## **Electronic Supporting Information**

### **Chimeric Microtubule Disruptors**

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**N.B.** All experiments with live animals were performed in compliance with the relevant laws and institutional guidelines, and were approved by the Imperial College Ethics Committee.

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#### General.

Reactions were carried out at room temperature (rt) unless otherwise stated. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded with either a JMN-GX270 at 270 and 67.5MHz respectively or a Varian Mercury VX400 spectrometer at 400 and 100.6 MHz. Chemical shifts are reported in parts per million (ppm,  $\delta$ ) relative to tetramethylsilane (TMS) as an internal standard and coupling constants in hertz (*J*). Mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, UK. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a SRS Optimelt MPA100 automated melting point system.

#### Materials

All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK), Fluka (Gillingham, UK) or Lancaster Synthesis (Morecambe, UK). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger and was stored in the refrigerator under positive pressure of  $N_2$  as a solution in toluene as described by Woo et al. [1] An appropriate volume of this solution was freshly concentrated *in vacuo* immediately before use. Column chromatography was conducted using an ISCO CombiFlash Rf automated system.

#### 6-(Benzyloxy)-7-methoxy-1,2,3,4-tetrahydroisoquinoline (4)

A mixture of 4-benzyloxy-3-methoxybenzaldehyde **3** (25 g, 103 mmol) and aminoacetaldehyde diethyl acetal (11.7 mL, 108 mmol) was stirred at 60°C for 8 hours then cooled to rt. The mixture was poured into ethanol (150 mL) and the resulting solution was treated with NaBH<sub>4</sub> (4.2 g, 110 mmol) in a portion wise manner. The mixture was then refluxed for 4 hours, cooled to rt and poured in ice/water. After 30 minutes stirring the organics were extracted with ethyl acetate and the organic layer was separated, washed with water, brine, dried (MgSO<sub>4</sub>), filtered and concentrated to give a light yellow oil (35.9 g, 97%) which was stirred in dioxane (40 mL) and 6M HCl (500 mL) at 45°C for 90 minutes. After cooling to 0°C the white solid was filtered, washed successively with cold water and diethyl ether then poured into water (200 mL). The suspension was stirred at 0°C and made alkaline with NaOH. After an additional 15 minutes stirring, the white solid was filtered off and washed with water then diethyl ether, dried under vacuum to give a white powder (23 g, 81%, 1 step). This white powder (23 g, 80.7 mmol) in DCM (100 mL) and TFA (50 mL) was stirred at 0°C and NaBH<sub>4</sub> (6.1 g, 161 mmol) was added in

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a portion wise manner. The mixture was stirred at rt for an o/n. After addition of water (200 mL) and DCM (200 mL), Na<sub>2</sub>CO<sub>3</sub> was added until alkaline pH. The organic layer was washed with water, brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure, yielding a cream colour powder which was stirred in diethyl ether, filtered, washed with diethyl ether and dried under vacuum to give an off-white powder (18.4 g, 66%, over 3 steps). mp 74-75 °C; <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.76 (1H, br s), 2.63 (2H, t, *J* 5.7 Hz), 3.07 (2H, t, *J* 5.7 Hz), 3.81 (3H, s), 3.92 (2H, s), 5.10 (2H, s), 6.52 (1H, s), 6.60 (1H, s), 7.27-7.44 (5H, m); <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>)  $\delta$  28.3, 43.6, 47.6, 55.5, 70.8, 109.3, 114.6, 126.2, 126.3, 126.9, 127.4, 128.3, 137.0, 146.2 and 147.7. HRMS [ESI] calcd. for C<sub>17</sub>H<sub>20</sub>NO<sub>2</sub> [M+H]<sup>+</sup>, 270.1489 found 270.1477.

#### General method for the synthesis of phenols (5a-c)

#### a) N-Benzylation of 6-(benzyloxy)-7-substituted-1,2,3,4-tetrahydroisoquinoline

A solution of 6-(benzyloxy)-7-methoxy-1,2,3,4-tetrahydroisoquinoline or 6-(benzyloxy)-7-ethyl-1,2,3,4-tetrahydroisoquinoline (1.5 mmol) and the appropriate benzyl chloride (390 mg, 1.8 mmol) in TEA (0.5 mL, 3.6 mmol) and ethanol (2.5 mL) was heated at 130°C for 60 minutes under microwave energy. After cooling to rt, the mixture was poured in water (20 mL) and ethyl acetate (80 mL), the organic layer was separated and washed with water, brine, dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The resulting solid was purified by flash chromatography (hexane/ethyl acetate or DCM/ethyl acetate) to give the desired compound.

#### 6-(Benzyloxy)-7-methoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline

white powder (520 mg, 77%). mp 144-145 °C. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.65-2.78 (4H, m), 3.55 (2H, s), 3.58 (2H, s), 3.82 (3H, s), 3.84 (3H, s), 3.85 (6H, s), 5.10 (2H, s), 6.53 (1H, s), 6.61 (2H, s), 6.63 (1H, s), 7.27-7.44 (5H, m), <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>)  $\delta$  28.8, 50.7, 56.0, 56.2, 61.0, 63.1, 71.2, 105.7, 110.2, 114.3, 126.3, 127.3, 127.5, 127.8, 128.6, 134.5, 136.9, 137.4, 146.8, 148.0 and 153.2. HRMS (ESI) calcd. for C<sub>27</sub>H<sub>32</sub>NO<sub>5</sub> [M+H]<sup>+</sup>, 450.2275 found 450.2270. Microanalysis: C: 71.8 (expected 72.14); H: 6.96 (expected 6.95); N: 3.06 (expected 3.12).

# b) Selective *O*-debenzylation of the 6-(benzyloxy)-7-substituted-2-trimethoxybenzyl-1,2,3,4-tetrahydroisoquinoline

A solution of 6-(benzyloxy)-7-substituted-2-trimethoxybenzyl-1,2,3,4-tetrahydroisoquinoline (1 mmol) in THF (20 mL) and methanol (20 mL) was treated with 10% Pd/C (40 mg) and stirred under an atmosphere of hydrogen. The reaction was monitored by TLC. Upon completion, the resultant suspension was filtered through celite, washed with ethyl acetate and then evaporated

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under reduced pressure. The crude mixture was purified by flash chromatography with (hexane/ethyl acetate or DCM/ethyl acetate) to yield the desired compound.

#### 6-Hydroxy-7-methoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline 5b

yellow powder (215 mg, 60%). mp 170-171 °C. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.66-2.81 (4H, m), 3.53 (2H, s), 3.59 (2H, s), 3.81 (3H, s), 3.84 (3H, s), 3.85 (6H, s), 5.49 (1H, br s), 6.48 (1H, s), 6.62 (2H, s), 6.66 (1H, s); <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>)  $\delta$  28.6, 50.8, 56.0, 56.1, 56.2, 61.0, 63.0, 105.7, 108.9, 114.3, 126.1, 127.1, 127.8, 134.4, 136.9, 144.1, 145.0 and 153.2. HRMS (ESI) calcd. for C<sub>20</sub>H<sub>26</sub>NO<sub>5</sub> [M+H]<sup>+</sup>, 360.1805 found 360.1814. Microanalysis: C: 66.7 (expected 66.83); H: 7.09 (expected 7.01); N: 3.66 (expected 3.90).

#### General method for the synthesis of sulfamates 6a-c

A solution of 6-hydroxy-7-substituted-2-trimethoxybenzyl-1,2,3,4-tetrahydroisoquinoline (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<sub>3</sub> (150 mg, 1.5 mmol) the reaction mixture was extracted into ethyl acetate (2 x 50 mL), the organic layers washed with water and brine, then dried (MgSO<sub>4</sub>) and evaporated. The crude product was purified by flash chromatography (hexane/ethyl acetate or DMC/ethyl acetate) and precipitated from diethyl ether if required.

#### 7-Methoxy-2-(3,4,5-trimethoxybenzyl)-6-O-sulfamoyl-1,2,3,4-tetrahydroisoquinoline 6b

yellow powder (125 mg, 57%), mp 143-144 °C. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  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 (3H, s), 7.07 (1H, s), <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>)  $\delta$  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<sub>20</sub>H<sub>27</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup>, 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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Plasmid pACA was transformed into *Escherichia coli* BL21(DE3) and cultured overnight in Luria–Bertani medium, supplemented with Kao and Michylek vitamin supplement, *E. coli* mineral supplement (5 g of EDTA, 0.5 g of FeCl<sub>3</sub>, 0.05 g of ZnCl<sub>2</sub>, 0.01 g of CuCl<sub>2</sub>, 0.01 g of CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.01 g of H<sub>3</sub>BO<sub>3</sub> and 1.6 g of MnCl<sub>2</sub>·6H<sub>2</sub>O in 800 ml of water, adjusted to pH 7.0 and sterile-filtered), 0.4% (w/v) glucose and 50 µg/ml ampicillin at 37 °C and 170 rev./min. Starter culture (14 ml) was used to inoculate 4×700 ml of the same medium and was grown until  $D_{600}$ ~0.8. The temperature was decreased to 30 °C and enzyme expression was induced with 0.25 mM isopropyl β-D-thiogalactoside and 1 mM ZnSO<sub>4</sub>. Cells were harvested after 5 h by centrifugation at 11300 g for 30 min at 4 °C.

Cells were resuspended in 20 mM Mes/NaOH, pH 6.2 (50 ml), and lysed using the 'one-shot' cell disruptor (Constant Systems, Low March, Northants, U.K.). After centrifugation at 9820 g, 4 °C, for 45 min, the extract was treated with 0.1% (v/v) polyethyleneimine, stirred for 15 min and centrifuged at 9820 g for 60 min at 4 °C. The crude cell extract was purified by using an S-Sepharose XL column with a 0–500 mM NaCl gradient in 100 ml of buffer. Fractions were analysed by SDS/PAGE, and the required fractions were concentrated to approx. 10 mg/ml using a Hi-Trap S-Sepharose column, eluting with 500 mM NaCl in 10 mM Mes/NaOH (pH 6.2).

#### Crystallization, data collection and structural determination

The hanging drop vapour diffusion method was used for the crystallization. Protein (2.5  $\mu$ l, ~10 mg/ml, ~0.3 mM) containing 0.5 mM Inhibitor **9** and 30 mM 2-mercaptoethanol was mixed with well buffer (2.5  $\mu$ l; 0.1 M Tris/HCl, pH 8.0, 1 mM ZnSO<sub>4</sub> and 2.49 M ammonium sulphate), with crystals appearing after 3–4 weeks at 4 °C.

X-ray diffraction data were collected at station I03 at the Diamond Light Source (U.K.) under cryogenic conditions with a 1 s exposure and a crystal to detector distance of 237.25 mm, with 200 frames of 1° oscillation collected. Before data collection, the crystals were flash-cooled at 100K in a cryoprotectant containing the reservoir solution and 30% of 7.0 M Sodium formate. Data were indexed and reduced with DENZO and SCALEPACK modules of the HKL suite[2] in the orthorhombic  $P2_12_12_1$  space group. The structure was determined by the molecular replacement method using CA II crystallized in the  $P2_12_12_1$  space group without the active-site zinc atom and any water molecules (1TTM.pdb[3]) as a search model with the program Phaser[4] from the CCP4i program suite[5]. Refinement was performed using Refmac5[6] from the program package CCP4i in addition to manual model building using the program COOT[7]. Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2010

Clear density for the active site Zinc, second Zinc and the inhibitor **9** were observed after the first round of refinement. The zinc atoms and inhibitor were introduced into the corresponding areas of electron density; with the topology files for inhibitor **9** generated using the Monomer Library Sketcher program from CCP4i. After alternating cycles of refinement and manual rebuilding the final model had a  $R_{\text{free}}$ =0.23 and  $R_{\text{cryst}}$ =0.21 (Table 1).

	Complex with inhibitor <b>9</b>	
Data collection		
Resolution range (Å)	50.00 - 1.49	
Space group	$P_{2_1}^{2_1} 2_1^{2_1}$	
Unit cell dimensions (Å)		
Highest-resolution shell (Å)	1.54 - 1.49	
Total no. of reflections	507016	
No. of unique reflections	37376	
Completeness (%)	93.3 (62.8)	
$R_{\rm sym}$ (%)	0.111(0.324)	
$I/\sigma(I)$	11.8 (2.1)	
Refinement		
No. of refined protein residues	258	
No. of refined water molecules	245	
No. of refined zinc atoms	2	
No. of refined inhibitor molecules	1	
Resolution range in refinement (Å)	25.93 - 1.49	
R <sub>free</sub>	0.23	
Test set size (%)	5.0	
R <sub>cryst</sub>	0.21	
Rms deviations		
Bond lengths (Å)	0.007	
Bond angles (deg)	1.142	
Average B value ( $Å^2$ )	20.53	
Primary $Zn^{2+}$ <i>B</i> -factor (Å <sup>2</sup> )	12.19	
Secondary $Zn^{2+}$ <i>B</i> -factor (Å <sup>2</sup> )	14.48	
Ligand <i>B</i> -factor range ( $Å^2$ )	14.68 - 34.50	
Water <i>B</i> -factor Average ( $Å^2$ )	29.2	
Ramachandran plot		
Most favoured (%)	87.2	
Additionally allowed (%)	12.3	
Generously allowed (%)	0.5	
Disallowed (%)	0.0	

Table 1 Crystallographic data for hCAII complex with Inhibitor 9



	First residue and atom	Second residue and atom	Distance in hCAII-9
			complex
А	His-94 NE2	Zn	2.03
В	His-96 NE2	Zn	2.03
С	His-119 ND1	Zn	2.08
D	Inhibitor SO <sub>3</sub> NH	Zn	1.98
Е	Inhibitor SO3NH O2	Zn	3.10
F	Inhibitor SO3NH O2	His-94 NE2	3.42
G	Inhibitor SO <sub>3</sub> NH	Thr-199, side chain OH	2.69
Н	Inhibitor SO3NH O1	Thr-199, main chain <u>N</u> H	3.06
Ι	Glu-106 OE1	Thr-199, side chain OH	2.59

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**Figure 1.** Binding of the inhibitors **9** in human CA II. Inhibitor and residues are shown as sticks and bound Zn(II) ions are shown as brown sphere. (**A**)  $2|F_o| - |F_c|$  simulated annealing omit maps (blue) contoured at 1.0. (**B**)  $|F_o| - |F_c|$  simulated annealing omit maps (green) contoured at 3.0 $\sigma$ . (**C**)  $2|F_o| - |F_c|$  maps (blue) contoured at 1.0 $\sigma$ . This diagram was prepared using PyMOL (http://www.delanoscientific.com).

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