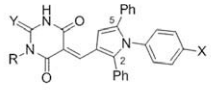
(also known as MDM4) was identified in 1996 and amplification (10%) or over-expression (17%) of MDMX has been found in many tumour types. Unlike MDM2, transcription of MDMX is not induced by DNA damage, and levels remain constant and the activity of the protein is regulated primarily by posttranslational 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.



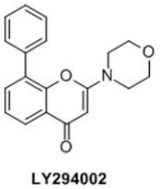
Compound	X	Y	R	MDM2 IC_{50} (nM)	MDMX IC_{50} (nM)
NU8324	NO_2	S	Me	168 ± 62	760 ± 140
NU8225	NO_2	O	H	153 ± 59	680 ± 180
NU8376	Br	S	Me	73 ± 2	-

443 POSTER

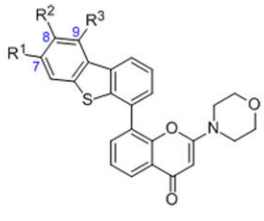
Development of potent inhibitors of DNA-dependent protein kinase (DNA-PK)

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]

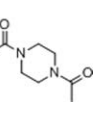


LY294002



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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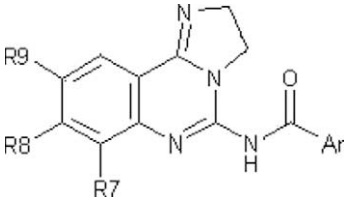
444 POSTER

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

W.J. Scott[1], M. Hentemann[2], B. Rowley[3], C. Bull[3], A.M. Bullion[2], J. Johnson[2], A. Redman[2], N. Liu[1], R. Jones[3], E. Sibley[2]. *[1]Bayer Schering Pharma AG, Global Drug Discovery, Berlin, Germany; [2]Former Bayer Research Center, Medicinal Chemistry, West Haven CT, USA; [3]Former Bayer Research Center, Cancer Research, West Haven CT, USA*

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

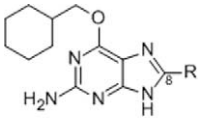
Structure-based design of C8-substituted O6-alkylguanine CDK1 and 2 inhibitors

B. Carbain[1], C. Roche[1], J.A. Endicott[2], B.T. Golding[1], I.R. Hardcastle[1], C. Cano[1], L. Zhen-Wang[1], D.R. Newell[1], M.E.M. Noble[2], R.J. Griffin[1]. *[1]Newcastle Cancer Centre Northern Institute for Cancer Research, School of Chemistry, Newcastle-upon-Tyne, United Kingdom; [2]Laboratory of Molecular Biophysics, Department of Biochemistry, Oxford, United Kingdom*

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

quantum mechanical energy calculations to identify suitable C-8 groups. Addition of a 2-methylphenyl or 2-chlorophenyl group at C-8 restored the potency of this series of 'reverse' binding mode compounds to that of NU2058, providing a novel starting point for inhibitor design. The synthesis, biological evaluation and structural biology of these CDK2 inhibitors will be discussed.



1; R = H
2; R = $CH(Me)_2$

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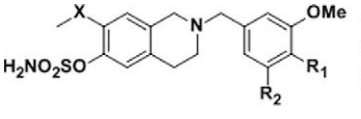
446 POSTER

Optimisation of tetrahydroisoquinoline based microtubule disruptors as anti-cancer agents

M.P. Leese[1], F. Jourdan[1], E. Ferrandis[2], S. Regis-Lydi[2], P.G. Kasprzyk[3], C. Stengel[4], S.P. Newman[4], A. Purohit[4], M.J. Reed[4], B.V.L. Potter[1].
[1]University of Bath, Pharmacy and Pharmacology, Bath, United Kingdom; [2]IPSEN, Institut Henri Beaufour, Les Ulis, France; [3]IPSEN, Biomeasure, Milford, USA; [4]Imperial College, Endocrinology and Metabolic Medicine, London, United Kingdom

We outline the discovery and optimisation of new microtubule disruptors with *in vivo* anti-tumor activity. Translation of the SAR from a steroidal series of microtubule disruptors led us to identify a series of tetrahydroisoquinoline (THIQ) based systems which exhibit a similar activity profile. Of this new series, 2-(3′,4′,5′-Trimethoxybenzyl)-7-methoxy-6-*O*-sulfamoyl THIQ 1 proved especially potent in both *in vitro* (GI_{50} [DU-145] 297 nM) and *in vivo* experiments. Herein, we describe the results of optimisation at C-6 and C-7 of the THIQ core and assessment of other polymethoxylated *N*-benzyl systems.
Variations at the N-2 and C-6 positions were achieved by alkylation, esterification and etherification. Friedel Crafts acylation of C-7 and functional group interconversion allowed access to various C-7 alkyl and alkoxy derivatives. The various dimethoxybenzyl compounds proved similar in activity to the lead compound 2 (GI_{50} 2.1 mM) in the *N*-mono-methoxybenzyl series while, apart from the 3′,4′,5′-trimethoxybenzyl compound 1, only 2′,4′,5′-trimethoxybenzyl substitution delivered sub-micromolar activity. Investigations of the effect of C-6 substitution proved more fruitful. In contrast to the SAR observed for 2 where the sulfamate group is essential for activity, the 6-OH, with the 6-*O*-acyl and 6-*O*-mesyl derivatives of 1 displayed similar or improved activity to the parent compound (GI_{50}s range from 650 to 220 nM). The 6-*O*-methyl derivative, in contrast, proved completely inactive, highlighting the importance of a H-bond donor directly attached to C-6 or a H-bond acceptor projecting further out from this position. The most pronounced improvement in activity was obtained from exploration of C-7 substitution. In the 3′,4′,5′-trimethoxybenzyl series isosteric replacement of methoxy with ethyl delivered a 7-fold improvement in activity (3 GI_{50} 41 nM). Intriguingly, the corresponding phenol proved significantly active suggesting different binding modes operate for the phenol and sulfamate derivatives since the H-bond acceptor properties of the C-7 substituent of the former are clearly important. Incorporation of a C-7 ethoxy group meanwhile proved detrimental for both sulfamate and phenol derivatives. The same transformations were made to 2, though no improvement in activity was obtained.
In order to establish the potential of these compounds as anti-tumor agents their activity in the RPMI-8226 multiple myeloma xenograft model was assessed. The >75% inhibition of tumor growth observed (3 p.o. 40 mg/kg, 28 d) in this preliminary study augers well for the development of this class of anti-cancer agents.



1 X = O, R_1 = R_2 =OMe
2 X = O, R_1 = R_2 = H
3 X = CH_2, R_1 = R_2 =OMe

447 POSTER

Stereoisomerism significantly impacts on the anticancer activity of novel oxaliplatin analogues in vitro and in vivo

U. Jungwirth[1], P. Heffeter[1], J. Gojo[1], S. Abramkin[2], K. Meelich[2], W. Körner[3], M. Micksche[1], M. Galanski[2], B.K. Keppler[2], W. Berger[1].
[1]Medical University Vienna, Institute of Cancer Research, Vienna, Austria; [2]University of Vienna, Institute of Inorganic Chemistry, Vienna, Austria; [3]University of Vienna, Institute for Geological Sciences, Vienna, Austria

Background: Aim of this study was to compare the anticancer properties of two oxaliplatin derivatives (KP1537, KP1691) in vitro and in vivo which differ only in the orientation of the methyl substituent at the cyclohexane ring (equatorial and axial, respectively).
Methods: Cytotoxic/antiproliferative effects were tested against selected human cancer cell lines by MTT analysis. Platinum accumulation was determined by ICP-MS. For in vivo experiments, cells were transplanted i.p. (leukemic) into immuno-competent or immuno-deficient mice.
Results: In vitro both oxaliplatin analogues exerted cytotoxic/cytostatic activity with IC_{50} values in the low μM range, with those for KP1691 beeing significantly lower in comparison to oxalipaltin. In contrast, KP1537 was moderately less active. KP1691 accumulated in tumour cells to the same extend as oxaliplatin. Surprisingly, KP1537 was taken up more rapidly and accumulated over time to 3-fold higher intracellular concentrations. However, the distribution between nuclear and cytosolic compartments was similar between all three platinum drugs. Remarkably, first in vivo experiments demonstrated that both novel substances were less toxic than oxaliplatin resulting in an altered therapeutic window. Generally, all compounds tested prolonged the survival of leukemia-bearing mice, but to different extents. KP1691 was least active, whereas oxaliplatin treatment resulted in an increase in life-span (ILS) by about 100% and 1/5 long-term survivor (LTS). Unexpectedly, the in vitro less active compound KP1537 induced a stronger ILS (>300%) and 3/5 LTS. Furthermore, the impact of the immune system was tested. As known for oxaliplatin, the novel compounds were more active in an immuno-competent background.
Conclusion: Taken together, these findings demonstrate that small sterical changes can have major impacts on the activity of anticancer metal complexes. Thus, the axial methylated KP1691 is more active in vitro but obviously does not efficiently reach its molecular target in vivo. In contrast, the equatorial methylated KP1537, which is less active in vitro, exerts very promising anticancer properties in vivo. Several aspects, including the higher accumulation rates, less adverse effects and the higher in vivo anticancer activity of KP1537 as compared to oxaliplatin, suggests further (pre)clinical development of this novel oxaliplatin analogue.
Supported by the Austrian Science Fund (FWF, grant L568, Translational-Research-Programm) and the "Translational Cancer Therapy Research Platform" of the University Vienna.

448 POSTER

Regulation of uridine phosphorylase-2 redox-control mechanism to improve capecitabine selectivity

G. Pizzorno[1], A. Ziemba[1], T. Le[1], S. Brotman[1], E. Hayes[1], S. Castronovo[1], Y. Gao[1], T. Roosild[1]. *[1]Nevada Cancer Institute, Drug Development, Las Vegas, USA*

The key for a successful chemotherapy agent is the selectivity and specificity for tumor tissue sparing the surrounding normal tissues from toxic anti-proliferative effects. Few agents have been designed to be selectively activated in tumor tissue, one of them is the 5-fluorouracil (5-FU) pro-drug Capecitabine. Capecitabine undergoes a 3-step activation process, initially hydrolysis of the carbamate side-chain by the hepatic carboxylesterases and eventually metabolized from 5′-deoxy-fluorouridine to 5-FU mainly in the tumor tissue that presents an increased phosphorolytic activity due to an elevated presence of the two phosphorolytic enzymes: uridine and thymidine phosphorylases. Several organs and tissues, including liver, express the same two phosphorolytic enzymes resulting in the activation to 5-FU with consequent toxic effects.
In mammalians Uridine Phosphorylase (UPP) is present in two isoforms: UPP1 and the more recently characterized UPP2. Human UPP2, a 317 aa. protein of 35 kD molecular mass, is 60% identical to human UPP1, while murine UPP2 is 85% identical to human UPP2. UPP-2 has broader substrate specificity than UPP1. In addition to uridine and deoxyuridine, UPP-2 utilizes thymidine as substrate. However, no phosphorolytic activity was detected when the enzyme was incubated with adenosine, cytidine, guanosine, deoxyadenosine, deoxycytidine or guanosine. In humans the protein is expressed in kidney, liver and spleen while in mouse UPP2 is present in liver and in much less amount in kidney and brain.
We have completed the crystallographic structure determination of hUPP2, having collected a 1.5Å dataset at SSRL and phased the data using Molecular Replacement, searching with a homology model of hUPP2 constructed