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Synthesis and *in vitro* antitumour activity of carboplatin analogues containing functional handles compatible for conjugation to drug delivery systems

Lisa-Maria Rečnik[a,b,d], Christophe Cantelli[a,b,d], Cyril Fersing[a,c], Céline Gongora[b], Jean-Pierre Pouget[b], Vincent Lisowski[a,*]

[a] Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, Université de Montpellier, ENSCM, UFR des Sciences Pharmaceutiques et Biologiques, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France

[b] Institut de Recherche en Cancérologie de Montpellier, INSERM, Université de Montpellier, Institut Régional du Cancer de Montpellier, 208 Avenue des Apothicaires, 34298 Montpellier Cedex 5, France

[c] Nuclear Medicine Department, Montpellier Cancer Institute (ICM), University of Montpellier, 208 Avenue des Apothicaires, 34298 Montpellier Cedex 5, France

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ABSTRACT

We describe herein the synthesis of a series of carboplatin derivatives with different functional groups at position 3 of the cyclobutane ring. This pharmacomodulation approach aims at facilitating the vectorisation of these analogues, *via* their subsequent conjugation to a drug delivery system. Five different derivatives bearing a hydroxy, keto, iodo, azido or amino function at position 3 were synthesised. One of these compounds was coupled to a bifunctional maleimide-containing linker. All compounds were tested *in vitro* for their cytotoxicity on four different cell lines including two platinum-resistant colorectal cancer cell line (SK-OV-3, HCT116, D3E2, D5B7) using an MTS assay. Overall, the tested compounds were up to six times more potent than carboplatin, especially on D5B7 human colorectal cancer cells. We demonstrated that these modifications led to potent analogues which are compatible with conjugation to a drug delivery system.

Introduction

Over 60 years ago Rosenberg *et al.* discovered the cytostatic activity of cisplatin (cis-[$Pt(NH_3)_2Cl_2$]) and its potential use as an anticancer agent (Fig. 1).[1,2] Cisplatin covalently binds to DNA and forms DNA crosslinks which cause DNA damage and subsequently induce apoptosis. The binding of cisplatin to the DNA is enabled by the loss of one chloride ion (leaving group) and the resulting formation of an aquo complex.[3] In 1978, cisplatin was first approved for medical use on the American market. Despite its great success in the treatment of testicular, ovarian, lung, breast, bladder cancer and many more, cisplatin is associated with a number of drawbacks.[4] The most common side effects are nephrotoxicity, neurotoxicity, ototoxicity and emetogenesis. Moreover, the solubility of cisplatin in aqueous solutions is quite limited.

To overcome these limitations, a second generation of platinum-based anticancer drugs with higher hydrosolubility, decreased side effects and improved antitumour activity was developed.[5] One of these derivatives is the clinically approved drug carboplatin which contains a bidentate cyclobutane-dicarboxyate (cbdc) ligand as leaving group (Fig. 1).[6] Although carboplatin is less potent than cisplatin, it benefits from reduced side effects, particularly a decreased nephrotoxicity, and a higher biological half-life.[7] It represents the drug of choice in the treatment of ovarian cancer and is also used for endometrial, lung, head and neck, bladder and other cancers management.

Up-to-date only a handful of derivatives of carboplatin which have been modified at position 3 of the cyclobutane ring are described in the literature. Bernhardt *et al.* synthesised carboplatin derivatives bearing a hydroxy, fluoro, chloro or bromo substituent in this position.[8] Introduction of a keto-group,[9] a nitrooxy group,[10] a dichloroacetate[11] or two methoxy groups[12] are also reported at position 3. It has been shown that these analogues have an improved or maintained antitumour activity compared to carboplatin. Modification at this position also allows for the attachment of a cell-targeting molecule in order to increase the drug concentration at the tumour site and reduce side effects. For example, Koide and coworkers installed a collagen-like triple-helical peptide as a drug carrier.[13] The group of Sinn attached cholic acid as a transporter fragment and were able to overcome resistance to platinum-

* Corresponding author.
E-mail address: vincent.lisowski@umontpellier.fr (V. Lisowski).
[d] Co-first author: these authors contributed equally to this work.

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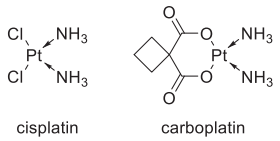


Fig. 1. Structures of cisplatin and carboplatin.

based drugs in an *in vitro* model.[14] Both examples involved the attachment of the cell-targeting molecule early in the synthesis *via* an alkyl spacer. Brunner *et al.* reported a porphyrin-containing carboplatin complex with the porphyrin unit bound to carboplatin *via* an ether bond.[15] Kratz and coworkers attached carboplatin to a maleimide-containing bifunctional linker *via* an ester bond.[16] All examples with conjugated drug delivery systems increased the antitumour activity of carboplatin.

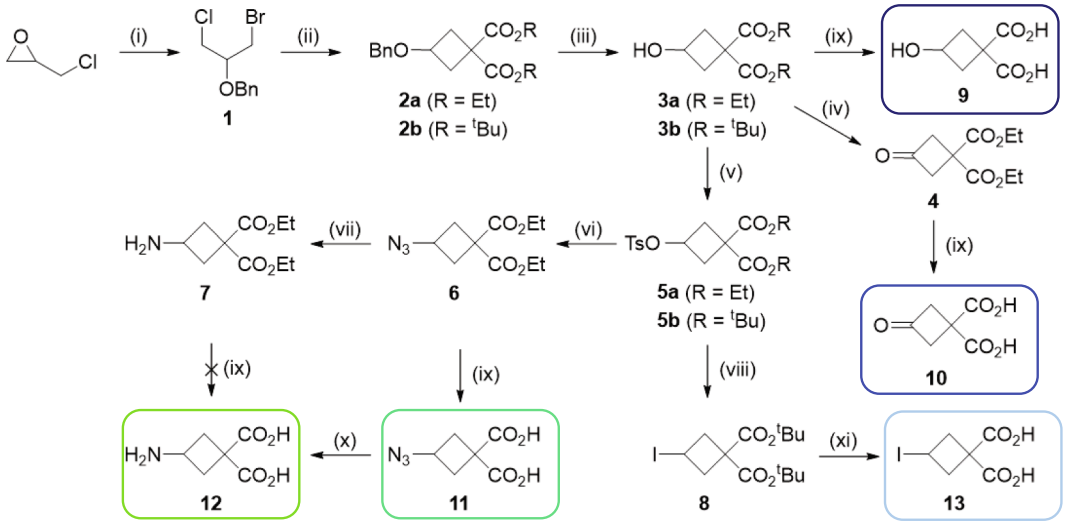
Our interest focused on the synthesis of carboplatin derivatives containing new functional handles at position 3 of the cyclobutane moiety. These functional groups can be used for attachment to various drug delivery systems such as peptides, monoclonal antibodies, nanoparticles or small molecules *via* a bifunctional linker. The functionalised analogues we chose to synthesise were the previously described hydroxy and keto derivatives[9,17] as well as the novel iodo, azido and amino analogues as they allow for quick and reliable methods of attachment *via* ester or ether bond formation (hydroxy group), metal-catalysed cross-coupling reactions (iodo group), Cu-catalysed alkyne-azide cycloaddition (CuAAC, azide group), amide or oxime bond formation (amino and keto group, respectively). In this work, five different carboplatin derivatives were synthesised and tested *in vitro* for their antitumour activity on ovarian cancer and colorectal cancer cell lines including a platinum-based drug-resistant cell line. To demonstrate the interest of functionalisation, one complex coupled to a bifunctional maleimide-containing linker *via* an amide bond was synthesised and evaluated *in vitro*.

In order to establish a library of carboplatin analogues, differently substituted cbdc ligands were synthesised (Scheme 1). According to a literature procedure, 3-hydroxycyclobutane dicarboxylate **3** was obtained in 3 steps.[18,19] Commercially available epichlorohydrin and benzyl bromide were heated overnight in the presence of $HgCl_2$ to give 2-benzyloxy-1-bromo-3-chloropropane **1** which was reacted with diethyl malonate and sodium hydride over 3 days to result in the formation of the benzyl-protected alcohol **2**. Deprotection of the alcohol with Pd $(OH)_2$ and H_2 at 50 psi gave alcohol **3** which was further oxidised to ketone **4** using pyridinium chlorochromate. Furthermore, alcohol **3** was also transformed to tosylate **5** using tosyl chloride, triethylamine and DMAP. The resulting tosylate **5** was then treated with sodium azide in the presence of Bu_4NHSO_4 in DMF to yield the azide-containing compound **6** which was further reduced to amine **7** using Pd/C under H_2 atmosphere. Tosylate **5** was also transformed to iodide **8** with NaI in acetone. The functionalised cbdc diethyl esters **3**, **4** and **6** were hydrolysed with LiOH in THF/H_2O to yield the corresponding free dicarboxylic acids **9**–**11**. Hydrolysis of amino-cbdc diethyl ester **7** to the amino-cbdc ligand **12** was not successful under these conditions most likely due to the formation of the hydrochloride salt which could not be isolated. However, hydrogenation of azido-cbdc **11** with Pd/C under H_2-atmosphere resulted in the formation of compound **12**. Iodo-cbdc ethyl ester **8** could not be hydrolysed to compound **13** under these conditions either, as the iodide was not stable under basic conditions. Therefore di-*t*-butyl ester **8** was synthesised using the same synthetic pathway but using di-*t*-butyl malonate instead of diethyl malonate. Ester **8** was then hydrolysed under acidic conditions using TFA in DCM to yield iodo-cbdc **13**.

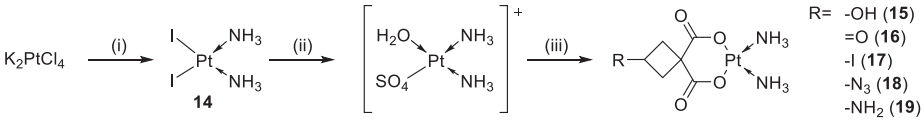
For the synthesis of the platinum complexes (Scheme 2), potassium tetrachloroplatinate K_2PtCl_4 was reacted with KI and ammonium hydroxide to give *cis*-diamminediiodoplatinum (II) **14**. The sulfato complex was prepared *in situ* by addition of $AgSO_4$ to the diiodo complex **14**. Further treatment with the barium salts of the cbdc ligands **9**–**13** resulted in the formation of complexes **15**–**19** bearing a hydroxy, oxo, iodo, azido or amino group at position 3 of the cbdc ligand.

One example of carboplatin derivatives coupled to a bifunctional linker was synthesised (Scheme 3). Aminocarboplatin **19** was coupled *via* an amide bond to a maleimide-containing linker used in several antibody-drug conjugates.[20] The coupling reaction of amino-cbdc with the linker was carried out prior to the complexation reaction. For this purpose, di-*t*-butyl 3-aminocyclobutyldicarboxylate **20** was synthesised according to the above procedure in five steps starting from *t*-butyl malonate and compound **1** and coupled to 6-maleimidocaproic acid. The *t*-butyl group was chosen as a protecting group for the carboxylic acid as the harsh basic deprotection conditions necessary for the hydrolysis of ethyl esters are not tolerated by maleimides. After hydrolysis of the *t*-butyl ester **21** with TFA the resulting dicarboxylic acid **22** was used for complexation with the platinum salt as described above to give compound **23**.

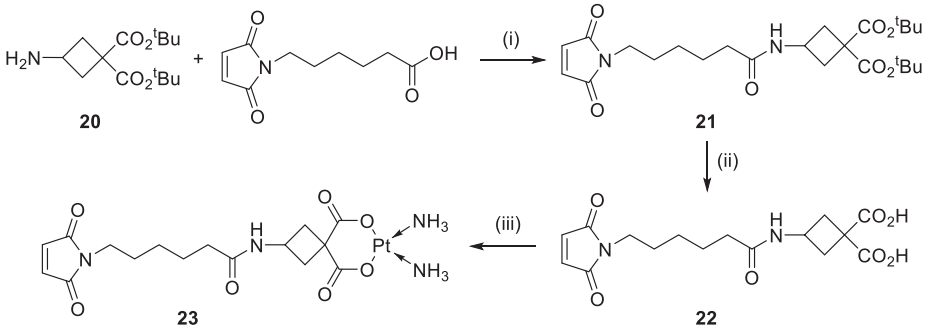
Compounds **15**–**19** and **23** were tested *in vitro* for their cytotoxicity in an MTS assay using SK-OV-3 (ovarian cancer cell line), HCT116



Scheme 1. Reagents and conditions: (i) BnBr, $HgCl_2$, 115 °C, 65%; (ii) diethyl malonate, NaH, 1,4-dioxane, 0 °C to r.t. to rf, 67% (2a), 63% (2b); (iii) $Pd(OH)_2$, H_2 (50 psi), EtOH, r.t., 97% (3a), 67% (3b); (iv) PCC, DCM, r.t., 91%; (v) TsCl, Et_3N, DMAP, DCM, r.t., 99% (5a), 71% (5b); (vi) NaN_3, Bu_4NHSO_4, DMF, 80 °C, 94%; (vii) Pd/C, H_2, EtOAc, r.t., 76%; (viii) NaI, acetone, rf, 34%; (ix) LiOH, THF, H_2O, r.t., 58% (9), 66% (10), 67% (11); (x) Pd/C, EtOAC, r.t., 74%; (xi) TFA, DCM, r.t., 68%.



Scheme 2. Reagents and conditions: (i) KI, H_2O, then NH_4OH, r.t., 89%; (ii) $AgSO_4$, H_2O, dark, r.t., not isolated; (iii) $Ba(OH)_2$, **9–13**, r.t., 79% (15), 52% (16), 39% (17), 31% (18); 3% (19).



Scheme 3. Reagents and conditions: (i) HATU, 2,6-lutidine, DMF, r.t., 89%; (ii) TFA, DCM, r.t., 92%; (iii) $Pt(NH_3)_2I_2$, $AgSO_4$, H_2O, then $Ba(OH)_2$, **23**, r.t., 30%

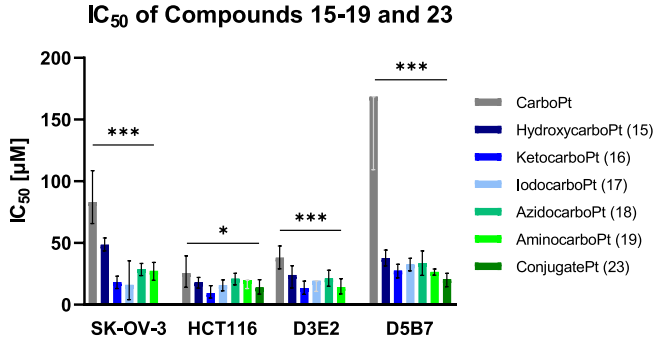


Fig. 2. IC_{50} Values of complexes **15–19** and **23** on SK-OV-3, HCT116, D2E3 and D5B7 cell lines in comparison to carboplatin. Results are means from experiments in triplicates. Error bars show the 95% confidence interval of non-linear regression. * $P < 0.005$, *** $P < 0.0001$.

(colorectal cancer cell lines) as well as the platinum-resistant colorectal cancer cell lines D3E2[21] and D5B7. All compounds were soluble in cell culturing media at the tested concentrations. Cells were incubated for 48 h in the presence of the drug and cell viability was determined after 72 h. IC_{50} values for compounds **15–19** and **23** were determined and are summarized in Fig. 2. All tested derivatives showed maintained or higher cytotoxicity on these four cell lines compared to carboplatin. On ovarian SK-OV-3 cells, iodocarboplatin **17** was the most active complex with an IC_{50} of 16.1 µM resulting in a cytotoxicity five times higher than carboplatin (83.1 µM). Values for hydroxycarboplatin **15** (48.6 µM) and ketocarboplatin **16** (18.3 µM) on SK-OV-3 cells are similar to those reported in literature (37.2 µM and 20.7 µM, respectively).[9,17] On HCT116 cells, the IC_{50} shows a similar trend. The synthesised complexes **15–19** have a slightly improved cytotoxic effect, with ketocarboplatin **16** displaying the lowest IC_{50} values (9.3 µM). The D3E2 and D5B7 cell lines are platinum-resistant cell lines that demonstrate resistance towards oxaliplatin. For D3E2, the obtained cytotoxicity results were similar to HCT116 and resistance towards carboplatin was not very distinct. In general, complexes **15–19** showed a 1,5 to 3-fold increased cytotoxicity, with ketocarboplatine **16** being the most cytotoxic compound (13.4 µM). D5B7 cells were significantly more resistant to carboplatin. However, this resistance was drastically reduced when a functional group was installed on the cbdc ligand of carboplatin. All derivatives **15–19** displayed a 4.5 to 6-fold increase in potency. Maleimide **23** displayed a similar antiproliferative activity as the corresponding amine **19** on HCT116 and D5B7 cell lines. Overall, among the tested cell lines complexes **15–19** and **23** showed a 2- to 4-fold improved cytotoxic activity on cell lines which are less sensible to carboplatin and a maintained cytotoxicity on platinum-sensitive cell lines.

In conclusion, 5 different carboplatin analogues **15–19** with functional groups at position 3 of the cbdc ligand were synthesised and one of them was conjugated to a bifunctional maleimide-containing linker *via* an amide bond. The cytotoxic profile of compounds **15–19** and conjugate **23** was determined using four different cell lines and all compounds showed maintained or increased cytotoxicity compared to commercially available carboplatin. Free aminocarboplatin **19** and aminocarboplatin coupled to a maleimide-bearing linker **23** showed the same cytotoxic profile on the four tested cell lines. All these experiments demonstrate that substitution at position 3 of the cbdc ligand are well tolerated and do not alter the cytotoxic behaviour of carboplatin. It was shown that the molecules can be attached to bifunctional linkers *via* an amide bond but further conjugations *via* ester bonds, hydroxime bonds, CuAAC reactions or transition-metal catalysed cross-coupling reactions can be envisaged. The bifunctional linker can then be attached to various drug delivery systems such as antibodies, nanoparticles, peptides or small molecules. These promising results are worth pursuing the development of carboplatin drug delivery systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127527.

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