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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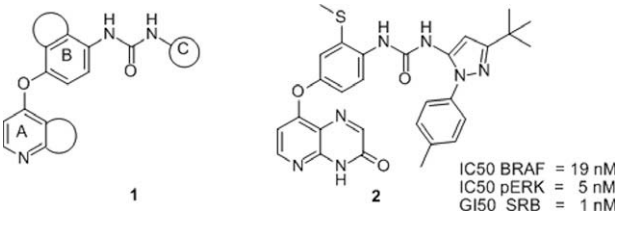
We describe the synthesis and optimisation of a series of new inhibitors of BRAF, a kinase whose mutant form (V600E) is implicated in several types of cancer, with particularly high frequency in melanoma. We designed and synthesised type II inhibitors interacting with the inactive conformation of the ^{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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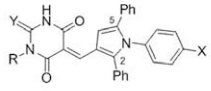
The p53 tumour suppressor acts as 'the guardian of the genome' playing roles in cell cycle progression, DNA repair and apoptosis. In normal cells the activity of p53 is tightly regulated by the MDM2 protein *via* a negative feedback loop. Inhibition of the MDM2−p53 protein–protein complex is expected to reactivate normal p53 pathways in cells over-expressing MDM2, resulting in anti-tumour activity. The MDM2 related protein MDMX

(also known as MDM4) was identified in 1996 and amplification (10%) or over-expression (17%) of MDMX has been found in many tumour types. Unlike MDM2, transcription of MDMX is not induced by DNA damage, and levels remain constant and the activity of the protein is regulated primarily by posttranslational modifications. MDM2 and MDMX appear to have different and complimentary activities, as both proteins inactivate p53. MDMX lacks a ubiquitin ligase function and acts by blocking the p53 transactivation domain. Importantly, over-expression of MDMX has been show to produce resistance to MDM2 inhibition with Nutlin-3.

Screening of commercially available compound libraries resulted in the discovery of novel and potent pyrrole inhibitors of the MDM2−p53 interaction exemplified by NU8324 (MDM2 IC_{50} = 168 nM). Structure–activity relationship (SAR) studies around the pyrrole scaffold have led to the identification of compounds with improved potency, e.g. NU8376 (MDM2 IC_{50} = 73 nM). Subsequently, the series was found to have potent MDMX−p53 activity. Regioselective syntheses of pyrroles bearing different 2- and 5- substituents have been developed and have generated further SARs. Key compounds with dual MDM2− and MDMX−p53 inhibitory activity have been investigated in cellular assays and the results will be reported.



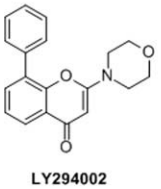
Compound	X	Y	R	MDM2 IC_{50} (nM)	MDMX IC_{50} (nM)
NU8324	NO_2	S	Me	168 ± 62	760 ± 140
NU8225	NO_2	O	H	153 ± 59	680 ± 180
NU8376	Br	S	Me	73 ± 2	-

443 POSTER

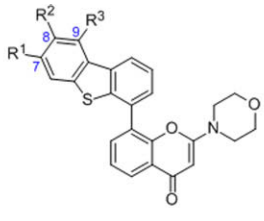
Development of potent inhibitors of DNA-dependent protein kinase (DNA-PK)

K.M. Clapham[1], T. Rennison[1], S. Rodriguez-Aristegui[1], J. Bardos[2], N.J. Curtin[3], B.T. Golding[1], I.R. Hardcastle[1], D.R. Newell[3], C. Cano[1], R.J. Griffin[1]. *[1]Newcastle Cancer Centre Northern Institute for Cancer Research, School of Chemistry Newcastle University, Newcastle-upon-Tyne, United Kingdom; [2]KuDOS Pharmaceuticals Ltd, 410 Cambridge Science Park Milton Rd, Cambridge, United Kingdom; [3]Newcastle Cancer Centre Northern Institute for Cancer Research, Paul O'Gorman Building Newcastle University, Newcastle-upon-Tyne, United Kingdom*

The cellular response to DNA double-strand break (DSB) formation is an essential component of normal cell survival, following exposure to DNA-damaging chemicals (e.g. doxorubicin) and ionising radiation.[1] The serine/threonine kinase DNA-dependent protein kinase (DNA-PK) is a member of the phosphatidylinositol 3-kinase related kinase (PIKK) family of enzymes, and plays an important role in DNA DSB repair *via* the non-homologous end-joining (NHEJ) pathway.[2] ATP-competitive DNA-PK inhibitors may, therefore, be useful as agents to improve the activity of radio- and chemo-therapy in the treatment of cancer.[3]

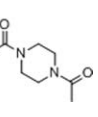


LY294002



NU7441; $R^1 = R^2 = R^3 = H$

NCL-00014518; $R^1 = R^2 = H$, R^3 =



In the absence of suitable structural biology information for DNA-PK, inhibitor design has been guided by a combination of structure–activity relationship (SAR) studies and homology modelling, based on the non-selective PIKK inhibitor LY294002. Identification of the lead dibenzothiophen-4-yl chromenone inhibitor NU7441 (DNA-PK; IC_{50} = 30 nM)[4] confirmed promising activity *in vitro* as a chemo- and radio-potentiator in a range of human tumour cell lines.[5] Further biological studies with NU7441 were hampered by sub-optimal pharmaceutical properties.

Subsequent substitution on the dibenzothiophen-4-yl moiety was investigated through the synthesis of novel analogues bearing a variety of groups at the 7-, 8- and 9-positions (e.g. R^1, R^2 or R^3 = Cl, OMe, OH, OR, NRR', SO_2Me, SO_2NMe_2). Interestingly, several of the newly synthesised compounds (e.g. NCL-00014518) showed high potency against the target enzyme (DNA-PK; IC_{50} = 0.29 nM). The synthesis and biological activity of these substituted dibenzothiophen-4-yl chromenone DNA-PK inhibitors will be discussed.

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[5] Y. Zhao *et al.*, *Cancer Res.*, 2006, 66, 5354.

444 POSTER

Novel 2,3-dihydroimidazo[1,2-c]quinazolines PI3K inhibitors: Discovery and SAR

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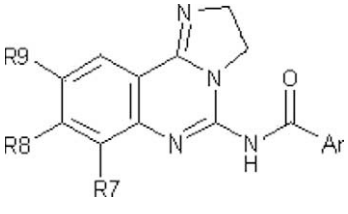
Herein we report on BAY 80-6946, a highly selective and potent pan class I PI3K inhibitor currently in phase I clinical trials. Phosphatidylinositol-3-kinase (PI3K) has become an increasingly important target for oncology research due to the involvement of the PI3K/Akt/mTOR signaling cascade in a wide variety of cancers. PI3K involvement is often marked by amplifications or activating mutations in the PIK3CA gene, which encodes the p110 subunit of PI3Kα. In addition, PI3K signaling is negatively regulated by the dual phosphatase PTEN. However, loss of function or deletions in the gene which encodes PTEN is a common occurrence in human cancers. Moreover, signaling through the PI3K/Akt/mTOR pathway has been shown to be an important pathway in the development of resistance mechanisms to a variety of anti-tumor treatments.

A novel class of 2,3-dihydroimidazo[1,2]quinazolines has been discovered as potent and selective PI3K inhibitors. Beginning with initial lead compounds, activity against PI3Kα and β isoforms was optimized using traditional and structure-based approaches. Herein is presented the SAR for the 2,3-dihydroimidazo[1,2]quinazolines, leading to the selection of BAY 80-6946. BAY 80-6946 is currently in phase I clinical trials.



445 POSTER

Structure-based design of C8-substituted O6-alkylguanine CDK1 and 2 inhibitors

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Defects in the functioning of members of the cyclin-dependent kinase (CDK) family that regulate mitotic progression compromise the normal cell cycle, and are associated with the molecular pathology of cancer [1,2]. As a consequence, small-molecule ATP-competitive CDK inhibitors have potential therapeutic value as antitumor agents.[3] Employing structure-aided design we have previously identified a series of CDK1/2-selective O^6-cyclohexylmethylguanines derived from NU2058 (1) (CDK2, IC_{50} = 16 mM).[4] C-8 substitution within this series demonstrated that the potency of the compounds decreases with increasing size of an alkyl substituent.

Further structural analysis revealed that, to avoid unacceptable steric clashes with Phe80, the C-8 isopropyl derivative (2) adopts a 'reverse' binding mode in which the purine backbone has flipped 180º compared to the binding mode of NU2058. This binding mode provided a platform from which to investigate the design of more potent CDK inhibitors, using