Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1686–1691

# Anticancer activity of a series of platinum complexes integrating demethylcantharidin with isomers of 1,2-diaminocyclohexane

Chun-Wing Yu,[a] Kay K. W. Li,[a] Siu-Kwong Pang,[a,b] Steve C. F. Au-Yeung[b] and Yee-Ping Ho[a,*]

[a]*School of Pharmacy, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China*
[b]*Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China*

Received 8 November 2005; revised 30 November 2005; accepted 2 December 2005
Available online 4 January 2006

**Abstract**—A series of platinum complexes derived from integrating demethylcantharidin (DMC) with different isomers of 1,2-diaminocyclohexane (DACH) has been synthesized and found to exhibit superior in vitro anticancer activity against colorectal and human hepatocellular cancer cell lines when compared with oxaliplatin, cisplatin, and carboplatin. Flow cytometric analysis revealed that the *trans*-DACH–Pt–DMC analogues showed similar behavior to oxaliplatin on affecting the cell cycle of the HCT116 colorectal cancer cell line, but distinct from that of cisplatin or carboplatin. The DACH component apparently dictates the *trans*-DACH–Pt–DMC complexes to behave mechanistically similar to oxaliplatin, whereas the DMC ligand appears to enhance the compounds' overall anticancer activity, probably by accelerating the cell cycle from G1 to S-phase with subsequent onset of G2/M arrest and accompanying apoptosis.
© 2005 Elsevier Ltd. All rights reserved.

Classical platinum (Pt)-based drugs such as cisplatin and carboplatin (Fig. 1) are among the most active anticancer agents and are widely used to treat various solid carcinomas.[1] However, intrinsic resistance in cancer cell lines such as hepatocellular carcinoma (HCC) and colorectal carcinoma, and the rapid emergence of acquired cisplatin resistance,[2] led to a global search for more effective Pt-based anticancer drugs.[3]

Oxaliplatin, a diaminocyclohexane (DACH)-containing third-generation platinum drug (Fig. 1), has a spectrum of activity and mechanisms of action and resistance that is clearly different from those of cisplatin and carboplatin,[4,5] and is the first Pt-based anticancer drug to demonstrate convincing clinical activity against colorectal cancer, the second leading cause of cancer-related deaths in the Western world.[6] Oxaliplatin was first launched for clinical use in France in 1996; rest of Europe in 1999 and recently USA in 2002, and its use has since increased dramatically.[7] It is highly plausible that oxaliplatin will forge a niche as a treatment option for those cancers that are unresponsive to other Pt-based drugs. For example, a recent clinical study has demonstrated that the FOLFOX regimen (oxaliplatin and infused fluorouracil plus leucovorin (5-FU/LV)) had superior activity over irinotecan, and bolus 5-FU/LV as a first-line therapy for metastatic colorectal cancer.[8]

The ability of oxaliplatin to circumvent cisplatin resistance caused by mismatch repair (MMR) deficiency is attributed to the presence of the 1,2-diaminocyclohexane (DACH) ligand.[4] It has been reported that the *trans*-(+)-(1*R*,2*R*-DACH) isomer of oxaliplatin is the most effective against cisplatin-sensitive and -resistant cancer cell lines.[5,9]

We recently reported the development of a novel series of Pt anticancer agents $[Pt(C_8H_8O_5)(NH_2R)_2]$ by integrating a Pt-moiety with demethylcantharidin (norcantharidin), a modified structural component of a traditional Chinese medicine (TCM).[10] The novel TCM–Pt compounds were found to be highly cytotoxic, particularly toward HCC; apparently able to overcome acquired cisplatin resistance; and demonstrated protein phosphatase 2A (PP2A) inhibitory activity: properties that were attributed to the inclusion of demethylcantharidin (DMC) in the chemical structures.[10,11] Our observations led us to infer a novel dual mechanism of

*Keywords*: Demethylcantharidin; 1,2-Diaminocyclohexane; Anticancer agents; Cell cycle.
* Corresponding author. Tel.: +852 2609 6831; fax: +852 2603 5295; e-mail: yeepingho@cuhk.edu.hk

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.019

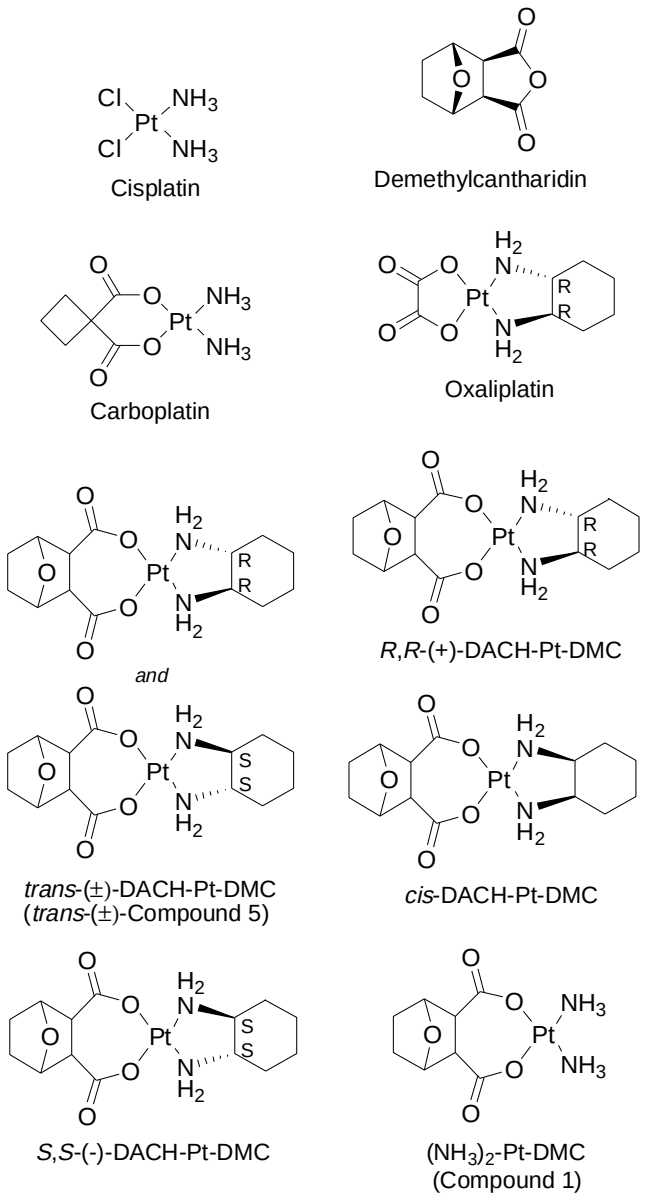


**Figure 1.** Chemical structures referred to in this study.

anticancer activity that encompassed a classical alkylating function by the Pt-amine moiety and inhibition of PP2A by the DMC component that is subsequently released from the complex.[11,12] We have also shown that DMC, as a PP2A inhibitor, can selectively target the nucleotide excision repair (NER) mechanism, thus enabling the circumvention of cisplatin resistance demonstrated by the TCM–Pt–DMC compounds.[11] A contemporary review by McCluskey et al.[13] that identified the potential for developing effective therapeutic strategies against many diseases including cancer by inhibition of protein phosphatase provided some support for our hypothesis of a novel dual mechanism of action.

Amongst our series of compounds, **5** (a racemate from *trans*-(±)-DACH; Fig. 1) has the closest structural resemblance to oxaliplatin and had exhibited the most potent anticancer activity.[10] In our continued quest for a superior Pt-based anticancer drug that is distinct from cisplatin or carboplatin, we herein describe the preparation of a new series of DACH–Pt–DMC analogues derived from using different isomers of DACH; report on the in vitro anticancer activity, specifically against HCC and colon cancer cell lines; and cell cycle analysis of adherent cells of HCT116 colorectal cancer cell line, selected because of its intrinsic resistance to cisplatin[14] but not to oxaliplatin, by flow cytometry. This study aimed to ascertain how the novel Pt-complexes that integrate DMC with stereochemically different DACH ligands might influence biological activity and to compare with oxaliplatin, the only DACH-containing Pt-based anticancer drug that is currently used clinically.

In brief, DMC was reacted with appropriate DACH–Pt-$(NO_3)_2$ intermediates, which were prepared from treatment of $K_2PtCl_4$ with appropriate diastereomeric DACH, followed by reaction with silver nitrate according to previously described methods.[10] Three new analogues, namely *trans*-(1*R*,2*R*)-, *trans*-(1*S*,2*S*)-, and *cis*-DACH–Pt–DMC (Fig. 1), were obtained in 73% yield for the *trans*-complexes and 33% yield for the *cis*-complex.[15–18] The novel compounds were characterized by $^1$H NMR, FAB-mass, and circular dichroism (CD) spectroscopy, and polarimetry. CD spectra clearly showed the optical activity of the novel DACH–Pt–DMC compounds, and the specific rotation $[\alpha]_D$ for *trans*-(1*R*,2*R*)-DACH–Pt–DMC was +55.57° and that for *trans*-(1*S*,2*S*)-DACH–Pt–DMC was −57.66°; whereas the original *trans*-(±)-DACH–Pt–DMC and the *cis*-DACH–Pt–DMC diastereomer were confirmed to be optically inactive.

The antiproliferative activity ($IC_{50}$) of the novel compounds against a range of human hepatocellular (Huh-7, PLC/5, and SK-Hep1) and colorectal (Colo320DM, HCT116, and HT29) carcinoma cell lines and two acquired cisplatin-resistant human hepatocellular sub-lines (Huh-7 and SK-Hep1) was measured by a standard tetrazolium (MTT) assay.[19] Acquired cisplatin-resistant sub-lines were developed by repeated exposure to cisplatin over a period of 12 months. Drug treatment was started 24 h after cells were seeded and $IC_{50}$ values were determined after cells were exposed to the drugs for 72 h (Table 1).

The in vitro results showed that the *trans*-analogues were consistently the most potent amongst all the compounds tested in both HCC and colon cancer cell lines: the *trans*-(+)-(1*R*,2*R*)-DACH–Pt–DMC complex, in particular, was the most effective diastereomer ($IC_{50}$ range of 2–5 μM in HCC and 0.4–0.8 μM in colon), thus concurring with previous reports of the biological behavior of different oxaliplatin isomers.[5,9] Amongst the control drugs, oxaliplatin was the most potent, demonstrating an $IC_{50}$ of ~1 μM in colon cancer cell lines and an $IC_{50}$ range of 7–11 μM against HCC cell lines. The results reflected the refractory nature of the selected cell lines toward cisplatin ($IC_{50}$ range of 10–50 μM in HCC and 2–6.5 μM in colon) and carboplatin ($IC_{50}$ range of 75–400 μM in HCC and 42–112 μM in colon). All of the diastereomeric DACH–Pt–DMC complexes and oxaliplatin were apparently able to circumvent

**Table 1.** Antiproliferative activity[a] of novel DACH–Pt–DMC complexes and reference drugs in selected human HCC, colorectal cancer cell lines, and human HCC cisplatin-resistant sub-lines

| Cell line | DMC | Cisplatin | Carboplatin | Oxaliplatin | *trans*-(±)-5 | *R*,*R*-(+)-5 | *S*,*S*-(−)-5 | *cis*-5 |
|---|---|---|---|---|---|---|---|---|
| Sensitive parent Huh-7 | 28.79 ± 3.45 | 10.21 ± 1.73 | 108.42 ± 13.25 | 7.46 ± 1.97 | 2.58 ± 0.28 | 2.33 ± 0.30 | 5.59 ± 0.71 | 15.21 ± 2.02 |
| Cisplatin-resistant Huh-7 subline[b] | 24.82 ± 3.03 (0.86) | 23.76 ± 2.75 (2.33) | 204.60 ± 21.29 (1.89) | 8.01 ± 1.99 (1.07) | 3.03 ± 0.43 (1.17) | 2.45 ± 0.28 (1.05) | 5.38 ± 0.85 (0.96) | 19.35 ± 1.85 (1.27) |
| PLC/5 | 25.68 ± 2.94 | 9.76 ± 1.71 | 75.23 ± 10.39 | 8.95 ± 1.56 | 3.39 ± 0.58 | 3.68 ± 0.30 | 6.76 ± 0.39 | 15.67 ± 2.54 |
| Sensitive parent SK-Hep1 | 20.23 ± 3.01 | 11.46 ± 1.98 | 121.58 ± 13.64 | 7.71 ± 2.03 | 3.89 ± 0.97 | 3.20 ± 0.54 | 6.91 ± 2.16 | 17.79 ± 2.88 |
| Cisplatin-resistant SK-Hep1 subline[b] | 19.63 ± 2.78 (0.97) | 52.04 ± 5.23 (4.54) | 402.55 ± 37.64 (3.31) | 11.47 ± 1.94 (1.49) | 5.92 ± 1.27 (1.52) | 5.36 ± 1.07 (1.68) | 10.24 ± 1.88 (1.48) | 20.05 ± 2.30 (1.13) |
| Colon-320DM | 19.92 ± 3.22 | 1.97 ± 0.59 | 42.75 ± 6.73 | 1.08 ± 0.52 | 0.81 ± 0.23 | 0.79 ± 0.24 | 1.60 ± 0.69 | 3.62 ± 0.61 |
| HCT116 | 23.20 ± 2.06 | 4.14 ± 0.80 | 69.43 ± 14.74 | 1.24 ± 0.15 | 0.81 ± 0.29 | 0.40 ± 0.25 | 2.04 ± 0.41 | 3.21 ± 0.58 |
| HT29 | 25.76 ± 2.55 | 6.47 ± 1.33 | 112.07 ± 11.12 | 1.31 ± 0.30 | 1.65 ± 0.49 | 0.77 ± 0.33 | 2.27 ± 0.94 | 8.52 ± 2.71 |

[a] $IC_{50}$ is the drug concentration effective in inhibiting 50% of the cell growth measured by the MTT assay after 72-h drug exposure ($IC_{50}$/μM ± SD; $n$ = 10–15 from two to three independent experiments).
[b] Resistance level ($n$-fold) in parentheses. The fold resistance equals the $IC_{50}$ of the resistant cells divided by the $IC_{50}$ of parental cells for various drugs.

cisplatin resistance in Huh-7 and SK-Hep1 sub-lines, thus reaffirming our earlier findings that the mechanism of antitumor action of *trans*-(±)-DACH–Pt–DMC is different from that of cisplatin or carboplatin, and more akin to that of oxaliplatin.[11] DMC, a PP2A inhibitor, also demonstrated a lack of cisplatin resistance and its $IC_{50}$ values were consistent at around 20–25 μM in all the cell lines tested.

Flow cytometric analysis (determined at 0, 6, 12, 18, 24, 48, and 72 h after drug treatment) of all *trans*-DACH–Pt–DMC complexes (at $IC_{50}$ and 5× $IC_{50}$ concentrations) once again showed distinct similarity to oxaliplatin, but different from cisplatin and carboplatin, in affecting the cell cycle of HCT116 colorectal cancer adherent cells.

Prominent differences were observed when HCT116 cells were subjected to drugs at 5× $IC_{50}$ concentrations (Figs. 2 and 3). Cisplatin caused a significant increase in the S-phase (49 ± 1) at 18 h, followed by G2/M-phase arrest (76 ± 5) from 48 to 72 h (Figs. 2c and d). The pattern for carboplatin was generally similar to that for cisplatin, except for a dramatic increase in the population of the sub-G1 phase (65 ± 3) at 72 h, which implied delayed apoptosis can occur with prolonged exposure to the drug (Fig. 2a). DMC was unique in that it exhibited an accumulation of S-phase (44 ± 2) at 6 h followed by G2/M-phase arrest (41 ± 3) after 12 h, with apparent accompanying apoptosis, as observed by a significant increase in the sub-G1 population from 6 h and reaching a maximum at 24 h (14 ± 2) (Fig. 2a). These findings are in agreement with those from other research groups where cantharidin-like PP2A inhibitors can accelerate the progression of the cell cycle from G1 to S-phase with ensuing G2/M arrest.[13,20]

In contrast, flow cytometric analysis revealed that the novel *trans*-DACH–Pt–DMC analogues and oxaliplatin followed a similar trend: that is, the compounds at 5× $IC_{50}$ concentrations all caused a significant decrease in the S-phase population within 18 h and at the same time induced G2/M arrest, but with no obvious apoptosis (Figs. 2a, c, and d). It was also observed that the G1 population remained more or less constant, which could imply that G1 arrest might be present and/or that a fraction of the cells were able to start another cycle due to activation of a repair mechanism.[21,22] These findings would suggest that the influence of the DACH structural component predominates over the DMC, in affecting the cell cycle of HCT116. However, the in vitro antiproliferative activity of the *trans*-(+)-*R*,*R*-DACH–Pt–DMC analogue is three times more potent than oxaliplatin in HCT116 cells (Table 1). Chemically, the two compounds have the same DACH-Pt moiety and differ only in the bidentate oxygenated-ligand; thus, it is highly probable that the superior anticancer activity is due to the presence of the DMC ligand.

In order to further substantiate a mechanistic role for DMC, we subjected an additional compound from our original series of DMC-Pt analogues, namely compound **1** (DMC-Pt-$(NH_3)_2$), to the same flow cytometric

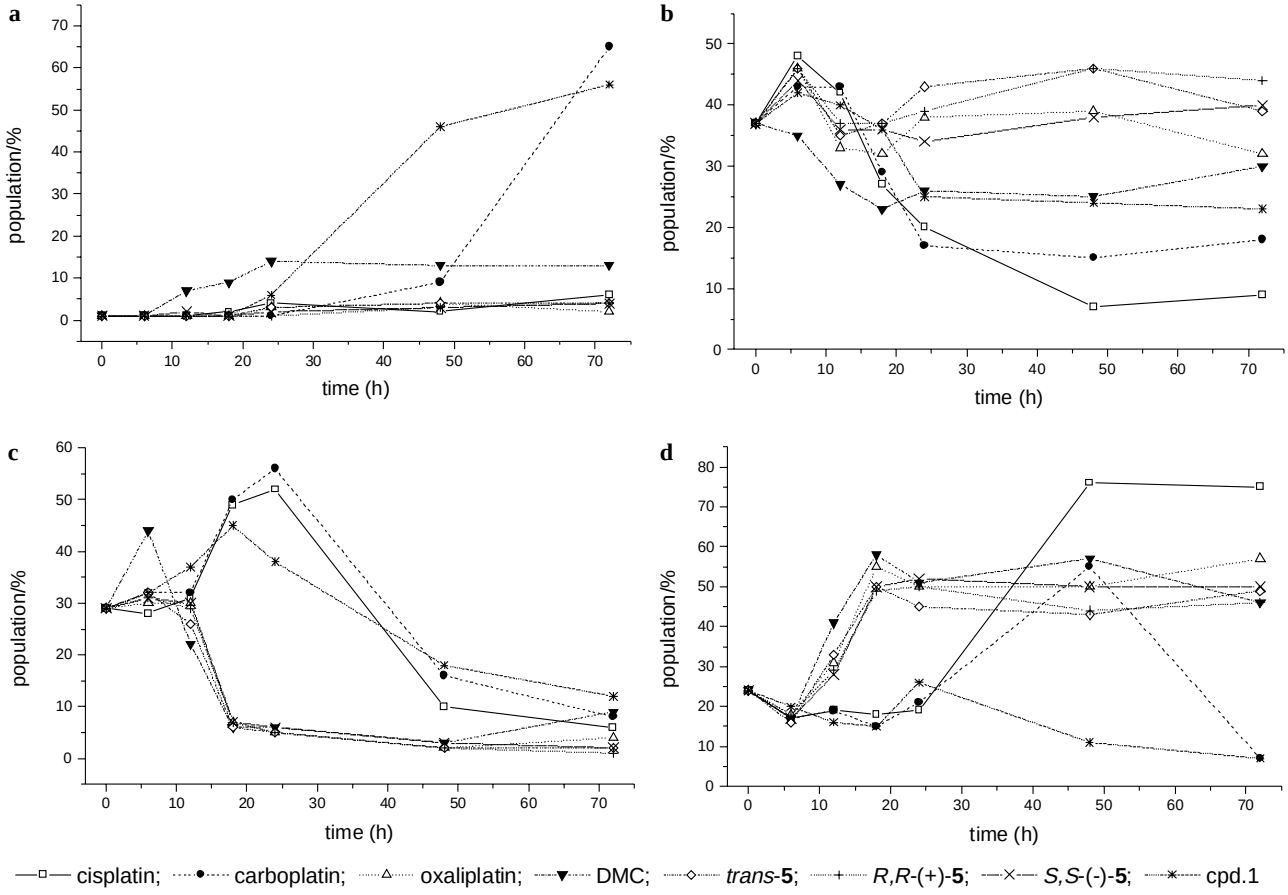


**Figure 2.** Population changes against time in the cell cycle of HCT116 after treatment with drugs at 5× $IC_{50}$ concentrations as determined by flow cytometry at (**a**) sub G1; (**b**) G1; (**c**) S; and (**d**) G2/M phases.

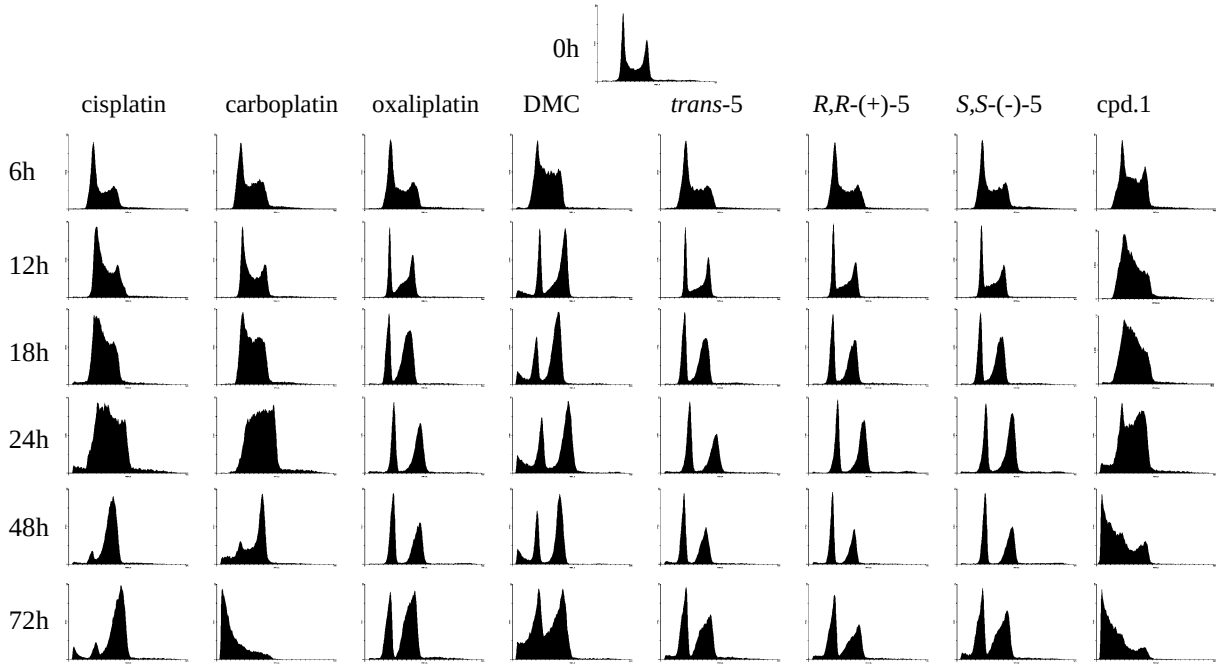


**Figure 3.** Representative DNA histograms of the effect of 5× $IC_{50}$ concentrations of cisplatin, carboplatin, oxaliplatin, DMC, *trans*-DACH–Pt–DMC analogues, and compound **1** on HCT116 cell cycle distribution determined by flow cytometry. Untreated (control) cells or cells treated for 6, 12, 18, 24, 48, and 72 h were harvested, fixed, stained with propidium iodide, and assessed for cell cycle distribution by FACS analysis, and quantified using WinMDI 2.8 software.

analysis. Compound **1** ($IC_{50}$ of $12.62 \pm 2.94$ μM in HCT116) was selected because we have previously shown it to dissociate in vitro, over a period of 24 h, to the diacid of DMC (endothall) and its inferred Pt-$(NH_3)_2$ moiety; the latter species being equivalent to the cytotoxic, alkylating Pt-moiety of cisplatin or carboplatin.[12] Interestingly, flow cytometry of compound **1** does indeed show an early sub-G1 phase accumulation from 24 h to 48 h ($46 \pm 2$) that is similar to DMC; *plus* an increase in the S-phase at 18 h ($45 \pm 3$) that resembles the effect due to cisplatin or carboplatin (Figs. 2a, c, and 3). Therefore, it is possible to deduce that the antiproliferative effect of **1** on HCT116 is due to a combination of: (i) the cisplatin- or carboplatin-like alkylation of DNA, a mechanism that is well established; and (ii) an acceleration or induction of apoptosis by the released diacid of DMC.

The structure of compound **1** can be compared directly with that of carboplatin: they both have the same $(NH_3)_2$-Pt moiety and again differ only in the dicarboxylato-ligand (Fig. 1). However, compound **1** is approximately five times more potent than carboplatin in HCT116 cells ($IC_{50}$ of $12.62 \pm 2.94$ μM and $69.43 \pm 14.74$ μM, respectively); thus, together with the cell cycle analysis, it provides further evidence of the DMC-ligand able to significantly enhance anticancer activity.

In conclusion, this is the first report of a study examining the mechanism of anticancer activity of new complexes that integrate demethylcantharidin (DMC), a modified component of a traditional Chinese medicine, with different isomers of 1,2-diaminocyclohexane (DACH). We have shown that the DACH and DMC components both contribute significantly to the compounds' potent anticancer activity, but with different mechanisms. Flow cytometric analysis has shown our *trans*-DACH–Pt–DMC analogues to influence the cell cycle of HCT116 with striking similarity to oxaliplatin, and it would appear that in these analogues, the DACH structural component predominates over the DMC, in dictating the mechanism of anticancer activity. However, the fact that the in vitro antiproliferative activity of the *trans*-(+)-(1*R*,2*R*)-analogue is three times more potent than oxaliplatin in HCT116, and that of compound **1** is five times more potent than carboplatin, does advocate that the bidentate DMC ligand has an important role in enhancing anticancer activity, probably by accelerating the progression of the cell cycle and early induction of apoptosis.

## Acknowledgments

We thank the Chinese University of Hong Kong (CUHK) for the provision of a studentship (to C.W. Yu). We also gratefully acknowledge the Research Grant Council of Hong Kong SAR for financial support (RGC Ref: 4181/02 M). Technical assistance from Ms. Jenny Hau, Department of Anatomy, CUHK, is also acknowledged.

## References and notes

1. Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451.
2. Fuertes, M. A.; Alonso, C.; Perez, J. M. *Chem. Rev.* **2003**, *103*, 645.
3. Ho, Y. P.; Au-yeung, S. C. F.; To, K. K. W. *Med. Res. Rev.* **2003**, *23*, 633.
4. Raymond, E.; Faivre, S.; Chaney, S. G.; Woynarowski, J. M.; Cvitkovic, E. *Mol. Cancer Ther.* **2002**, *1*, 227.
5. Di Francesco, A. M.; Ruggiero, A.; Riccardi, R. *Cell. Mol. Life Sci.* **2002**, *59*, 1914.
6. Giacchetti, S.; Perpoint, B.; Zidani, R.; Le Bail, N.; Faggiuolo, R.; Focan, C.; Chollet, P.; Llory, J. F.; Letourneau, Y.; Coudert, B.; Bertheaut-Cvitkovic, F.; Larregain-Fournier, D.; Le Rol, A.; Walter, S.; Adam, R.; Misset, J. L.; Levi, F. *J. Clin. Oncol.* **2000**, *18*, 136.
7. Graham, J.; Muhsin, M.; Kirkpatrick, P. *Nat. Rev. Drug Discov.* **2004**, *3*, 11.
8. Goldberg, R. M.; Sargent, D. J.; Morton, R. F.; Fuchs, C. S.; Ramanathan, R. K.; Williamson, S. K.; Findlay, B. P.; Pitot, H. C.; Alberts, S. R. *J. Clin. Oncol.* **2004**, *22*, 23.
9. Pendyala, L.; Kidani, Y.; Perez, R.; Wilkes, J.; Bernacki, R. J.; Creaven, P. J. *Cancer Lett.* **1995**, *97*, 177.
10. Ho, Y. P.; To, K. K. W.; Au-yeung, S. C. F.; Wang, X.; Lin, G.; Han, X. *J. Med. Chem.* **2001**, *44*, 2065.
11. To, K. K. W.; Wang, X.; Yu, C. W.; Ho, Y. P.; Au-yeung, S. C. F. *Bioorg. Med. Chem.* **2004**, *12*, 4565.
12. To, K. K. W.; Ho, Y. P.; Au-Yeung, S. C. F. *J. Chromatogr. A* **2002**, *947*, 319.
13. McCluskey, A.; Sim, A. T. R.; Sakoff, J. A. *J. Med. Chem.* **2002**, *45*, 1151.
14. Aebi, S.; Kurdi-Haidar, B.; Gordon, R.; Cenni, B.; Zheng, H.; Fink, D.; Christen, R. D.; Boland, C. R.; Koi, M.; Fishel, R.; Howell, S. B. *Cancer Res.* **1996**, *56*, 3087.
15. *Synthesis of trans-(±)-DACH–Pt–DMC.* $K_2PtCl_4$ (2 g, 4.818 mmol) was dissolved in deionized water (10 mL). *trans*-(±)-1,2-Diaminocyclohexane (*trans*-(±)-DACH) (0.55 g, 4.82 mmol) was dissolved in deionized water (2 mL) and added dropwise to the reaction mixture slowly at room temperature and a yellow precipitate (product) gradually formed. The above reaction mixture was stirred at room temperature overnight, then filtered and washed with ice water (5 mL), EtOH (5 mL), and diethyl ether (5 mL). The product was dried at 65 °C in an oven. Yield obtained: 1.73 g (94%). *trans*-(±)-DACH-$PtCl_2$ (1.63 g, 4.29 mmol) was suspended in deionized water (15 mL), $AgNO_3$ (1.46 g, 8.57 mmol) was added to the reaction mixture at room temperature. The reaction mixture was stirred in the dark at room temperature overnight, after which undissolved AgCl (white ppt) was filtered off. To the filtrate, demethylcantharidin (0.72 g, 4.29 mmol) and NaOH (0.34 g, 8.57 mmol) were added at room temperature and the reaction mixture was further stirred at room temperature overnight to give a white-gray precipitate. The reaction mixture was cooled to 0 °C after the reaction, filtered, and washed with ice water (5 mL), EtOH (5 mL), and diethyl ether (5 mL), and the product (white solid) dried at 65 °C. Yield obtained: 1.51 g (72%). Proton NMR for *trans*-(±)-DACH–Pt–DMC: $^1$H NMR ($D_2O$) δ 1.18 (m, 2H; $Hf_3$ and $Hf_4$), 1.31 (m, 2H; $He_3$ and $He_4$), 1.59 (m, 2H; $Hf_1$ and $Hf_2$), 1.76 (m, 4H; $Ha_1$, $Ha_2$, $Ha_3$ and $Ha_4$), 2.06 (m, 2H; $He_1$ and $He_2$), 2.38 (m, 2H; $Hd_1$ and $Hd_2$), 3.98 (d, $J = 10.8$ Hz, 1H; $Hc_1$ or $Hc_2$), 4.01 (d, $J = 10.8$ Hz, 1H; $Hc_1$ or $Hc_2$), 4.85 (m, 2H; $Hb_1$ and $Hb_2$). IR (KBr) *v*: 3195, 3150 (N–H), 1646, 1608 (C=O); 1396 (COO) 1266, 1215, 1066, 940 (C–C–C). HRMS (FAB): calcd for $C_{14}H_{22}O_5N_2Pt$ [$MH^+$] 494.1249, found 494.12527.

16. *Synthesis of trans-(R,R)-DACH–Pt–DMC.* The method used was as described for the preparation of *trans*-(±)-DACH–Pt–DMC, except that *trans*-(*R*,*R*) 1,2-diaminocyclohexane was used. *trans*-(*R*,*R*)-$PtCl_2$DACH was obtained in 95% yield and *trans*-(*R*,*R*)-5 in 73% yield. $[\alpha]_D$ +55.57°.
17. *Synthesis of trans-(S,S)-DACH–Pt–DMC.* The method used was as described above, except that the starting material was *trans*-(*S*,*S*) 1,2-diaminocyclohexane. *trans*-(*S*,*S*)-$PtCl_2$DACH was obtained in 95% yield and *trans*-(*S*,*S*)-5 in 73% yield. $[\alpha]_D$ −57.66°.
18. *Synthesis of cis-DACH–Pt–DMC.* The method used was as described above, except that the starting material was *cis*-1,2-diaminocyclohexane (*cis*-DACH). Yield of *cis*-$PtCl_2$DACH was 75% and that of *cis*-DACH–Pt–DMC was 33%.

19. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
20. Sakoff, J. A.; Ackland, S. P.; Baldwin, M. L.; Keane, M. A.; McCluskey, A. *Invest. New Drugs* **2002**, *20*, 1.
21. Reardon, J. T.; Vaisman, A.; Chaney, S. G.; Sancar, A. *Cancer Res.* **1999**, *59*, 3968.
22. Arnould, S.; Guichard, S.; Hennebelle, I.; Cassar, G.; Bugat, R.; Canal, P. *Biochem. Pharmacol.* **2002**, *64*, 1215.