low dosages, these compounds could stimulate the expression of some p53 pathways genes. A comprehensive study showing the differential expressions of all possible p53 pathways genes has not been reported.
Materials and Methods: The drugs were added at low dosages to isogenic p53 knock-out HCT116 colon adenocarcinoma cell lines for 16 hours. Total RNA from the cells was extracted and the expression profiles of the treated cells were compared to untreated cells by hybridization to the Illumina microarrays. Flow cytometry was performed concurrently to demonstrate cell cycle arrest and apoptosis. Genotoxic effect was determined by measuring double-stranded DNA breaks using H2AX assay. Array results were analyzed using TIGR array analysis software and interactions of differentially expressed genes were mapped using Ingenuity program. Array results were confirmed by quantitative polymerase chain reactions on selected genes.
Results: By comparing the expression profiles of the p53 isogenic cell lines, microarray analysis revealed that ActD and LMB were able to activate p53-dependent pathways at dosage of not more than 10 nM. Reduction in the number of cells in S phase was also observed at these dosages. Higher dosages of these compounds led to accumulation of cells at sub G1 phase and differential expression of genes not related to p53 pathways. The activation of p53 pathways at low dosages is similar to treatment with Nutlin. No DNA fragmentation was observed at all dosages used.
Conclusions: ActD and LMB at low dosages are able to stimulate p53-dependent pathways without a general toxic effect.

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SRJ09, a lead compound in anticancer drug design: in vitro, in vivo and mechanistic studies

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Background: The search for more effective and selective anticancer drugs is currently being researched actively involving the various entities of the drug discovery programme. We have shown andrographolide (AGP), a compound isolated from a local herb, *Andrographis paniculata*, to have anticancer activity *in vitro* and *in vivo*. In order to improve the antitumour properties of AGP, semisynthetic derivatives of this compound were synthesised in our laboratory, with the aim of identifying the most promising anticancer compounds and to elucidate their mechanism(s) of action.
Materials and Methods: Cell viability assays (MTT and SRB) were used to determine the *in vitro* growth inhibitory properties of compounds. Nude mice were utilised for the *in vivo* antitumour study. Flow cytometry was performed to assess cell cycle arrest and apoptosis. Western blotting was used to determine the cellular protein levels.
Results: SRJ09 (3,19-(2-bromobenzylidene)andrographolide) displayed better antitumour activity when compared with AGP and other derivatives (SRJ11 and SRJ23). In the NCI *in vitro* anticancer screen the compound showed selectivity towards melanoma, colon, renal and breast cancers. The antitumour activity of AGP, SRJ09, SRJ11 and SRJ23 was shown to be not compromised by P-glycoprotein activities in MES-SADx5 multidrug resistant cell line. The *in vivo* antitumour study showed SRJ09 delayed quadruple tumour growth by 4 days in HCT-116 colon cancer xenografted mice treated with 400 mg/kg dose (q4dx3) when compared with control. SRJ09 induced a G_1 arrest in MCF-7 breast and HCT-116 colon cancer cells and the effect was attributed to decreased CDK-4 and increased of p21 expressions without affecting the expression of cyclin D1. Apoptosis was the main mode of cell death induced by SRJ09 and was p53 and bcl-2 independent.
Conclusions: In conclusion, SRJ09 emerged as the lead anticancer agent given its ability to induce G_1 specific cell cycle arrest and apoptosis and to have *in vivo* antitumour activity. Additionally, NCI's *in silico* SOM analysis indicated this compound might have a novel molecular target. Therefore, further studies in improving the anticancer properties of SRJ09 by chemical modification will be advantageous.

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Discovery of potent and selective focal adhesion kinase inhibitors

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Background: Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that regulates multiple cell functions and it is known as a key driver of tumor cell proliferation, migration, and survival. NVP-TAE226, known as a dual inhibitor of FAK and IGF-IR with 2-phenylamino-pyrimidin-4-ylamino-benzamide scaffold, demonstrated the inhibition of growth of 4T1 murine breast tumor cells and metastasis to the lung in an orthotopic model in a dose-dependent manner. However, a potential effect on the glucose metabolism through Insulin receptor (Ins-R) kinase inhibition was suspected because of the modest selectivity of NVP-TAE226 over Ins-R kinase (approx. 8-fold). Under a particular condition in C57BL6 mice, which exhibit high sensitivity to glucose metabolism interference, an increase of insulin and glucose levels was observed at a dose of 100 mg/kg p.o..
Material and Methods: To discover FAK inhibitors with higher selectivity over Ins-R kinase than NVP-TAE226, the scaffold was modified on the basis of the structural information of FAK and Ins-R kinase. With this approach, 2-phenylamino-pyrimidin-4-ylamino-2,3-dihydro-isoindol-1-one and 2-phenylamino-pyrimidin-4-ylamino-3,4-dihydro-2*H*-isoquinolin-1-one were found to be potential scaffolds to show high selectivity not only for Ins-R kinase but also other tyrosine kinases.
Results: Among the synthesized compounds, compound **1** showed higher selectivity over Ins-R kinase than NVP-TAE226 (more than 150-fold). As a result of further optimization studies of these series, compound **2** and **3**, which exhibited more than 780-fold selectivity over Ins-R, did not show any effect on the insulin and glucose levels in the sensitive model using C57BL6 mice. Furthermore, theyshowed equivalent or more potent anti-tumor activities compared with NVP-TAE226 in the *in vivo* studies.

	IC_{50} [μmol/L]				
	FAK	CDK1	IGF-1R	Ins-R	c-Src
NVP-TAE226	0.0053	0.56	0.12	0.044	2.3
1	0.0011	2.9	0.5	0.19	7.4
2	0.0042	>10	>10	>3.3	>10
3	0.0012	>10	1.9	1.8	2.3

Conclusions: These novel classes of selective and small molecule FAK inhibitors have potential clinical applications with potent *in vivo* anti-tumor activities and high tolerability.

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The rational design of inhibitors of the telomere-hnRNP A1 interaction

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The heterogeneous nuclear ribonucleoparticle (hnRNP) A1 and A2 proteins are multi-functional proteins that associate with telomeres, stimulate telomerase activity, participate in mRNA transport, and are involved in pre-mRNA splicing. They have sequence-specific RNA and single-stranded DNA binding activity, via tandem RNA recognition motifs (RRM). A1 and A2 are required for the viability of transformed human cells, but are dispensable for the growth of normal cells. We undertook a rational design approach to develop small molecules capable of inhibiting binding of A1 and A2 to telomeric single stranded DNA. Based on published x-ray structures, we chose to target the core of the RRM binding pocket that interacts with the nucleotides TAG within the TTAGGG telomeric repeat. Using Biacore (TM) analysis we determined that the TAG oligo retained good affinity for A1, and using x-ray crystallography confirmed that its binding to A1 was analogous to the full telomeric repeat. Using the TAG trinucleotide as a