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Discovery of CYT997: a structurally novel orally active microtubule targeting agent

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ABSTRACT

CYT997 was discovered as a potent tubulin polymerization inhibitor possessing potent cytotoxic activity against a range of cancer cells. Details of SAR studies, pharmacokinetic investigations and synthesis of compounds leading to the discovery of CYT997 are reported.

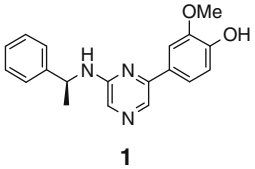
Compounds that bind to tubulin and effect normal microtubule function and dynamics have proven to be some of the most successful cytotoxic anti-cancer drugs discovered. Thus, for example, the structurally diverse natural products paclitaxel, vincristine, vinblastine, and the epothilones,[1] all exert their cytotoxic effects predominantly through disruption of microtubule dynamics. In recent years there has been significant activity in the discovery and development of novel small molecule tubulin binding drugs devoid of some of the drawbacks associated with conventional agents.[2] Thus, a number of compounds that potently bind tubulin and that also possess improved pharmaceutical properties (including solubility and metabolic stability), have reduced affinity for drug efflux pumps such as Pgp and are orally bioavailable have been identified. Furthermore, several small molecule tubulin binding compounds have recently been shown to possess profound anti-vascular effects on the immature vasculature of tumours.[3] These compounds are now known as vascular disrupting agents and clinical trials with a number of these agents are currently underway.[4]

As part of a program focused on the discovery of novel small molecule cytotoxic agents, we conducted a cell based anti-proliferative screen of Cytopia's small molecule library using two prostate cancer cell lines, DU145 and PC3. From this screen we identified the *S* α-methylbenzylamino pyrazine derivative **1** (Fig. 1) as a low micromolar inhibitor of cell proliferation (Table 1).

We conducted a focused analogue program on this compound and found that increasing the chain length of the alkyl substituent on the benzylic carbon led to a significant increase in cellular activity (Table 1).[5] Extending the benzylic methyl substituent on **1** by one carbon to an ethyl chain (**2**) reduced activity in the DU145 cellular assay by more than 3 fold. However, lengthening the ethyl substituent by a further carbon to a propyl group **3**, increased the inhibitory potency of the molecule by 15-fold on DU145 cells and by more than 50 fold on PC3 cells compared to the methyl analogue **1**.

To simplify the synthesis and maximize efficiency, we investigated the SAR around the left and right-hand aromatic rings of the lead compound **3** using racemic material. We followed up compounds of interest in the *S* enantiomeric series.

Substitution at the 4 position of the benzylic aryl ring with an electron donating substituent (Table 2, **5**) or an electron withdrawing substituent (**6** and **7**) reduced potency compared to the unsubstituted phenyl compound **4**. However, although an electron



1

Figure 1. Initial screening hit **1**.

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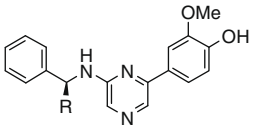
Table 1
Chain elongation of the benzylic carbon alkyl substituent

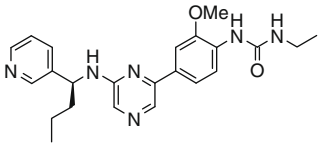


Compound No.	R	DU145 IC_{50} (μM)	PC3IC_{50} (μM)
1	CH_3	3	10
2	CH_2CH_3	10	10
3	$CH_2CH_2CH_3$	0.2	0.16

donating substituent at position 3 of the benzylic aryl ring (**8**) was not favourable, electron withdrawing substituents at this position (**9** and **10**) were tolerated. Replacement of the phenyl ring in **4** with a 3-pyridyl ring (**11**) not only gave comparable potency but also lowered the lipophilicity of the series.

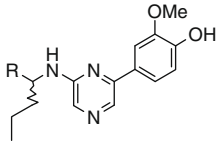
With the benzylic aromatic ring fixed as a 3-pyridyl moiety, investigations into the aromatic ring directly attached to the pyrazine core indicated the requirement of a 3,4-disubstitution pattern. Although we had shown the hydrogen bond donor/acceptor characteristic of the 4-hydroxy substituent to be essential for retention of potency in this series, we found the 4-hydroxyl group to be a metabolic liability. In vivo glucuronidation of this group followed by rapid excretion limited oral bioavailability in this context. Attempts to reduce glucuronidation by increasing the size of the flanking substituent from the 3-methoxy group of **11** with 3-ethoxy (Table 3, **12**) or 3-isopropoxy (**13**) did not lead to greater metabolic stability. We next turned our attention to isosteres of the phenolic hydroxyl and found that replacement of the 4-hydroxyl group with an amide (**14** and **15**) led to a loss in potency and also introduced another potential metabolic liability in a hydrolysable amide bond. Thus, replacement of the 4-hydroxyl with an ethyl urea moiety (**16** and **17**) retained the crucial hydrogen bond donor/acceptor characteristics required at this position, furthermore delivering a metabolically robust functional group. While the ethyl urea led to an increase in potency within this series, placing a hydrophilic solubilizing group on the terminal end of the ethyl urea **18** led to significant loss of potency.

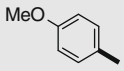
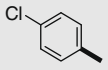
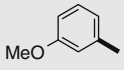
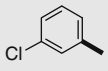
The *S* enantiomer (**19**) of compound **17** was highly active in both DU145 and PC3 cellular assays with IC_{50} values of 4 and 3 nM, respectively.



19

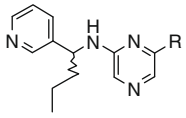
Table 2
Alterations to the benzylic aromatic ring

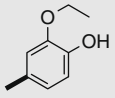
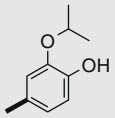
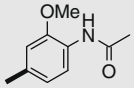
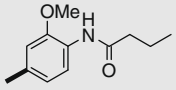
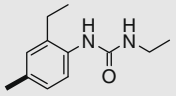
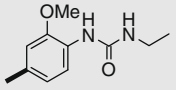
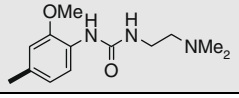


Compound no.	R	DU145 IC_{50} (μM)	PC3 IC_{50} (μM)
4		0.5	0.16
5		3	3
6		10	20
7		4	ND
8		1	10
9		0.6	1
10		0.3	0.6
11		0.6	0.3

ND = Not determined.

Table 3
Alterations to the RHS aromatic ring



Compound no.	R	DU145 IC_{50} (μM)	PC3 IC_{50} (μM)
12		ND	1
13		10	10
14		ND	5
15		ND	0.6
16		0.1	0.16
17		0.06	0.04
18		1	1

ND = Not determined.

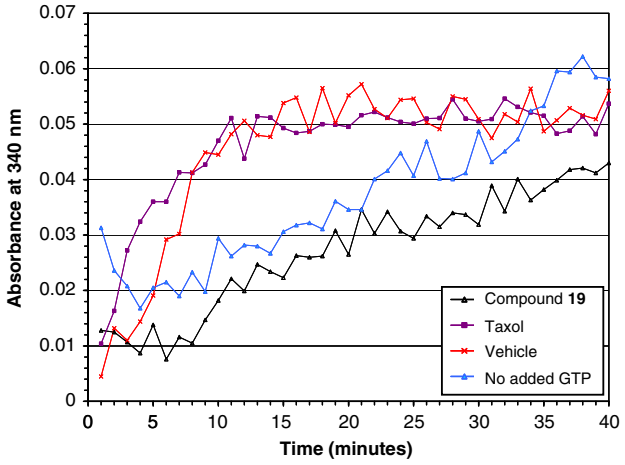


Figure 2. Compound **19** exhibits inhibition of tubulin polymerization at 3 μM. Tubulin polymerization was determined by absorption of light at 340 nm by the reaction mixture due to microtubule formation.

Table 4
Pharmacokinetic data of **19** following iv (5 mg/kg) co-administration in rats with solubilising agent Captisol™

Compound	$t_{1/2}$ (iv) (h)	V_z (mL/kg)	Cl (mL/min/kg)
19	0.6	7100	127.1

Investigations utilizing confocal microscopy showed that in PC3 cells, compound **19** caused profound disruption of the cellular microtubule network within 1 h of exposure and led to cell cycle arrest at the G_2/M phase. These activities are consistent with the compound acting as a microtubule disruptor.[2] In vitro studies confirmed that compound **19** inhibited polymerization of purified bovine tubulin at 3 μM as compared to taxol-induced or GTP-induced polymerization (Fig. 2).[6] This level of inhibition is similar to that reported for the known microtubule polymerization inhibitor combretastatin A4.[7]

Table 5
Alterations to the 2,6-disubstituted pyrazine core.

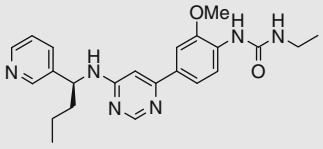
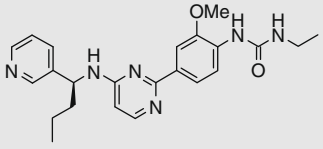
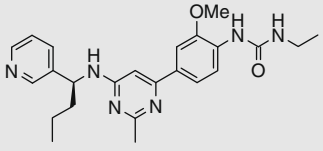
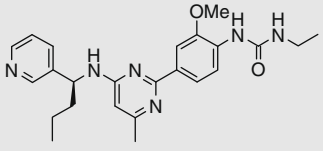
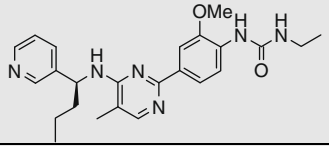
Compound no.	Structure	DU145 IC_{50} (μM)	PC3 IC_{50} (μM)
20		0.1	0.1
21		0.2	0.15
22		0.1	0.08
23		0.2	0.2
24		0.06	0.06

Table 6
Pharmacokinetic data of the mesylate salt of **24** upon iv (5 mg/kg) and po (25 mg/kg) administration in rats

Compound	$t_{1/2}$ (iv) (h)	V_z (mL/kg)	Cl (mL/min/kg)	C_{max} (po) (μM)	F (%)
24	1.6	5200	36.8	9.4	78

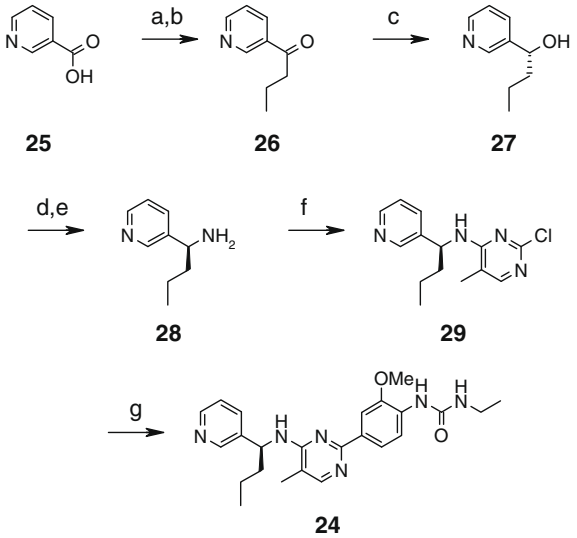
Despite **19** displaying profound cytotoxicity across a panel of diverse cell lines, the compound exhibited poor pharmacokinetic properties (Table 4) and limited solubility in aqueous systems.

To address the poor pharmacokinetics and to increase the aqueous solubility of **19**, we investigated the core heteroaromatic ring of this series (Table 5). Replacement of the 2,6-disubstituted pyrazine of **19**, with a heterocycle more susceptible to ionization at physiological pH such as 4,6-disubstituted pyrimidine (**20**) or 2,4-disubstituted pyrimidine (**21**) gave compounds with improved aqueous solubility and acceptable potency in cell based anti-proliferative assays. Introduction of a methyl substituent onto the central heteroaromatic ring led to compounds **22**, **23** and **24**, all of which retained potency across a panel of cancer cell lines.

In conjunction with being one of the most potent compounds in cellular assays, **24** also displayed an excellent pharmacokinetic profile as shown in Table 6.

Compound **24** was active when dosed orally in a number of mouse xenograft studies[8] and importantly demonstrated vascular disrupting properties in vitro and in vivo.[9] On the basis of these results and other positive preclinical studies, we selected **24** as a clinical candidate (CYT997) and are currently conducting Phase II clinical trials for the treatment of selected cancers.[10,11]

We synthesized CYT997 (**24**) in multi-gram quantities as shown in Scheme 1. Nicotinic acid **25** was converted to butanone **26** via Grignard addition to the Weinreb amide. Asymmetric reduction



Scheme 1. Reagents and conditions: (a) *N*,*O*-Dimethylhydroxylamine hydrochloride, EDAC, 4-pyrrolidinopyridine, NEt_3, CH_2Cl_2, rt, 75 h, 85%; (b) PrMgCl, THF, 0 °C to rt, 2 h, 83%; (c) $BH_3.SMe_2$, (*S*)-(−)-α,α-diphenyl-2-pyrrolidinemethanol, $B(OMe)_3$, THF, 0° to rt, 20 h; (d) diphenylphosphoryl azide, DBU, THF, 0° to rt, 68 h; (e) PPh_3, toluene, H_2O, 80°, 2 h, 92% over 3 steps[16,17]; (f) 2,4-dichloro-5-methylpyrimidine, DIPEA, EtOH, 70°, 16 h, 54%; (g) 1-ethyl-3-[2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)phenyl]-urea, $Pd(PPh_3)_4$, Na_2CO_3 (aq), *n*-PrOH, toluene, 100 °C, 44 h, 61%.

of **26** with borane dimethyl sulfide complex utilizing a chiral pyrrolidine catalyst[12] yielded the *R* alcohol **27**. Conversion of the *R* alcohol to the *S* amine **28** proceeded via azide formation and Staudinger reduction,[13] with concomitant stereoinversion. Base catalysed addition of the weakly nucleophilic amine **28** to 2,4-dichloro-5-methylpyrimidine gave **29**. Subsequent palladium catalysed Suzuki–Miyaura reaction[14] with the appropriate boronate yielded compound **24** as a pale beige solid.[15]

In summary, we have discovered CYT997 (**24**), a potent small molecule tubulin polymerization inhibitor and vascular disrupting agent that has completed Phase I trials by both intravenous[18] and oral[19] routes and is now in Phase II clinical trials for the treatment of selected cancers.[10,11]

References and notes

1. Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259–296.
2. Carlson, R. O. *Expert Opin. Investig. Drugs* **2008**, *17*, 707–722.
3. Tozer, G. M.; Kanthou, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423–435.
4. Kanthou, C.; Tozer, G. M. *Expert Opin. Ther. Targets* **2007**, *11*, 1443–1457.
5. DU145 cells [ATCC HTB-81, human prostate carcinoma] (2×10^4 cells/mL) and PC-3 cells [ATCC CRL-1435 human prostate adenocarcinoma] (2×10^5 cells/mL) were cultured in microtest plates in DMEM and Ham's F12 K medium, respectively, supplemented with 10% FBS and the test compound for 72 h at 37 °C in 5% CO_2. Cell proliferation was assessed using Alamar Blue™ (Serotec).
6. Tubulin assembly was conducted in MES buffer (100 mM MES, pH 6.4, 1 mM EGTA, 0.5 mM $MgCl_2$) using microtubules purified from bovine brain. Glycerol (5%) and GTP (1 mM) were added to the tubulin mixture before the addition of **19** in order to promote polymerization. In the case of the negative control no GTP was added. The assay was commenced by addition of 10 μL of **19** (3 μM in 1% DMSO) to 100 μL of the tubulin reaction mixture (~72 μg tubulin/reaction) in a pre-warmed 96-well plate. Absorbance was read every 60 s at a wavelength of 340 nm for a period of 40 min using a BMG Polarstar plate reader at 37 °C.
7. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209–211.
8. Burns, C. J.; Fantino, E.; Phillips, I. D.; Su, S.; Harte, M. F.; Bukczynska, P. E.; Dublevic, V.; Frazzetto, M.; Joffe, M.; Kruszelnicki, I.; Wang, B.; Wang, Y.; Wilson, N.; Dilley, R. J.; Wan, S.; Charman, S. A.; Shackleford, D. M.; Fida, R.; Malcontenti-Wilson, C.; Christophi, C.; Wilks, A. F., submitted for publication.
9. Burns, C. J.; Fantino, E.; Phillips, I. D.; Segal, D.; Su, S.; Harte, M. F.; Dublevic, V.; Joffe, M.; Malcontenti-Wilson, C.; Christophi, C.; Shnyder, S. D.; Wilks, A. F., in preparation.
10. Efficacy study of CYT997 in combination with carboplatin and etoposide in glioblastoma. ClinicalTrials.gov Identifier NCT00650949.
11. Efficacy study of CYT997 in multiple myeloma. ClinicalTrials.gov Identifier NCT00664378.
12. Moriyasu, M.; Shioiri, T. *Synlett* **1997**, 273–274.
13. Gololobov, Y. G.; Zhmurova, I. N.; Kasukhin, L. F. *Tetrahedron* **1981**, *37*, 437–472.
14. Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, 9633–9695.
15. All compounds were characterized by ^{1}H NMR and/or reverse phase LC–MS and MS. *Compound* ***24***: A mixture of 6-chloro-*N*-[(1*S*)-1-pyridin-3-ylbutyl]pyrazin-2-amine **29** (5.0 g, 18.1 mmol), 1-ethyl-3-[2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)phenyl]-urea (6.94 g, 21.7 mmol) and aqueous sodium carbonate (13.6 mL, 2 M, 27.1 mmol) in toluene (120 mL) and *n*-propanol (40 mL) was degassed with nitrogen for 15 min. After this time tetrakis(triphenylphosphine)palladium(0) (2.09 g, 1.81 mmol) was added and the mixture was heated at reflux for 44 h. The mixture was allowed to cool to room temperature and then diluted with ethyl acetate (150 mL). The organic solution was washed with water (3 × 100 mL), brine (100 mL), dried (Na_2SO_4) and filtered through Celite to give a dark brown foam which was purified by flash chromatography (silica, ethyl acetate/methanol), followed by hot methanol charcoal filtration and trituration with diethyl ether to give *N*-[2-methoxy-4-(6-{[(1*S*)-1-pyridin-3-ylbutyl]amino}pyrazin-2-yl)phenyl]-*N*′-ethylurea **24** (4.7 g, 60%) as a pale beige solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (t, *J* 7.2 Hz, 3H), 1.19 (t, *J* 7.2 Hz, 3H), 1.36–1.53 (m, 2H), 1.83–2.05 (m, 2H), 2.11 (d, *J* 0.9 Hz, 3H), 3.28–3.37 (m, 2H), 3.92 (s, 3H), 4.63 (t, *J* 4.8 Hz, 1H), 4.77 (d, *J* 6.6 Hz, 1H), 5.29–5.36 (m, 1H), 6.83 (br s, 1H), 7.22–7.26 (m, 1H), 7.68 (dt, *J* 7.8, 1.8 Hz, 1H), 7.72 (d, *J* 1.8 Hz, 1H), 7.86 (dd, *J* 8.7, 1.8 Hz, 1H), 8.06 (s, 1H), 8.07 (d, *J* 8.7 Hz, 1H), 8.47 (dd, *J* 5.5, 1.8 Hz, 1H), 8.69 (d, *J* 2.1 Hz, 1H).
16. The *S* amine **28** was obtained with an ee of 80%.
17. We required multi-kilogram amounts of **24** to support clinical trials. However, the asymmetric reduction of **26** and conversion to amine **28** via the azide was not considered viable on large scale. Therefore, we obtained the *S* amine **28** in bulk quantities as its racemate and resolved it into its pure enantiomer through salt formation with mandelic acid.
18. Phase I dose-escalation study for CYT997. ACTR number: ACTRN12605000793617. www.anzctr.com.au
19. Phase I accelerated dose-escalation study of CYT997 given as an oral capsule every two weeks in patients with advanced solid tumours. ACTR number: ACTRN12606000520538. www.anzctr.com.au