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SYNTHESIS AND BIOLOGICAL PROPERTIES OF 1069C: A NEW SYNTHETIC ANTITUMOUR AGENT WITH VERY LOW CROSS-RESISTANCE POTENTIAL

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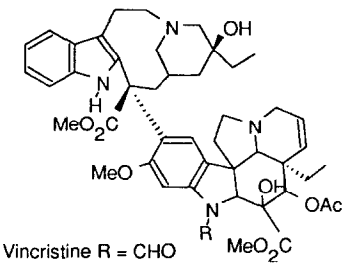
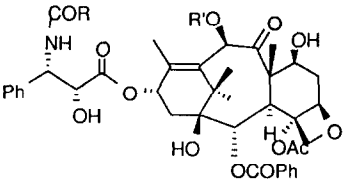
Abstract: *A novel imidazopyridazine carbamate, 1069C, is a potent microtubule inhibitor which binds at the Colchicine site on tubulin and is effective in vivo against murine tumours made resistant to clinically used antitumour drugs.*

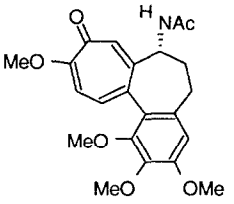
Advances over existing cancer treatments have often been made with compounds which work by new mechanisms. The clinically important *vinca* alkaloids, Vincristine and Vinblastine,[1] disrupt microtubule (MT) assembly, important for cell division, by interacting with the α- and β-tubulin dimers. Although the precise mechanism of how microtubule assembly is blocked is not clear it is apparent that specific high and low affinity tubulin binding sites[2,3,4] exist for the *vincas*. Further development of *vincas* has led to the semi-synthetic analogue, Navelbine, which has shown promising clinical activity.[5] Currently there is great interest in another series of naturally derived MT inhibitors, Taxol[6] and a semi-synthetic analogue, Taxotere.[7] In contrast to the *vinca* alkaloids, Taxol[8,9] disrupts MT function through the promotion of tubulin polymerisation and stabilisation of MTs. Thus far, Taxol in Phase 2 studies has shown activity in acute myelogenous leukaemia,[10] ovarian cancer,[11] malignant melanoma,[12] and breast cancer.[13] The drawback, however, to Taxol's use has been the limited supply available from the bark of the Pacific yew tree. Extensive resources are being channelled into alternative methods for production of Taxol and Taxotere which may involve semi-synthetic chemistry.[14] Considering the current interest in novel MT inhibitors, it is timely to report our work on the discovery of a synthetic MT inhibitor, 1069C, which binds to the Colchicine site on mammalian tubulin, is a potent anti-proliferative agent *in vitro*, and appears to have a low potential for cross-resistance to other antitumour agents, *in vivo*



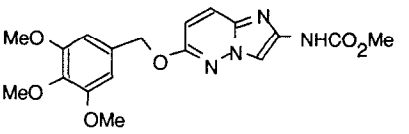
Vincristine R = CHO
Vinblastine R = Me



Taxol R = Ph , R' = Ac
Taxotere R = t-BuCO , R' = H

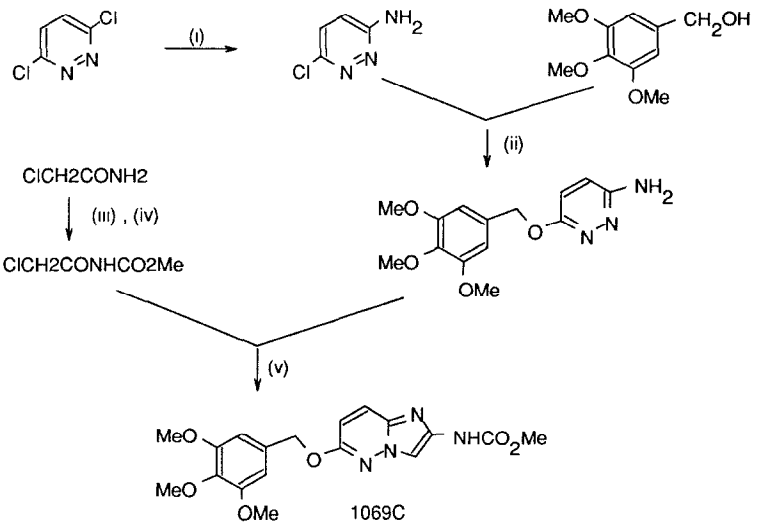


Colchicine



1069C

1069C was synthesised according to the scheme below,[15] from cheap, readily available, starting materials



Reagents. (ii) NH_3, H_2O, (ii) $KOBu^t$, DME; (iii) $(COCl)_2$; (iv) MeOH; (v) DMF.

When compared against other MT inhibitors (Table 1) 1069C is a more potent inhibitor in a standard assay[16] of mammalian brain tubulin polymerisation *in vitro* than Colchicine, and is similar to Vincristine. When $P388D_1$ leukaemia cells were incubated for 1hr with various MT inhibitors (Table 1) and the MT's visualised by immunofluorescence techniques[17] it was shown that 1069C disrupted the mitotic spindles at very low concentrations, and 1069C appeared to be more potent than Vincristine. Furthermore, the concentrations required by 1069C to disrupt MT's in intact P388 cells were similar to those which inhibit proliferation (*vide infra*) suggesting that the mode of action for the antitumour activity of 1069C is *via* the disruption of the MT's.

TABLE 1 MICROTUBULE INHIBITORY EFFECTS *IN VITRO*

Compound	Tubulin Polymerisation IC_{50} ($X10^{-6}M$)	P388 Cell Spindle Disruption EC_{50} ($X10^{-6}M$)
1069C	0.31	0.006 - 0.009
Vincristine	0.22	0.02 - 0.04
Colchicine	1.5	-

TABLE 2 *in vitro* EFFECTS AGAINST $P388D_1$ CELLS

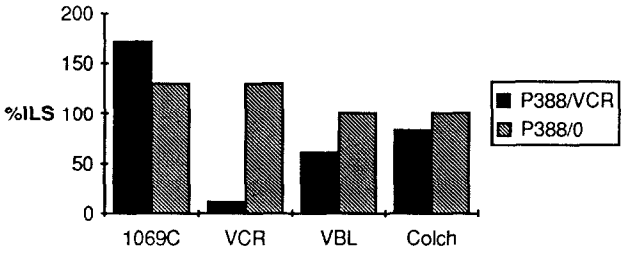
Compound	Proliferation IC_{50} ($X10^{-6}M$)	Colony Formation IC_{50} ($X10^{-6}M$)
1069C	0.0089	0.013
Vincristine	0 0047	0.19
Vinblastine	0.0017	0.0082
Colchicine	0.00062	0.071

Competition experiments with mammalian (equine) brain tubulin between 1069C and ^{3}H-Colchicine or ^{3}H-Vinblastine showed that 1069C is a competitive inhibitor*, at the Colchicine site, with K_i = 0.75 μM,[18] and had no affect at the *vinca* alkaloid site.

1069C is a potent anti-proliferative agent against growing murine leukaemia P388 cells *in vitro*[19] (Table 2) with a potency in a similar range to other MT inhibitors. Colchicine appears to be about an order of magnitude more active than 1069C. However, when evaluated in a colony-forming assay[20] (where the P388 cells are allowed to grow into colonies over 14 days in the absence of compound) 1069C retains high potency, whilst Colchicine, Vincristine and Vinblastine lose activities of some 100, 40 and 5-fold, respectively. Effectiveness of a compound in a colony-forming assay may be particularly relevant when considering the clinical situation where the drug has access to the tumour mass for a limited time.

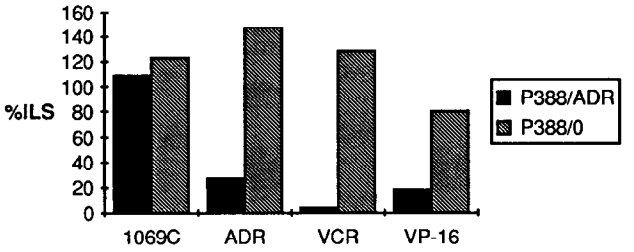
Using the established, NCI-type, P388 *in vivo* model,[21] 1069C at 10mg/kg, produced a 147 ± 13% mean increase in life span (ILS) compared to the controls.[22] When evaluated against P388 tumours, which are resistant to clinically used antitumour drugs,[23] 1069C showed a remarkable lack of cross-resistance . in Figure 1, 1069C is as effective against the P388/Vincristine resistant tumour *in vivo* as the parent P388/0 whereas Vincristine and Vinblastine clearly are not so effective against P388/VCR. In Figure 2, 1069C is fully effective against the P388/Adriamycin resistant tumour whereas Adriamycin, Vincristine and VP-16 are only weakly active Given that the P388/ADR tumour is insensitive to a range of clinically-used antitumour drugs[24] with different structures and mechanisms of action it would appear that 1069C has the potential to be effective against multi-drug-resistant (MDR) tumours.

Figure 1 - P388/VCR Resistant Tumour Studies



* *1069C has some structural and biological similarities to the benzimidazole antimitotics [See E Hamel in "Microtubule Proteins," J Avila, Ed. CRC Press, Boca Raton, FL, pp 89-192]*

Figure 2 - P388/ADR Resistant Tumour Studies



In summary, 1069C has a very interesting profile · it is a potent MT inhibitor at the Colchicine site, a very active antiproliferative agent and is fully effective against murine P388 tumours *in vivo* which are insensitive to a range of different clinically used antitumour drugs. Furthermore, 1069C may be synthesised in a few stages from readily available materials.

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15. All compounds were characterised by the usual analytical and spectroscopic methods. 1069C : mp 217-220°C, δ_H (d_6 DMSO) 10.36 (1H, br, s, NH), 7.87 (1H, J_{AB} 8.8Hz), 7.85 (1H, s, 3-H), 6.87 (1H, J_{AB} 8.8Hz, 7-H), 6.85 (2H, s, PhH), 5.25 (2H, s, CH_2), 3.79 (3H, s, COOMe), 3.79, 3.70 and 3.68 (9H, s, $(OMe)_3$)
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22. 10^6 P388 cells injected IP on day O Compound dosed IP on day 1, 5 and 9. At a dose of 10mg/kg IP on day 1,5 and 9 there were 8/126 60 day survivors produced. These survivors were not used in the mean % ILS value.
23 10^6 P388/resistant tumour cells injected IP on day 0 Each compound dosed at its optimum level IP on days 1,5 and 9 and results expressed as a % increase in life span of the tumour bearing mice versus the compound untreated controls
Figures 1 and 2 compare results of the efficacies of compounds (at their optimum doses) against the P388/resistant tumour and the normal P388/0 tumour.
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