Bioorganic & Medicinal Chemistry Letters 21 (2011) 6386–6388

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Antitumor dinuclear platinum(II) complexes derived from a novel chiral ligand

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ARTICLE INFO

Article history:
Received 28 May 2011
Revised 15 August 2011
Accepted 24 August 2011
Available online 28 August 2011

Keywords:
1*R*,2*R*-Diaminocyclohexane derivative
Dinuclear platinum(II) complexes
Cytotoxicity

ABSTRACT

A new chiral ligand, 2-(((1*R*,2*R*)-2-aminocyclohexyl)amino)acetic acid (HL), was designed and synthesized to prepare a series of novel dinuclear platinum(II) complexes with dicarboxylates or sulfate as bridges. The evaluation of these metal complexes in vitro cytotoxicity against human HCT-116, MCF-7 and HepG-2 cell lines were made. All compounds showed antitumor activity to HCT-116 and MCF-7. Particularly, compounds M3 and M5 not only exhibited better activity than carboplatin against MCF-7 and HepG-2, but also showed very close activity to oxaliplatin against HCT-116.

As molecular structures different from classical mononuclear platinum-based drugs such as cisplatin and carboplatin, multinuclear platinum complexes have attracted much attention since 1990's, especially rising of BBR3464.[1,2] For carrying high positive charges (+4) and flexible alkyl chains, BBR3464 could form a range of interstrand GG adducts dictated by the sequence of DNA bases, while the cytotoxicity of cisplatin is due to the formation of mainly mono-functional Pt(G) and intrastrand bi-functional Pt(GG) adducts which have been proved to cause a local distortion of DNA.[3–5] Thus, multinuclear platinum complexes are expected to be able to circumvent the inherent or acquired cisplatin-resistance in a panel of human tumor models.[6–8] So far a number of various diamines have been involved as bridges to connect platinum units in previous researches including BBR3464, however, results for phase II studies of BBR3464 were not so inspiring[9] and severe dose limiting side-effects such as diarrhea and vomiting limited its clinic use.[10]

Since 1*R*,2*R*-diaminocyclohexane (DACH) has been believed to play an important role in the success of oxaliplatin,[11,12] we have designed to modify the DACH skeleton by selectively introducing a functional group to one of its amino moieties so that it can act as a tridentate ligand to prepare dinuclear platinum(II) complexes with dicarboxylates as bridges. Upon this design, we have recently reported a series of dinuclear platinum complexes with 2-(((1*R*,2*R*)-2-aminocyclohexylamino)methyl)phenol as ligand.[13] Primary in vitro tests exhibited that those compounds showed cytotoxicity towards some selected human cell lines comparable to that of carboplatin, but our further work indicated that the steric hindrance and lipotropy of the aromatic group may reduce their antitumor activity. So, rather small and hydrophilic carboxylic acids have been considered as functional groups to combine with one of amino nitrogen atoms of DACH deliberately. In this paper, we report such a new ligand, 2-(((1*R*,2*R*)-2-aminocyclohexyl)amino)acetic acid (HL).With HL as carrier group and dicarboxylates/sulfate as bridges, five novel dinuclear platinum(II) complexes were prepared and their cytotoxicity was evaluated against three human cell lines.

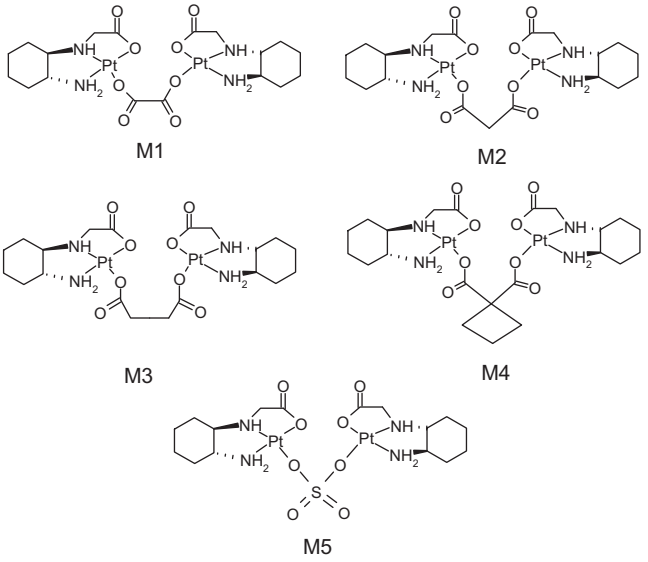

Figure 1. Chemical structures of dinuclear platinum(II) complexes.

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doi:10.1016/j.bmcl.2011.08.100

Notably, the targeted dinuclear platinum complexes differ from the previously reported multinuclear platinum complexes which have employed various diamines as bridges (including BBR3464). Structures of the dinuclear complexes are shown in Figure 1.

Mono-Boc protecting DACH[14] was used as starting material to prepare the ligand as before, since it is difficult to directly get the monsubstituted derivative due to the equivalent reactivity of the two amino groups in DACH. The synthetic process of HL is shown as following. Reaction of mono-Boc protecting DACH with ethyl 2-chloroacetate offered intermediate 1, which was then removed the protecting group to give intermediate 2.2 was hydrolyzed in the presence of NaOH, leading to the formation of intermediate 3, which was finally neutralized by aqueous HCl solution to give a free ligand, HL (Scheme 1).

Before preparing the targeted dinuclear platinum complexes, important intermediate [PtLI][15] was firstly prepared, which was then used to react with different silver salts (dicarboxylates/sulfate) to afford compounds M1–M5.[16,17]

Intermediate [PtLI] and targeted dinuclear platinum complexes were characterized by IR, ^{1}H NMR, ESI-MS spectra and microanalysis together with TG-thermal analysis. Found values for each compound in the elemental analysis were in good agreement with calculated values. In the IR spectra, bands of $\nu_{NH/NH2}$ and $\delta_{NH/NH2}$ in the platinum complexes shifted to lower frequencies than those of free primary amine and secondary amine. Carboxylate anions binding with Pt(II) were confirmed by the examination of the C=O absorptions shifting from free carboxylic acids near 1700 cm^{-1} to bands near 1646–1584 cm^{-1}. The complexes showed $[M+H]^+$ or $[M+Na]^+$ corresponding to their formula weights and relative fragment peaks in their ESI mass spectra. Typical isotopes of Pt element: ^{194}Pt (33%), ^{195}Pt (34%), ^{196}Pt (25%), were found with three protonated ion isotopic peaks. The ^{1}H NMR spectral of all prepared complexes in Figure 1 were all consistent with their corresponding protons both in the chemical shifts and in the number of protons. TG-thermal analytic data of compounds M2 and M4 showed that they had analogous thermal processes, in which the weight loss began at about 200–230 °C, and then kept steady until about 660–700 °C. The final residue, corresponding to 53–56% weight loss, can be attributed to platinum element.

Poor aqueous solubility is a severe problem for some platinum anticancer drugs in clinic use. Compared with cisplatin (1.0 mg/ml) and oxaliplatin (7.0 mg/ml), the aqueous solubility of all resulting platinum complexes has been greatly improved (30–50 mg/ml at 25 °C) for introducing hydrophilic carboxyl to DACH as expected.

MTT assay was carried out to evaluate the in vitro cytotoxicity of the resulting dinuclear platinum complexes as described by Mosmann et al.,[18–21] using HCT-116, MCF-7 and HepG-2 cell lines, respectively. The IC_{50} values of the platinum complexes with carboplatin and oxaliplatin as positive controls are given in Table 1

As we can see, all dinuclear platinum complexes showed cytotoxicity towards HCT-116 cell line with IC_{50} values varying from 5.1 to 27.5 μM, the order of the potency is oxaliplatin > M5 > M3 > M2 > M1 > M4. Clearly, cytotoxicity increased along with the extending carbon chain of the carboxylate bridge, but the cyclobutyl chain in compound M4 had a reverse effect. Among these dinuclear platinum complexes, compounds M3 and M5, which have succinate and sulfate as bridges, respectively, showed very close activity to oxaliplatin.

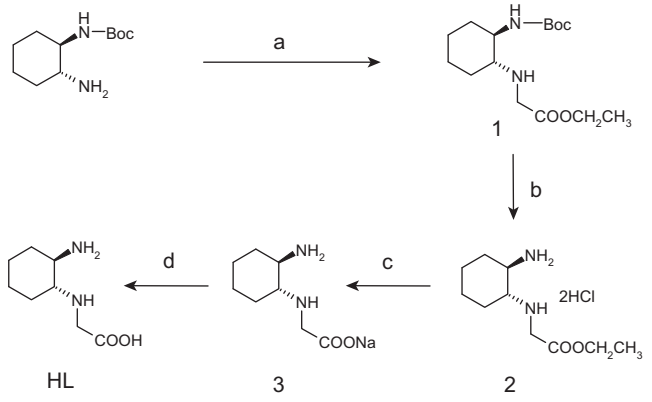
When tested against MCF-7 cell line, all complexes exhibited affirmative cytotoxicity, the order of IC_{50} values is M5 < M3 < carboplatin < M2 < M1 < M4. Both compounds M3 and M5 showed better activity than carboplatin, and the cytotoxicity of compound M5 was even two times as potent as that of carboplatin. Again, cytotoxicity increased along with the addition of the carbon chain in the carboxylate bridge, which was observed in HCT-116 cell line with these compounds.

When the cell line was HepG-2, only compounds M3 and M5 showed significant cytotoxicity that was better than carboplatin.

Based on the structure–activity relationship of these compounds, we have got an interesting finding that sulfate as a bridge may significantly improve the cytotoxicity of the dinuclear platinum complex. It is noted from Figure 2 that the cytotoxicity of compound M5 is better than carboplatin against HepG-2 and MCF-7 cell lines and very close to oxaliplatin against HCT-116 cell line, that is the best performance in all resulting dinuclear platinum complexes. When succinate was selected as a bridge, compound M3 also showed interesting activity, in spite of being lower than compound M5. Other compounds which owned oxalate, malonate and cyclobutane-1,1-dicarboxylate as bridges showed weak antitumor activity.

It is noted that the cytotoxicity of compounds with the bridging dicarboxylates increased with the linear carbon chain length of dicarboxylate (M3 > M2 > M1), however, compound M4 with 1,1-cyclobutyldicarboxylate had the weakest activity among these complexes. We suppose that the balance between lipotropy and hydrophily of the platinum-based compounds is closely relative to their antitumor activity. In fact, aqueous solubility of compounds M3 and M5 are in the middle of these five compounds (aqueous solubility order: M1 > M2 > M5 > M3 > M4). The hydrophily of compound M4 has been remarkably reduced with the introduction of cyclobutyl moiety.

Moreover, we have ever prepared a number of mononuclear Platinum compounds of the ligand (HL) with several monodentate carboxylates as leaving group and made in vitro biological tests,



Scheme 1. Reagents: (a) ethyl 2-chloroacetate, K_2CO_3, acetonitrile; (b) HCl/EtOAc; (c) NaOH, EtOH/H_2O; (d) HCl/H_2O.

Table 1
In vitro cytotoxicity of complexes M1 to M5

Complex	IC_{50}[a] (μM)		
	HCT-116[b]	MCF-7[c]	HepG-2[d]
M1	17.2	23.5	37.5
M2	13.1	16.8	31.2
M3	5.8	9.9	8.2
M4	27.5	31	>50
M5	5.1	7.1	6.8
Carboplatin	Not tested	15.3	9.7
Oxaliplatin	4.3	Not tested	Not tested

[a] All IC_{50} values (drug concentration giving 50% survival) calculated based on the Pt content are means ± SD (SD <9% of the mean value) from at least three separated experiments.
[b] Human colorectal cancer cell.
[c] Human breast cancer cell.
[d] Human hepatoma cell.

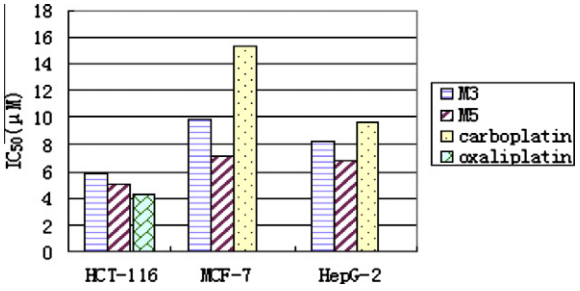


Figure 2. Cytotoxicity of compounds M3 and M5 with carboplatin or oxaliplatin as positive controls.

but the IC_{50} values of those mononuclear compounds against the same tumor cells were disappointing.

In conclusion, a new N-monosubstituted chiral DACH ligand, 2-(((1*R*,2*R*)-2-aminocyclohexyl)amino)acetic acid, has been designed and synthesized to prepare a series of novel dinuclear platinum(II) complexes which own dicarboxylates/sulfate as bridges, and that differ from the previously reported multinuclear platinum complexes which have employed various diamines as bridges. In vitro cytotoxicity tests indicated that all resulting dinuclear platinum(II) complexes exhibited antitumor activity to HCT-116 and MCF-7 cell lines. Compounds M3 and M5 not only showed very close activity to oxaliplatin against HCT-116 but also showed better cytotoxicity than carboplatin against MCF-7 and HepG-2 cell lines. Furthermore, all compounds showed much better aqueous solubility than oxaliplatin. Consequently, compounds M3 and M5 may be deserved for further investigation as leading compounds.

Acknowledgments

We are grateful to the National Natural Science Foundation of China (Project No.20971022) and the Six Top Talents Funding of Jiangsu Province (Project No. 2008046)) for the financial aids to this work.

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15. Synthesis of [PtLI]: To a stirring aqueous solution of KI (80 mmol), K_2PtCl_4 (12 mmol) in water (40 ml) was added. The blending solution was stirred at 25 °C for 30 min under a nitrogen atmosphere to get a black solution of K_2PtI_4. Then an aqueous solution (40 ml) of HL (12 mmol) and NaOH (12 mmol) was added dropwise under stirring in the dark at 25 °C. After 24 h, the dark yellow precipitate was filtered, washed sequentially with water, ethanol and ether, and then dried in vacuum.
Data for [PtLI]: Yield: 60%, dark yellow solid. IR (ν, cm^{-1}): 3546s (br), 3176s, 2934m, 2858m, 1642vs, 1586m, 1445m, 1334s, 1291m, 1076w, 1010m, 911w, 648w, 459m; ^1H NMR (D_2O/TMS): δ 1.10–1.50 (m, 6H, 3CH_2 of DACH), 1.80–2.07 (m, 2H, CH_2 of DACH), 2.30–2.81 (m, 2H, 2*CH* of DACH), 3.35–3.85 (m, 2H, NHCH_2COO^-). ESI-MS: *m*/*z* $[M+Na]^+$ = 516 (25%), $[M+K]^+$ = 532 (60%). Anal. ($C_8H_{15}IN_2O_2Pt$) C, H, N.
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17. General preparation of the complexes: M1, M2, M3, M4 and M5: A suspension of the corresponding silver dicarboxylate or Ag_2SO_4 (5 mmol) and [PtLI] (10 mmol) in 100 ml water was stirred at 60 °C under a nitrogen atmosphere in the dark for 24 h, the resulting yellow deposit was filtered off and washed with water for two times. The filtrate was then evaporated to nearly dryness and light yellow solids precipitated, which were washed with small icy water for two times, and dried in vacuum.
Data for M1: Yield: 29%, pale yellow solid. Formula: $C_{18}H_{30}N_4O_8Pt_2$, Mol.Wt: 820. C 26.41(26.34), H 3.81(3.66), N 6.73(6.83), Pt 47.38(47.56). IR (ν, cm^{-1}): 3421s(br), 3179s, 2935s, 2860s, 1634vs, 1448m, 1392s, 1309s, 1073w, 1013w, 916w, 809m, 777s, 649m, 562m, 518m, 460m. ^1H NMR(D_2O/TMS): δ 1.09–1.42(m, 8H, 4CH_2 of 2DACH), 1.59–1.60 (m, 4H, 2CH_2 of 2DACH), 2.02–2.83 (m, 8H, 2CH_2 of 2DACH and 4*CH* of 2DACH), 3.37–3.82 (m, 4H, 2NHCH_2COO^-). ESI-MS *m*/*z*: $[M+H]^+$ = 821 (32%).
Data for M2 Yield: 22%, pale yellow solid. Formula: $C_{19}H_{32}N_4O_8Pt_2$, Mol.Wt: 834. C 27.40(27.34), H 3.89(3.84), N 6.66(6.71), Pt 46.62(46.76). IR (ν, cm^{-1}): 3410s(br), 3183s, 2936s, 2861m, 1591vs, 1352vs, 1254m, 1175m, 1073m, 1017m, 928m, 701s, 594s(br). ^1H NMR (D_2O/TMS): δ 1.09–1.61 (m, 12H, 6CH_2 of 2DACH), 2.02–2.78 (m, 8H, 2CH_2 of 2DACH and 4*CH* of 2DACH), 3.21–3.96 (m, 6H, 2NHCH_2COO^- and $CH_2(COO^-)_2$). ESI-MS *m*/*z*: $[M+H]^+$ = 835 (25%).
Data for M3 Yield: 31%, pale yellow solid. Formula: $C_{20}H_{34}N_4O_8Pt_2$, Mol.Wt: 848. C 28.40(28.30), H 3.96(4.01), N 6.68(6.60), Pt 45.81(45.99). IR (ν, cm^{-1}): 3404s(br), 3197s, 2934s, 2859m, 1632vs, 1570vs, 1394vs, 1290s, 1173m, 1074w, 1011w, 878w, 650m(br), 458m(br). ^1H NMR (D_2O/TMS): δ 1.09–1.36 (m, 8H, 4CH_2 of 2DACH), 1.55–1.57 (m, 4H, 2CH_2 of 2DACH), 1.75–2.69 (m, 12H, 2CH_2 of 2DACH, 4*CH* of 2DACH, 4H of $(CH_2COO^-)_2$), 3.09–3.67 (m, 4H, 2NHCH_2COO^-). ESI-MS *m*/*z*: $[M+H]^+$ = 849 (30%), $[M+Na]^+$ = 871 (70%).
Data for M4 Yield: 36%, pale yellow solid. Formula: $C_{22}H_{36}N_4O_8Pt_2$, Mol.Wt: 874. C 30.08(30.20), H 4.18(4.12), N 6.52(6.41), Pt 44.48(44.62). IR (ν, cm^{-1}): 3419vs(br), 3198vs, 2941s, 2863s, 1584vs, 1337vs, 1251m, 1158m, 1119m, 1021w, 911m, 888m, 762m, 707m, 615m, 571m. ^1H NMR (D_2O/TMS): δ 1.16–1.93 (m, 14H, 6CH_2 of 2DACH and CH_2 of cyclobutyl group), 2.03–2.95 (m, 12H, 2CH_2 of 2DACH, 4*CH* of 2DACH and 2CH_2 of cyclobutyl group), 3.21–3.77 (m, 4H, 2NHCH_2COO^-). ESI-MS *m*/*z*: $[M+H]^+$ = 875 (25%), $[M+Na]^+$ = 897 (30%).
Data for M5 Yield: 28%, pale yellow solid. Formula: $C_{16}H_{30}N_4O_8SPt_2$, Mol.Wt: 828. C 23.11(23.19), H 3.66(3.62), N 6.63(6.76), Pt 47.01(47.10). IR (ν, cm^{-1}): 3433m(br), 3184m, 2935m, 2853m, 1646s, 1455m, 1394m, 1338m, 1293m, 1184s, 1110vs, 1012m, 990m, 927w, 619vs. ^1H NMR (D_2O/TMS): δ 1.09–1.37 (m, 8H, 4CH_2 of 2DACH), 1.53–1.76 (m, 4H, 2CH_2 of 2DACH), 1.96–2.08 (m, 4H, 2CH_2 of 2DACH), 2.44–2.70 (m, 4H, 4*CH* of 2DACH), 3.48–3.75 (m, 4H, 2NHCH_2COO^-). ESI-MS *m*/*z*: $[M+H]^+$ = 829 (30%).
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