(3) assays of candidate drug activity, including electrophoretic mobility shift assay (EMSA), RNAse protection assay (RPA), and assays of cellular apoptosis and toxicity, and (4) confirmation of anti-tumor in nude mouse xenograft models.
Results: (1) G-quartet forming oligonucleotides (GQ-ODN), named T40214 and T40231, were developed as potent agents, which specifically inhibit Stat3 DNA-binding activity in several human cancer cell lines, such as hepatoma (HepG2), prostate (PC-3), breast (MDA-MB-468), and head and neck (167, B4B8, 1968) cancer cells, holding promise for the systemic treatment of many forms of human cancer. (2) We have constructed a model of GQ-ODN/Stat3 complex and established a structure-activity relationship (SAR) between GQ-ODN and Stat3 dimer for drug design and screening. (3). We have also developed a novel and effective intracellular delivery system for GQ-ODNs. This delivery system greatly increased the delivery efficiency and drug activity of GQ-ODNs within cells. Also this system was capable of delivering G-quartet inhibitors into tissues and tumors in xenograft animal models. (4) Our *in vivo* data demonstrated that T40214 and T40231 suppressed the growth of prostate and breast tumors *in vivo* by inhibiting Stat3 activation, resulting in a dramatic increase in apoptosis of tumor cells. The mean size of the breast tumor xenografts of placebo-treated mice increased from 11 fold over 18 days while the mean sizes of both T40214 and T40231-treated mice remained unchanged ($p<0.001$). The mean size of the prostate tumor xenografts of placebo-treated mice increased from 9 fold over 10 days while mean sizes of both T40214 and T40231-treated mice were only increased by 2.2 and 4 fold, respectively ($p<0.05$). The results also demonstrated that the mean levels of phosphorylated Stat3 (p-Stat3), Bcl-x_L and Bcl-2 were decreased by 9, 4.3 and 10-fold, respectively, and caspase 3 cleavage products increased 3-fold in the tumors from drug-treated animals compared to tumors from placebo-treated mice. The percentage of apoptotic cells was increased nearly 8-fold in the tumors of drug-treated mice (83.6±1.0%) compared to the tumors of placebo-treated mice (11.2±10.1%).
Conclusion: GQ-ODNs as novel anti-cancer agents specifically inhibited Stat3 activation among other STAT protein members and suppressed the expression of Stat3–regulated anti-apoptotic genes, such as *Bcl-x_L*, *Bcl-2* and *Mcl-1*. GQ-ODN also suppressed the growth of human tumors where Stat3 is activated and significantly increased apoptosis of tumor cells. Therefore, GQ-ODNs as a new class of potent anti-cancer agents hold promise for the systemic treatment of many forms of human cancer.

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Synthesis and cytoxicity studies of platinum nucleobase adducts

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We are studying interaction of cisplatin analogs with DNA nucleobases. A series of novel platinum (IV) nucleobase monoadducts of the type [Pt^{IV}(DACH)*trans*-(X) $_2$LCl]NO_3 (where DACH = *trans*-1*R*,2*R*-diaminocyclohexane, L = adenine, guanine, and 9-ethylguanine and X = acetato ligand) have been synthesized and characterized by elemental analysis and by NMR spectroscopic technique. The crystal structure of the model nucleobase complex [Pt^{IV}(*trans*-1*R*,2*R*-diaminocyclohexane) *trans*-(acetate)$_2$(9-ethgua)Cl]$NO_3 \cdot H_2O$ was determined using a single crystal X-ray diffraction method. The complex crystallized in the monoclinic space group $P2_1/c$, with a = 10.446(2) Å, b = 22.906(5) Å, c = 10.978(2) Å, Z = 4, and R = 0.0569, based upon the total of 11570 collected reflections. In this complex, platinum had a slightly distorted octahedron geometry owing to the presence of a geometrically strained five-member ring. The two adjacent corners of the platinum plane were occupied by the two amino nitrogen of DACH, whereas, the other two equatorial positions occupied by chloride ion and 9-ethylguanine. The remaining two axial positions were occupied by the oxygen atoms of acetato ligands. The DACH ring was in a chair configuration. An intricate network of intermolecular hydrogen bonds held the crystal lattice together. Such DACH-Pt-DNA adducts have good in vitro cytotoxic activity against the cisplatin-sensitive human cancer ovarian A2780 cell line (IC50 = 1–8 μM). Interestingly, a substituted nucleobase (9-ethylguanine) adduct was over 6-fold more potent than regular adducts. The cross-resistance factor against the 44-fold cisplatin-resistant 2780CP/clone 16 cells was about 3–9; thus, the cytotoxicity of adducts was indicative of low potency, but the resistance factors were also substantially low. These results suggest that DNA adducts of DACH-Pt are cytotoxic with low cross-resistance. (Supported by NCI CA 77332 and CA 82361)

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Synthesis, anti-proliferative and anti-angiogenic effects of sulfamoylated 2-methoxyestradiol analogues

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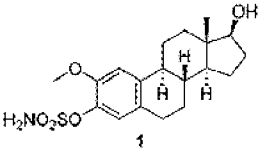
The ability of 2-methoxyestradiol (2-MeOE2), an endogenous estrogen metabolite, to inhibit both the proliferation of human cancer cells and angiogenesis is well established. Sulfamoylated derivatives of 2-MeOE2, such as 2-methoxy-3-*O*-sulfamoyl estradiol **1** (2-MeOE2MATE), display enhanced activity and, in contrast to 2-MeOE2, cause an irreversible cell cycle arrest. In this study we report on the structure activity relationships of the family of *mono*- and *bis*-sulfamoylated 2-substituted estradiols as anti-proliferative agents. Efficient multi-step chemical syntheses of these compounds have been developed allowing a determination of synergistic effects of 2-, 3- and 17-substituents. To rationalize the activities observed in this series we have applied computational modelling techniques to identify the likely site of interaction of these molecules with tubulin. Novel compounds were evaluated against the proliferation of human breast (MCF-7) and ovarian (A2780) cancer cells *in vitro*. Optimal anti-proliferative activity in the simple estradiol-3-*O*-sulfamate series was afforded by the 2-methoxy, 2-ethyl and 2-methyl sulfanyl functions. The bioisosteric nature of these 2-substituents is illustrated by the anti-proliferative activities observed for 2-methoxy-, 2-ethyl- and 2-methyl sulfanyl-estradiol-3,17-*O*, *O*-*bis* sulfamates **2**–**4** which caused 50% growth inhibition in A2780 ovarian cancer cells at concentrations of 0.24, 0.26 and 0.23mM respectively. Subsequent experiments on the bioisosteric replacement of the 17-sulfamate group delivered several further active series which caused 50% growth inhibition (A2780) at concentrations as low as 0.04 μM. Further evaluation of these compounds showed that these compounds show highly promising anti-angiogenic activity. Compound **2** inhibited HUVEC proliferation at 0.33 μM, cord formation at 0.06 μM and chemotaxis at 0.36 μM in *in vitro* studies. Results obtained in the NCI hollow fibre assay and Lewis Lung model, as well as in *in vivo* models of angiogenesis underline the therapeutic potential of sulfamoylated 2-methoxyestradiol analogues as drug candidates with a multi-targeted mode of action.



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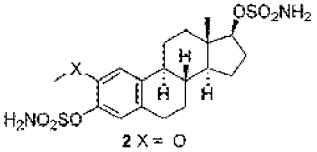

2 X = O
3 X = CH_2
4 X = S

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Identification of inhibitors of the MDM2–p53 interaction using a virtual screening approach with multiple binding modes

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Rational structure-based molecular design can be used to improve lead compounds. This requires that the structure of the intermolecular complex formed between the target protein and the lead compound is known. Ligand-protein docking studies can be used to overcome a lack of experimental structural data. Whilst it is critical for any following experiment that the correct binding mode is selected, it can be difficult to distinguish a single docking solution as preferred. Failure to identify the correct binding mode will spoil subsequent design efforts.
The impact of considering multiple binding modes from docking studies has previously been statistically quantified. We made use of the approach in a virtual screen of reagents on a lead scaffold (Figure 1) known to inhibit the protein-protein interaction between MDM2 and p53. The aim was two-fold: first, to improve the affinity and drug-likeness of the compounds through the use of substituents with an increased level of functionality; second, to narrow down the number of putative binding modes by introducing stronger directionality in the interaction.
A small number of geometrically diverse and high-scoring binding modes were selected from a large pool of docking solutions of the lead compound. The scaffold was extracted from the selected solutions and reagents