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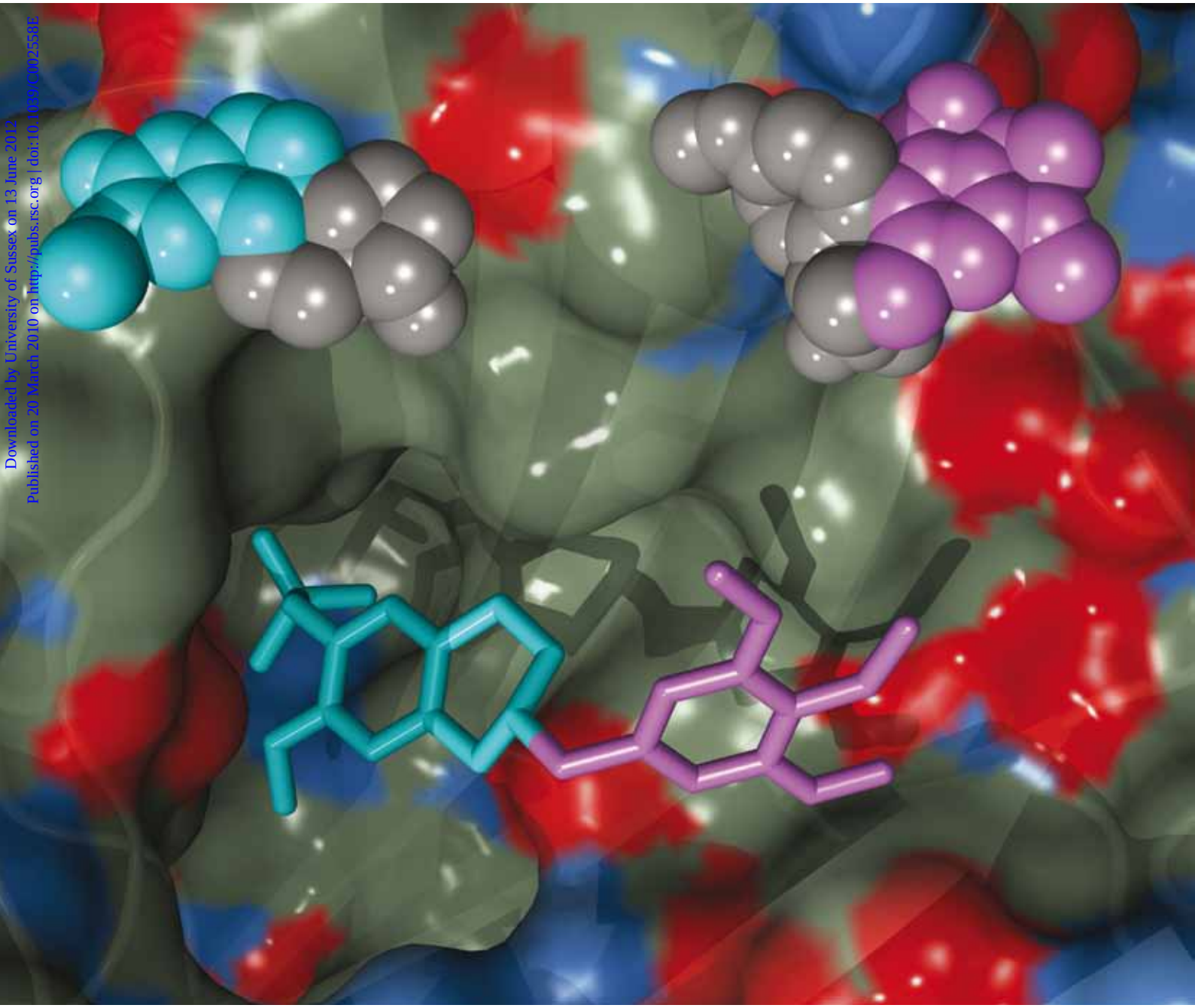

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Chimeric microtubule disruptors

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A chimeric approach is used to discover microtubule disruptors with excellent *in vitro* activity and oral bioavailability; a ligand–protein interaction with carbonic anhydrase that enhances bioavailability is characterised by protein X-ray crystallography. Dosing of a representative chimera in a tumour xenograft model confirms the excellent therapeutic potential of the class.

Microtubule disruptors inhibit the normal dynamic assembly of α,β-tubulin into polymeric microtubules, a process key to mitosis, triggering cell cycle arrest and ultimately cell death.[1] Their cytotoxic effects are thus targeted towards rapidly dividing cancer cells. Although arguably the most successful class of anti-cancer drugs, issues with structural complexity, oral bioavailability, therapeutic window, formulation and acquired resistance remain.[2] Herein, we outline a chimeric approach to the discovery of simple small molecule inhibitors of both tubulin polymerization and angiogenesis which overcome many of the problems associated with existing clinical agents.

Our design strategy integrates pharmacophore elements from two series of colchicine site binding natural products that disrupt microtubule dynamics. 2-Methoxyestradiol (2-MeOE2, **1**) is an anti-tumor agent of poor oral bioavailability with dual anti-proliferative and anti-angiogenic actions.[3] Its apparent lack of toxicity and mechanism of action led to numerous clinical trials, yet these were hampered by rapid inactivating metabolism and conjugative clearance.[4] Colchicine **2** is one of a number of potent natural product microtubule disruptors incorporating a trimethoxyaryl motif.[5] Its application as an anti-cancer agent is compromised by a low therapeutic index. By incorporation of the sub-pharmacophores (Fig. 1) from **1** and **2** into a single chimera, we envisaged generation of novel agents with enhanced drug-like properties that might

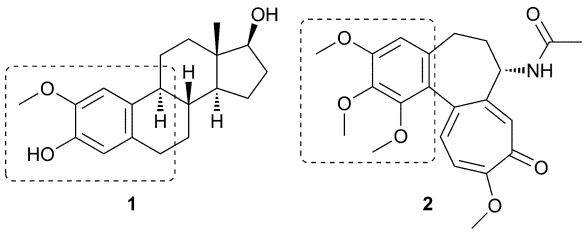
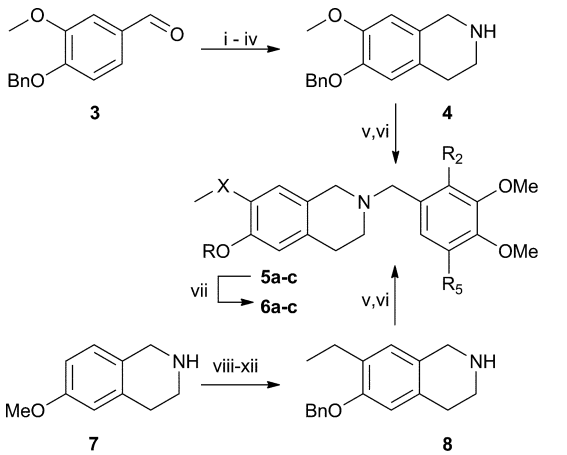


Fig. 1 Structures of 2-methoxyestradiol **1** and colchicine **2**. The pharmacophore elements used to construct the chimera are boxed.

overcome the limitations of parent compounds. Thus, a bicyclic motif bearing elements of the steroidal AB-rings of 2-MeOE2 was attached to a trimethoxyaryl group. Nitrogen incorporation in the bicycle provided a logical point through which the trimethoxyaryl motif could be attached and also allows for salification and enhanced aqueous solubility.

A tetrahydroisoquinoline (THIQ) mimic was thus targeted and synthesized from benzyl vanillin **3** (Scheme 1). The desired THIQ **4** core was accessed *via* a Pomeranz–Fritsch approach.[6] *N*-Benzylation of **4** followed by hydrogenation delivered the prototypical chimeras **5a** and **5b**. The compounds were evaluated for their ability to inhibit DU-145 (prostate cancer) and MDA MB-231 (breast cancer) cell proliferation (Table 1). While **5a** showed no significant activity at concentrations up to



Scheme 1 Synthesis of THIQ derivatives. *Reagents and conditions*: (i) $(MeO)_2CHCH_2NH_2$ (ii) $NaBH_4$, MeOH (iii) 6 M HCl, dioxane (iv) $NaBH_4$, TFA (v) $(MeO)_3BnCl$, Et_3N, EtOH, MW 130 °C, 1 h (vi) H_2, Pd/C (vii) H_2NSO_2Cl, DMA (viii) AcCl, $NaHCO_3$, DCM (ix) $AlCl_3$, AcCl, DCM (x) Et_3SiH, TFA (xi) BnBr, NaH, DMF (xii) NaOH, EtOH.

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Table 1 GI_{50}s in μM against the proliferation of human prostate[a] and breast[b] cancer cell lines *in vitro*

Entry	R	X	R_2	R_5	DU-145[a]	MDA MB-231[b]
5a	H	O	OMe	H	>100	>100
5b	H	O	H	OMe	0.650	0.620
5c	H	CH_2	H	OMe	3.85	5.41
6a	SO_2NH_2	O	OMe	H	17.0	11.9
6b	SO_2NH_2	O	H	OMe	0.297	0.329
6c	SO_2NH_2	CH_2	H	OMe	0.041	0.052

100 μM, **5b** showed excellent activity with GI_{50} values of 650 and 620 nM, respectively, validating the design concept.

Having previously pioneered the sulfamoylation of phenols to improve oral bioavailability (*vide infra*), and considering that the 6-hydroxyl group would be prone to conjugation, we next synthesized the 6-*O*-sulfamates **6a** and **6c**[7] from **5a** and **5b** using sulfamoyl chloride in dimethyl acetamide. The *N*-(2,3,4-trimethoxy)benzyl derivative **6a** showed improved, but still modest, anti-proliferative activity while **6b**§ proved *ca.* 2-fold more active than **5b**. Exchange of the methoxy for the isosteric ethyl group at C-7 was next explored. 6-Methoxytetrahydroisoquinoline **7** was thus acylated at N-2; then a Friedel–Crafts acylation effected concomitant 7-acylation and demethylation of the 6-methoxyl group.

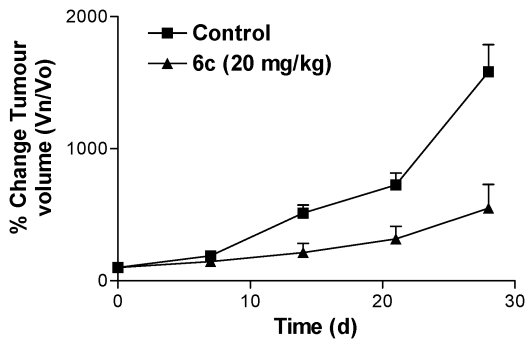

Fig. 2 Effects of **6c** on the growth of MDA MB-231 xenografts.

Reduction of the C-7 acyl group followed by benzyl protection delivered the doubly protected THIQ **8** that could then be converted to the target phenol **5c** and sulfamate **6c** under standard conditions (Scheme 1). In the case of **5c** substitution of the C-7-methoxy group for ethyl proved detrimental to activity, with the 6-fold reduction in activity illustrating the importance of the methoxy oxygen in the phenol series. Conversely, sulfamate **6c** proved *ca.* 100-fold more active than its parent phenol **5c** and 7-fold more active than **6b** against the DU-145 line, thus indicating a divergence in the SAR of the 6-hydroxy and 6-*O*-sulfamoyl compounds. The relative activities of 2-MeOE2 **1**, prototypical chimera **5b** and modified chimera **6c** (DU-145 GI_{50}s 1.22 μM, 650 nM and 41 nM) illustrate the power of the chimeric approach and value of subsequent optimization.

The ability of the active chimeras to inhibit the Taxol stimulated polymerization of tubulin was established and confirmed the mechanism of action; the activities of **5b**, **6b** and **6c** in this assay correlate with their relative anti-proliferative effects. Inhibition of angiogenesis alongside cell proliferation by co-administration of a microtubule disruptor and an angiogenesis inhibitor[8] offers a dual, clinically validated approach against cancer. The activity of **6c** against HUVEC cell proliferation *in vitro* (GI_{50} 6 nM), a marker of anti-angiogenic potential, indicates that the chimeras retain 2-MeOE2's dual mechanism of action.

An important challenge for new agents is the circumvention of acquired resistance that often stems from upregulation of drug efflux pumps. The MCF-7_{DOX} cell line, by upregulation of P-glycoprotein (PgP), is resistant to the DNA intercalator doxorubicin and the taxanes.[9] **6c** similarly inhibited proliferation of both wild type human breast cancer MCF-7 cells (GI_{50} 20 nM) and their resistant MCF-7_{DOX} counterparts (GI_{50} 10 nM), indicating that the chimera is not a substrate for the PgP pump and has potential for use in the therapy of taxane resistant tumors.

Having established a desirable *in vitro* profile **6c** was progressed to *in vivo* evaluation. The effects of **6c** on the

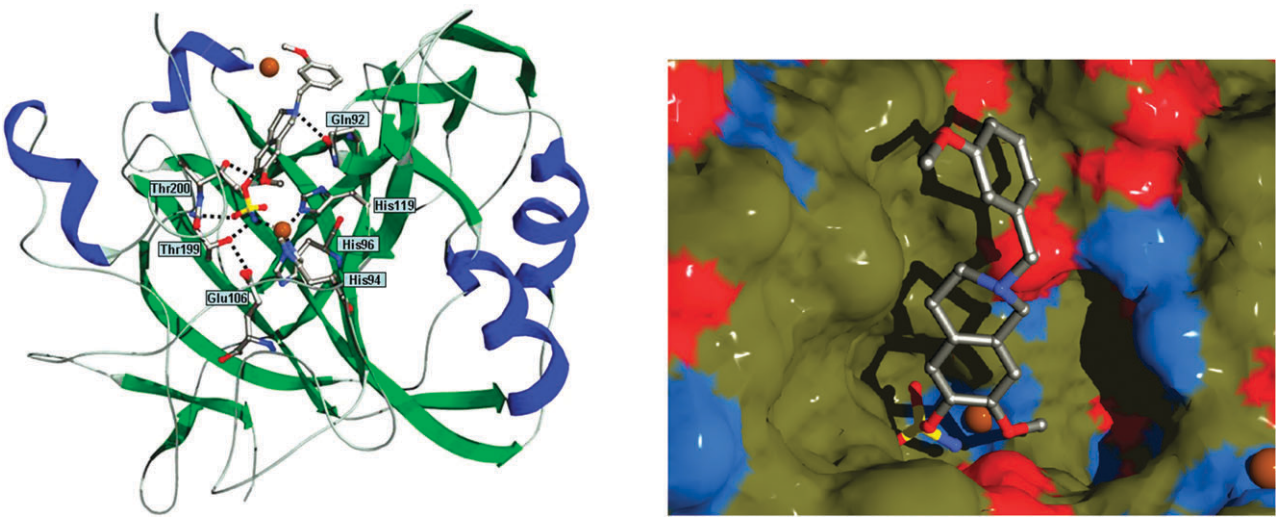

Fig. 3 Protein X-ray crystal structure of hCAII-**9** complex; left panel: active site funnel region of hCAII-**9** complex; right panel: ligand **9** shown viewing into active site, showing potential for further elaboration of pendant aryl group, with the protein shown as a molecular surface; acidic residues are coloured red, basic residues in blue and the zinc ions are shown in brown.

growth of MDA MB-231 xenografts in athymic female nude mice were investigated. Oral dosing at 20 mg kg^{-1} was carried out over 28 days using a simple aqueous medium. This initial experiment (Fig. 2) revealed that, even at a non-optimized dose or formulation, **6c** greatly inhibits the growth of the breast cancer xenograft and with no apparent signs of toxicity.

The high oral bioavailability of selected aryl sulfamates is believed to arise from sequestration by red blood cell (rbc) carbonic anhydrase (hCAII)[10] which allows avoidance of first pass liver metabolism. We defined the interaction of the sulfamoylated chimeras and hCAII using protein crystallography. Of a panel of inhibitors complexed with hCAII, crystals obtained with THIQ derivative **9** (the *N*-[3-methoxybenzyl] analogue of **6a**) were of suitable quality for X-ray analysis. The protein crystal structure (Fig. 3) shows the co-ordination of the catalytic Zn by the sulfamate nitrogen and one of its oxygens, whilst the flanking methoxy group H-bonds to Thr-200. The THIQ nitrogen also appears to pick-up a bonding interaction (to Gln-92), while the *N*-benzyl group projects into a hydrophobic region that tantalizingly could be further utilized to enhance potency for this carrier.

Since this new class of orally available agent overcomes numerous issues limiting the optimal clinical application of microtubule disruptors it will be attractive to optimise the best representatives towards early clinical entry in oncology.

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Notes and references

§ Preparation of 7-methoxy-2-(3,4,5-trimethoxybenzyl)-6-*O*-sulfamoyl-1,2,3,4-tetrahydroisoquinoline **6b**: a solution of 6-hydroxy-7-methoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (180 mg, 0.5 mmol) and sulfamoyl chloride (1 mmol) in DMA (1 mL) was stirred at rt under nitrogen for 24 hours. After addition of water (5 mL) and $KHCO_3$ (150 mg, 1.5 mmol) the reaction mixture was extracted into ethyl acetate (2 × 50 mL), the organic layers washed with water and brine, then dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (DCM/ethyl acetate gradient) and precipitated from diethyl ether to give the desired product as a yellow powder (125 mg, 57%), mp 143–144 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.70–2.84 (4H, m), 3.58 (2H, s), 3.61 (2H, s), 3.81 (3H, s), 3.83 (3H, s), 3.84 (6H, s), 5.10 (2H, br s), 6.61 (1H, s), 6.63 (1H, s) and 7.07 (1H, s), ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 28.1, 50.4, 55.7, 56.2, 56.4, 61.0, 62.8, 105.7, 111.2, 124.2, 127.6, 133.7, 134.6, 137.0, 137.4, 149.4 and 153.3. HRMS (ESI) calcd for $C_{20}H_{27}N_2O_7S$ $[M + H]^+$, 439.1533; found 439.1525. Microanalysis: C: 54.3% (expected 54.78%); H: 5.99% (expected 5.98%); N: 6.30% (expected 6.39%).

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