MDA-MB-468, T47-D ductal carcinoma cells, and mitozatrone (MITX) and paclitaxel (Tx400) resistant MCF-7 cells. Cultures of human mammary epithelial cells and fibroblasts were used as normal controls. Cell viability assays (MTT, crystal violet, phosphatase and colony-forming assays) were used to assess the effect of fisetin on breast cancer cell viability. The mechanism of fisetin's cytotoxic effect was explored using assays for apoptosis/necrosis, i.e., Annexin V-propidium iodide staining, DNA fragmentation measured by JAM assay, and necrosis measured by lactate dehydrogenase-release assay. A pan-caspase inhibitor was used to determine the role of caspase activation while flow cytometric analysis of $DiOC_6$ and dihydroethidium-stained cells was used to assess mitochondrial membrane stability and reactive oxygen species (ROS) production, respectively.
Results: Cell viability assays demonstrated a variable cytotoxic effect of fisetin on the breast cancer cell lines. Typically, a 23% (T47-D) to 81% (MDA-MB-468) decrease in cell viability was observed following 72 h exposure to 100 μM fisetin. The majority of fisetin-treated breast cancer cells died by apoptosis, although some breast cancer cells underwent necrosis following fisetin treatment. MITX and Tx400 cells were resistant to the fisetin's cytotoxic effect, suggesting that fisetin is a target for drug efflux pumps. Fisetin-induced apoptosis involved mitochondrial membrane destabilization and ROS production but was caspase-independent.
Conclusion: Although fisetin shows promise as a possible treatment for breast cancer, additional research is required to further delineate fisetin's mechanism of action. Future studies will establish the in vivo activity of fisetin in immune-deficient mice bearing breast cancer xenografts.

Structure–activity relationship

441 POSTER

The discovery of novel, highly potent inhibitors of BRAF

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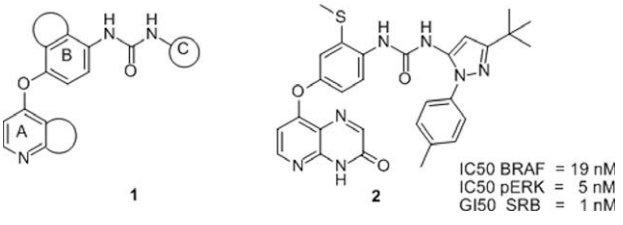
We describe the synthesis and optimisation of a series of new inhibitors of BRAF, a kinase whose mutant form (V600E) is implicated in several types of cancer, with particularly high frequency in melanoma. We designed and synthesised type II inhibitors interacting with the inactive conformation of the ^{V600E}BRAF. The inhibitors present a tripartite A-B-C structure (See Figure 1) where A is a hinge binding heterocyclic system, B is an aryl spacer group lying in the hydrophobic pocket and C a heteroaromatic group which protrudes into the pocket created by the DFG-out position in the inactive BRAF conformation. The most effective inhibitors are potent (IC50 < 50 nM) against isolated ^{V600E}BRAF *in vitro* and in cellular assays (the reduction of phosphorylation of extracellular regulated kinase [pERK] and proliferation [SRB] assays in ^{V600E}BRAF-dependent cells) (an example is shown in Figure 2). Substituted and unsubstituted pyrido-[4,5-b]-imidazolone and pyrido-[2,3-b]-pyrazinone hinge binders feature in the most active compounds. 2-Fluorophenyl, 2-thiomethylphenyl and naphthyl moieties (1,4-substituted) provide high cellular activities to the inhibitors. Substituted pyrazoles, particularly 3-*tert*-butyl-1-aryl-1*H*-pyrazoles, increase the cellular potencies without detrimental effects on the potency on isolated ^{V600E}BRAF. In summary, compounds have been designed that inhibit isolated ^{V600E}BRAF at low nanomolar concentrations. In mutant BRAF-dependent cells, these inhibitors prevent downstream signaling of pERK and inhibit proliferation. Concomitant benefits are good oral bioavailability, low metabolism and high plasma concentrations *in vivo*.



442 POSTER

Triarylpyrroles, dual inhibitors of the MDM2−p53 and MDMX−p53 protein–protein interactions

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The p53 tumour suppressor acts as 'the guardian of the genome' playing roles in cell cycle progression, DNA repair and apoptosis. In normal cells the activity of p53 is tightly regulated by the MDM2 protein *via* a negative feedback loop. Inhibition of the MDM2−p53 protein–protein complex is expected to reactivate normal p53 pathways in cells over-expressing MDM2, resulting in anti-tumour activity. The MDM2 related protein MDMX