polymerase. Experiments with DNA in vitro confirm the threading binding mode and slow complex dissociation kinetics, and measurements using DNA microarrays have shown these agents to be powerful inhibitors of mRNA synthesis at cytotoxic doses. These findings confirm the importance of linker rigidity in kinetically stabilizing the DNA-ligand complex, and the importance of linker rigidity and slow kinetics in conferring template inhibition of transcription. Currently, we are exploring ways of enhancing these properties within the bis(9-aminoacridine-4-carboxamide) paradigm by structural modifications to the chromophore, the threading side-chain, and the linker itself. In the work described here, we report the synthesis and biological activity of a series of compounds in which a benzene ring has been fused to the acridine chromophore at the 5,6 position. In previous studies with monomeric 9-aminoacridine-4-carboxamides, this substitution has been shown to enhance DNA affinity, and to slow complex dissociation rates: effects attributed to enhanced stacking interactions between the intercalated chromophore and the DNA base pairs. In the dimer series, we find the benzacridine substitution enhances both cytotoxic potency and the life-time of the DNA complex.

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Cytotoxic activity of 4'-hydroxychalcone derivatives against Jurkat cells and their effects on mammalian DNA topoisomerase I

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Chalcones (1,3-diaryl-2-propen-1-ones) are alfa, beta-unsaturated ketones with cytotoxic and anticancer properties. Several reports have shown that the compounds with cytotoxic properties may also interfere with DNA topoisomerase functions. We synthesized five derivatives of 4'-hydroxychalcones and carried out cytotoxicity tests against transformed human T (Jurkat) cells as well as plasmid supercoil relaxation experiments using mammalian DNA topoisomerase I. The compounds synthesized was 3-aryl-1-(4'-hydroxyphenyl)-2-propen-1-one. The aryl part was phenyl, p-methylphenyl, p-methoxyphenyl, p-chlorophenyl and 2- thienyl for the compounds I-V respectively. The order of the cytotoxicity of the compounds was; IV > III > II > I > V. The compound IV, 3-(4-chlorophenyl)-1-(4'-hydroxyphenyl)-2-propen-1-one, had the highest Hammett and log P values (0.23 and 4.21, respectively) and exerted both highest cytotoxicity and strongest DNA topoisomerase I inhibition. The compounds I and II gave moderate interference with the DNA topoisomerase I while the remaining ones did not interfere with the enzyme.

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Biochemical and cellular effects of a novel cyclin-dependent kinase inhibitor

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Cyclin-dependent kinases (CDK) are essential components of the cell-cycle regulatory system and due to their frequent deregulations in cancer cells they have become important targets for drug development. We have recently prepared novel group of potent and selective CDK inhibitors based on pyrazolo[4,3-d]pyrimidine scaffold. The prototype derivative LGR1404 is an isomer of roscovitine, which is a well known CDK inhibitor. We therefore directly compared effects of both compounds in biochemical and cellular assays. As expected, compound LGR1404 was found to potently inhibit cyclin-dependent kinases CDK2, CDK5 and CDK9 in enzyme assays, with IC50 values in submicromolar range. Being more potent CDK inhibitor than roscovitine, the compound also demonstrated much stronger anti-proliferative activities in human cancer cell lines, including standardized NCI60 panel. An average GI50 for roscovitine is 19,3 μM, while LGR1404 has GI50 about 7 μM. Cells treated with LGR1404 show a dose-dependent decrease of phosphorylation of the retinoblastoma protein and cell cycle arrest. Moreover, the compound increases cellular level of the tumor suppressor protein p53, stabilizes its nuclear localization and, subsequently, activates transcription of some p53-regulated genes; this effect probably results from inhibition of CDKs involved in transcription. Finally, LGR1404 causes apoptosis in treated cells, as assessed by activation of caspases, fragmentation of PARP and nuclei condensation. In conclusion, all biochemical and cellular effects of the compound are fully consistent with direct inhibition of CDKs, both cell cycle and transcriptional. The novel prototype inhibitor significantly exceeds activities of roscovitine and, thus, demonstrates the qualities of all other pyrazolo[4,3-d]pyrimidine inhibitors with potential pharmacological applications in oncology.

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Dysregulation of defence systems by 5-fluorouracil in colon cancer HT-29 cells

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A primary cause of cancer treatment failure and patient relapse is an acquired or intrinsic resistance to anticancer therapies. Acquisition of drug resistance can be attributed to various factors including inhibition of apoptosis, altered expression of multidrug resistance-associated proteins, altered drug metabolism or uptake, and/or overexpression of defence systems. Since various anticancer drugs are potential inducers of defence pathways, this could have a marked incidence on cancer cell resistance. Using colon HT29 cells, we found that 5-fluorouracil (5-FU), widely used in the treatment of colorectal cancer, induced the expression of mRNAs encoding glutathione transferases M3 and S1 and antioxidants enzymes such as NAD(P)H:quinone oxidoreductase 1, heme oxygenase-1 and γ-glutamylcysteine synthetase. To further determine the mechanisms involved in 5-FU effects, we investigated whether it activates the Nrf2/antioxidant response element (ARE) pathway which is implicated in the regulation of several genes involved in cell defense systems. Translocation of Nrf2 into the nucleus after 5-FU exposure was demonstrated by immunolocalization and western blot assays. By using an ARE driven-reporter gene (luciferase) assay, activation of the luciferase activity by 5-FU was evidenced and this effect was inhibited by co-transfecting a vector expressing a dominant negative Nrf2. Moreover, transfection of Nrf2 siRNA into HT-29 cells increased 5-FU cytotoxicity. In conclusion, these results demonstrate that 5-FU activates the Nrf2/ARE pathway which modulates the chemosensitivity of colon cancer HT29 cells and might represent a potential therapeutic target in 5-FU treatment.

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Heat shock protein 90 inhibitors modulate choline phospholipid profiles and metabolizing enzymes in human melanoma cells

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Heat shock protein 90 (Hsp90) inhibition is a novel anticancer strategy permitting simultaneous depletion of many oncogenic proteins (eg CRAF & HIF-1α) and many Hsp90 targeted drugs such as 17-AAG and 17-DMAG are now in clinical trial. Here we use magnetic resonance spectroscopy (MRS), a non-invasive technique for studying cell metabolism, to assess whether Hsp90 inhibition in human melanoma cells is associated with metabolic alterations that may serve as biomarkers of target modulation in the clinic.

SKMEL28 human melanoma cells were treated with equipotent concentrations of 17-AAG (100 nM), 17-DMAG (200 nM) or our novel agent CCT018159 (30 microM) for 48h then extracted in methanol, chloroform and water (1:1:1), and aqueous fractions analysed by 31P MRS. Western blotting for expression of CRAF and Hsp70 (known to be induced upon Hsp90 inhibition) was used to confirm drug action.

^{31}P MRS analysis indicated that exposure of cells to 17-AAG resulted in an increase in the level of metabolites involved in membrane phospholipid turnover. Cellular phosphocholine (PC), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE) content increased by ~3, 4 and 2.6-fold respectively (n=4, $p \leq 0.02$). Furthermore, nucleoside triphosphates (NTP) and PC/NTP were also increased by 2 and 1.7-fold respectively ($p \leq 0.049$), concomitant with CRAF depletion and Hsp70 induction.

Similar changes were seen with 17-DMAG (PC, GPC and PC/NTP up by 2.8, 4.8 and ~2-fold respectively) and CCT018159 (PC, GPC & PC/NTP up by 2.4, 3 and 1.4-fold respectively).

We next assessed the effect of Hsp90 inhibitors on the activity of enzymes involved in the breakdown of the major membrane phospholipid phosphatidylcholine (PtdCho). Amplex Red spectrophotometric assay of PtdCho specific phospholipase C (PtdCho-PLC) showed a decrease in the enzyme's specific activity to 45±19% of controls (n=4, p=0.015) in 17-AAG treated cells. Western blotting showed a marked reduction in phosphorylated (activated) cytoplasmic phospholipase A2 (cPLA2) but not total cPLA2 in cells treated with all three inhibitors.

Our results indicate that inhibition of Hsp90 in human melanoma cells results in altered choline phospholipid metabolism that is associated with