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Synthesis and Cytotoxic Evaluation of Novel Platinum(II) Complexes with C_2-Asymmetric and C_2-Symmetric Chiral Vicinal Diamines†

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A series of new platinum(II) complexes with C_2-asymmetric and C_2-symmetric 1,2-diamines were designed and synthesized by convenient methods, involving samarium diiodide induced reductive coupling as the key step. The results of cytotoxicity showed that compounds (*R*,*R*)-**11a** and (*S*,*S*)-**11a**, two novel platinum(II) complexes with asymmetric 1,2-diamines, exhibited more potent cytotoxicity than that of oxaliplatin against all leukemia cell lines. Interestingly, (*R*,*R*)-**11a** and (*S*,*S*)-**11a** demonstrated less potent activity against three solid cancer cell lines than that of oxaliplatin, which indicated that these two compounds may only selectively inhibit the leukemia cell lines. In contrast, (*R*,*R*)-**15a** and (*S*,*S*)-**15a**, two platinum(II) complexes with symmetric 1,2-diamines, showed similar cytotoxicity to that of oxaliplatin against all leukemia cell lines and more potent activity against solid cancer cell lines. Further flow cytometry data indicated that (*R*,*R*)-**11a** could obviously arrest leukemia K562 cells in G2/M phases.

Keywords platinum(II) complexes, chiral vicinal diamines, cytotoxicity, leukemia cell

Introduction

Cisplatin (Figure 1) is a widely used chemo-therapeutic agent for the treatment of testicular cancer, and is also used in combination regimens for a variety of other tumors, including ovarian, cervical, bladder, lung, and those of the head and neck.[1-3] However, the significant side effects, such as cumulative toxicities of nephrotoxicity, ototoxicity, and peripheral neuropathy, restrict the clinical success of cisplatin.[4,5] Moreover, the therapeutic efficacy of cisplatin is limited by inherent or treatment-induced resistant tumor cell sub-populations. Therefore, much more attention has been focused on designing new platinum compounds with improved pharmacological properties and a broader range of antitumor activity. Among the over 30 platinum agents, which have entered clinical trials, only carboplatin and oxaliplatin have received worldwide approval so far. Others, such as nedaplatin, lobaplatin and heptaplatin, only have gained regionally limited approval. Although several new candidates were abandoned during the clinical trials due to the low efficacy, high toxicity and no advantage over cisplatin and carboplatin, the sterically hindered complex *cis*-aminedichloro (2-methylpyridine)-platinum(II) (ZD0473)[6,7] and multinuclear complexes BBR-3464[8] have been still continued for evaluation. To our best knowledge, chiral ligands are important for the cytotoxicity of oxaliplatin.[9] Further, Noji reported that *trans* isomers of 1,2-cyclohexanediamine platinum(II) complexes were more efficacious than corresponding *cis* isomers against leukemia P388.[10] Gust also reported the synthesis of platinum(II) complexes with chiral diamines and their cytotoxicities against breast cancer.[11] During the course of our research, we have developed an efficient method for the preparation of optically pure C_2-asymmetrical and C_2-symmetrical vicinal diamines by samarium diiodide (SmI_2) induced reductive coupling for the synthesis of

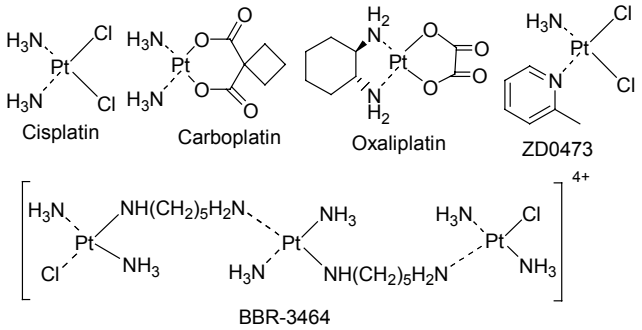

Figure 1 Reported platinum complexes.

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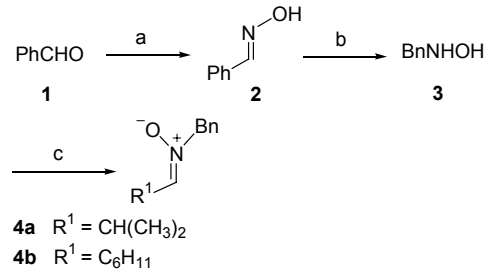
platinum(II) complexes.[12] The advantages of this method could provide chiral 1,2-diamines with high *ee* values and high yields. With these amines in hand, we prepared two types of new platinum(II) complexes with C_2-asymmetric and C_2-symmetric 1,2-diamines. Herein, we report the synthesis and cytotoxic evaluation of these analogs against four leukemia cell lines and three solid cancer cell lines. The preliminary study of structure-activity relationship was also discussed.

Results and Discussion

The important intermediates nitrones (**4a** and **4b**) were synthesized by using benzaldehyde (**1**) as the starting material, as illustrated in Scheme 1. Benzaldehyde (**1**) was first transformed into oxime (**2**) by using hydroxylamine hydrochloride. The obtained oxime (**2**) was then reduced by adding sodium cyanoborohydride and stirred at room temperature overnight to afford *N*-benzyl hydroxylamine (**3**) in 80% yield. Compound **3** was reacted with aldehydes to obtain their corresponding nitrones (**4a** and **4b**) in 85% and 90% yields, respectively.

Scheme 1 Synthesis of compounds **4a**—**4b**

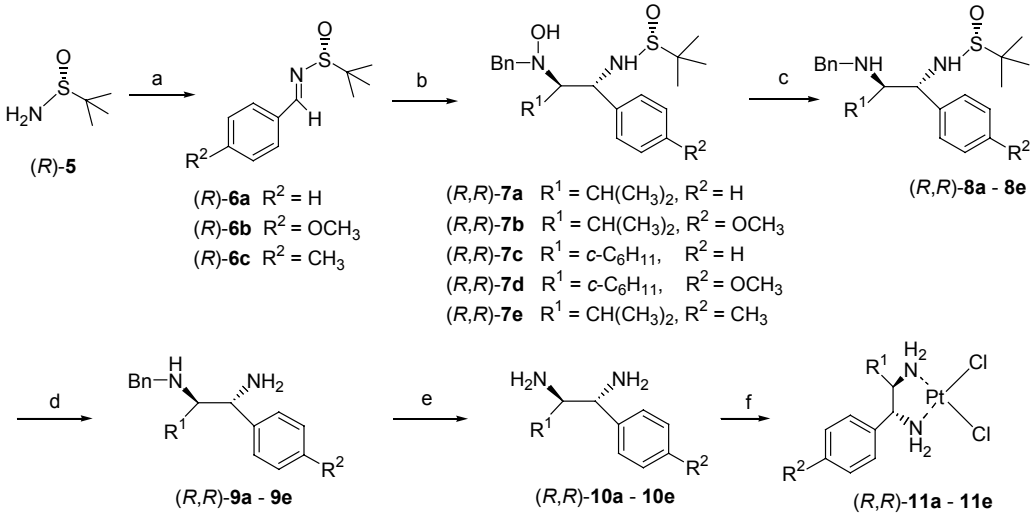


Reagents and conditions: (a) NH_2OH•HCl, r.t., 30 min; (b) $NaBH_3CN$, r.t., overnight; (c) R^1CHO, $MgSO_4$, r.t., 5 h

With **4a** and **4b** in hand, the synthesis of desired targets (*R*,*R*)-**11a**—**11e** was accomplished by the route shown in Scheme **2**.[12] (*R*)-(+)-2-Methyl-2-propanesulfinamide **5** reacted with aromatic aldehydes to obtain (*R*)-*N*-*tert*-butylsulfinyl imine **6a**—**6c** in 60%—70% yields. After applying the SmI_2 induced reductive coupling reaction, a series of optically pure C_2-asymmetrical vicinal diamine precursors (*R*,*R*)-**7a**—**7e** were efficiently obtained by reacting nitrone with (*R*)-*N*-*tert*-butanesulfinyl imine in THF at −78 ℃ in the presence of SmI_2 and *tert*-butyl alcohol. After removing the hydroxy group on nitrogen in (*R*,*R*)-**7a**—**7e** with acetic acid, $Cu(OAc)_2$ and zinc powder at room temperature, the corresponding intermediates (*R*,*R*)-**8a**—**8e** were achieved in 80%—85% yields. The sulfinyl groups in (*R*,*R*)-**8a**—**8e** were further removed by using 4 mol/L HCl in 1,4-dioxane at room temperature in the presence of MeOH. Then, optically pure asymmetric (*R*,*R*)-1,2-diamines **10a**—**10e** were prepared by catalytic debenzylation with 10% $Pd(OH)_2$ under hydrogen. Finally, the corresponding (*R*,*R*)-1,2-diamine platinum(II) complexes **11a**—**11e** were obtained by reacting **10a**—**10e** with potassium tetrachloroplatinate in 65%—75% yields. By adopting the similar methods for (*R*,*R*)-**11a**—**11e**, the synthesis of (*S*,*S*)-**11a**—**11e** was accomplished from (*S*)-(−)-2-methyl-2-propanesulfinamide **5** in six steps.

The reductive homocouplings of (*R*)-*N*-*tert*-butylsulfinyl imine **12a**—**12c** were successfully proceeded in the presence of SmI_2 and HMPA in THF at −78 ℃ to produce (*S*,*S*)-**13a**—**13c**.[13] The sulfinyl groups in (*S*,*S*)-**13a**—**13c** were further removed by using 4 mol/L HCl in 1,4-dioxane at room temperature in the presence of MeOH to yield C_2-symmetric (*S*,*S*)-1,2-diamines **14a**—**14c**. With **14a**—**14c** in hand, (*S*,*S*)-1,2-diamine platinum(II) complexes **15a**—**15c** were synthesized simply by reacting with potassium tetrachloroplatinate at room

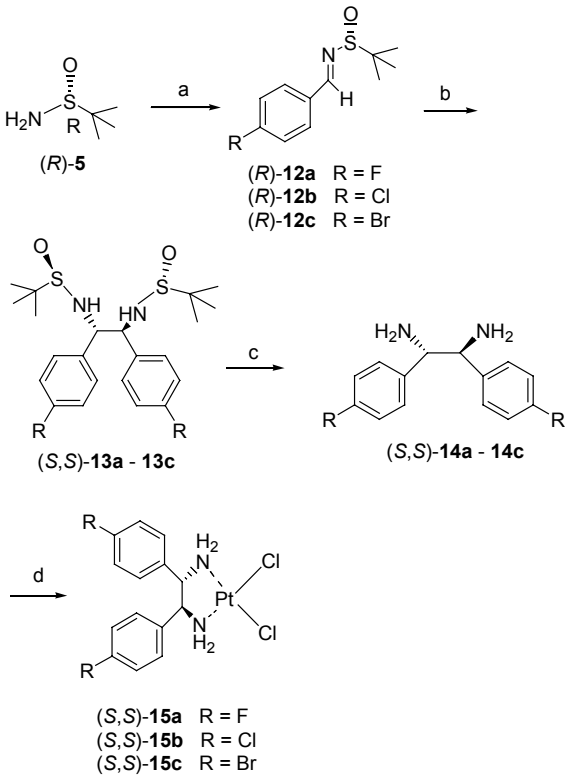
Scheme 2 Synthesis of the title compounds (*R*,*R*)-**11a**—**11e**



Reagents and conditions: (a) $KHSO_4$, R_2PhCHO, toluene, 45 ℃; (b) **4a/4b**, 2 equiv. SmI_2, *t*-BuOH, THF, -78 ℃; (c) Zn, $Cu(OAc)_2$, AcOH, EDTA-2Na, r.t.; (d) HCl, MeOH, r.t.; (e) H_2, $Pd(OH)_2$/C, r.t.; (f) K_2PtCl_4, r.t., overnight

temperature overnight. Compounds (*R*,*R*)-**15a** − **15c** were also synthesized from (*S*)-*N*-*tert*-butylsulfinyl imine **12a**−**12c** in a same manner as that for (*S*,*S*)-**15a** −**15c** (Scheme 3).

Scheme 3 Synthesis of the title compounds (*S*,*S*)-**15a**−**15c**



Reagents and conditions: (a) $KHSO_4$, RPhCHO, toluene, 45 ℃; (b) SmI_2, HMPA, THF, –78 ℃; (c) HCl, MeOH, r.t.; (d) K_2PtCl_4, r.t., overnight

The *in vitro* cytotoxicities of platinum(II) complexes were evaluated against four leukemia cell lines (THP-1, K562, KASUMI-1 and NB4) and three solid cancer cell lines (MDA-MB-231, OMC685, A-549) by MTT or SRB assay (Table 1).

For the first series of analogs [(*R*,*R*)-**11a**−**11e** and (*S*,*S*)-**11a** − **11e**], (*R*,*R*)-**11a** and (*S*,*S*)-**11a** exhibited more potent activity than that of oxaliplatin against all leukemia cell lines. Interestingly, when the isopropyl was substituted by cyclohexyl group, analogs, such as (*R*,*R*)-**11c** and (*S*,*S*)-**11c**, abolished their potencies, which indicated that increased steric bulky groups could be unfavorable to their cytotoxicity. Furthermore, when the *p*–position of phenyl group was substituted by electron-donating group, *i.e.* methoxyl, (*R*,*R*)-**11b** lost its cytotoxicity against K562 and NB4 cell lines, though still maintained moderate activity against THP-1 and KASUMI-1 cell lines. However, for its (*SS*) analog, (*S*,*S*)-**11b** only slightly decreased its potency. For three solid cancer cell lines, all novel platinum(II) complexes with C_2-asymmetric 1,2-diamines exhibited weaker activity than that of oxaliplatin, indicating that this type of compounds may only inhibit leukemia cell lines selectively, and not inhibit solid cancer cell lines. It was found that the cytotoxicities of (*S*,*S*)-**11a**, **11b**, **11e** against K562 and NB4 were more active than those of (*R*,*R*)-**11a**, **11b**, **11e**.

For the second series of novel platinum(II) complexes with C_2-symmetric 1,2-diamines, (*R*,*R*)-**15a** and (*S*,*S*)-**15a** showed slightly decreased activity compared with that of oxaliplatin against all leukemia cell lines, while both (*R*,*R*)-**15b** and (*S*,*S*)-**15c** diminished their cytotoxicity against all leukemia cell lines. In contrast to platinum(II) complexes with C_2-asymmetric 1,2-di-

Table 1 Cytotoxicities (IC_{50}, μmol•L^{-1}) of platinum(II) complexes with chiral 1,2-diamines

Compound	THP-1	K562	KASUMI-1	NB4	MDA-MB-231	OMC685	A-549
(*R*,*R*)-**11a**	0.23	0.99	0.27	1.15	168.55	88.73	237.92
(*S*,*S*)-**11a**	0.34	0.32	0.41	0.24	354.39	107.44	358.89
(*R*,*R*)-**11b**	2.87	＞10	5.28	＞10	182.11	278.44	167.35
(*S*,*S*)-**11b**	1.70	2.46	9.09	3.88	384.98	207.22	249.39
(*R*,*R*)-**11c**	＞10	＞10	＞10	5.22	126.61	114.35	＞600
(*S*,*S*)-**11c**	＞10	＞10	＞10	＞10	427.28	＞600	526.33
(*R*,*R*)-**11d**	＞10	＞10	＞10	＞10	149.66	234.82	166.83
(*S*,*S*)-**11d**	14.88	＞10	6.00	11.32	178.46	107.75	172.68
(*R*,*R*)-**11e**	2.84	＞10	3.45	＞10	98.02	86.21	284.85
(*S*,*S*)-**11e**	0.63	1.63	1.03	1.58	332.22	169.15	281.51
(*R*,*R*)-**15a**	3.21	2.17	0.61	2.99	47.66	95.09	75.43
(*S*,*S*)-**15a**	1.27	1.58	1.21	1.59	23.53	100.94	85.03
(*R*,*R*)-**15b**	＞10	＞10	＞10	＞10	52.16	157.58	68.70
(*S*,*S*)-**15b**	4.79	＞10	1.42	＞10	110.52	87.09	77.03
(*R*,*R*)-**15c**	1.08	＞10	＞10	3.51	56.83	106.09	50.91
(*S*,*S*)-**15c**	＞10	＞10	＞10	＞10	116.54	153.11	95.15
Oxaliplatin	0.49	1.46	0.53	1.81	61.31	59.20	76.67

amines, (*R*,*R*)-**15a**—**15c** and (*S*,*S*)-**15a** showed more potent activity than that of oxaliplatin against MDA-MB-231, and (*R*,*R*)-**15a**—**15c** showed more potent activity than that of oxaliplatin against A549. All these platinum(II) complexes with C_2-symmetric 1,2-diamines exhibited weaker activity against OMC685 than that of oxaliplatin. On the four leukemia and three solid cancer cell lines, platinum(II) complexes with C_2-symmetric 1,2-diamines showed no enantioselectivity, consistent with the result of Gust.[11]

The ability of compound (*R*,*R*)-**11a** to inhibit the cell cycle was also investigated. After administration of various concentrations of (*R*,*R*)-**11a** for 48 h, K562 cell cycle was measured by flow cytometry. The results of (*R*,*R*)-**11a** on K562 cell cycle progression were shown in Figure 2. As compared to the control, G2/M phases of K562 cells administrated with (*R*,*R*)-**11a** (0, 1, 2 and 4 μmol•L^{-1}) for 48 h were increased from (23.57±2.6)% to (34.92±8.2)%, whilst S phase cells were decreased from (51.75±5.4)% to (35.48±0.28)%. Almost all cells treated with 4 μmol•L^{-1} of (*R*,*R*)-**11a** for 48 h died. The results showed that (*R*,*R*)-**11a** could arrest K562 cells in G2/M phases ($p<0.05$).

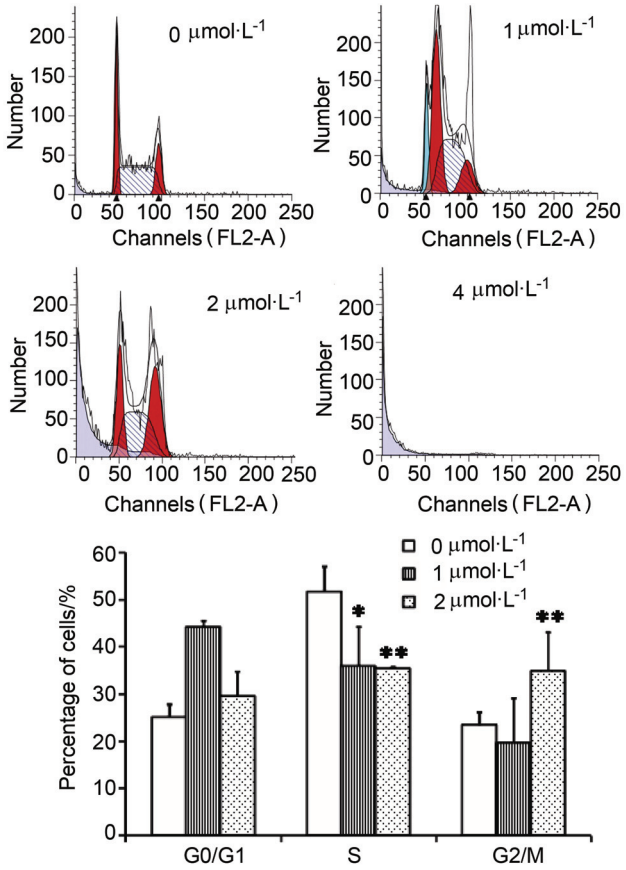


Figure 2 G2/M cell cycle arrest in K562 cells by (*R*,*R*)-**11a**. K562 cells were seeded into 6-well plates at a density of 1×10^4 cells per well. Then, the cells were treated with (*R*,*R*)-**11a** (0, 1, 2 and 4 μmol•L^{-1}) for 48 h and analyzed by flow cytometry. Quantitative analysis of the apoptotic nuclei was shown in the follows. Data are expressed as means±SD, *$p<0.05$ vs. control group, **$p<0.01$ vs. control group.

Flow cytometry was used to quantitatively detect the apoptotic rate. Cells (5×10^3/well) were seeded into 6-well plates and exposed to (*R*,*R*)-**11a** at various concentrations (0, 1, 2 and 4 μmol•L^{-1}) for 48 h, and then harvested and washed with phosphate buffered saline (PBS). Staining went along with 195 μL bonding buffer containing 5 μL Annexin V-FITC in the dark at room temperature for 10 min, and then added 10 μL PI in the dark at 4 ℃ for 10 min. The apoptotic cells were analyzed with FACScan flow cytometry (BD FACSCalibur) (Figure 3).

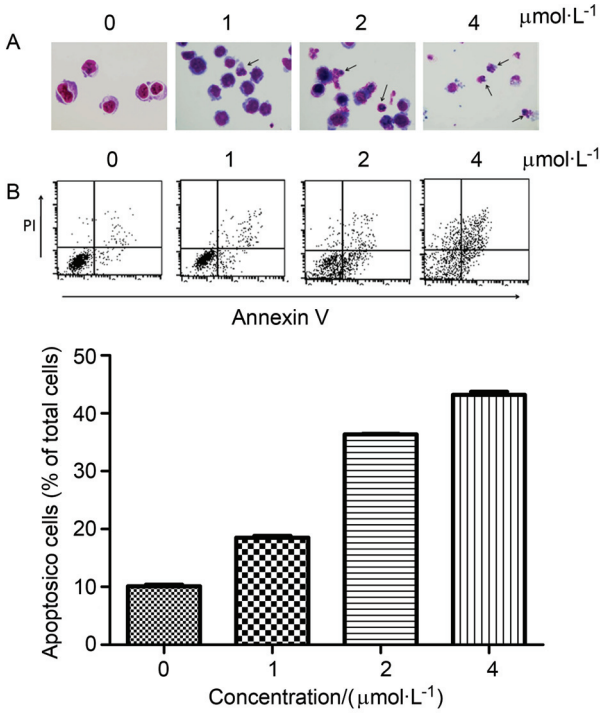


Figure 3 (*R*,*R*)-**11a** induced the apoptosis of K562 cells. Apoptotic cells were analyzed by flow cytometry with Annexin V-FITC and PI staining. Early apoptotic cells (Annexin-V+) were displayed in the lower right quadrant and late apoptotic cells (Annexin-V+and PI+) were shown in the upper right quadrant. Necrosis cells were shown in the upper left quadrant (PI+). The percentages of apoptotic cells were indicated by Annexin-V+ cells shown as the mean±SD of three independent experiments. *$p<0.05$ vs. control group, **$p<0.01$ vs. control group.

Conclusions

Ten platinum(II) complexes with C_2-asymmetric diamines **11a**—**11e** (*RR* or *SS*) and six platinum(II) complexes with C_2-symmetric diamines **15a**—**15c** (*RR* or *SS*) were synthesized by convenient methods, involving samarium diiodide induced reductive coupling as the key step. The results of cytotoxicity showed that (*R*,*R*)-**11a** and (*S*,*S*)-**11a** exhibited more potent activity against all leukemia cell lines and less potent activity against three solid cancer cell lines than that of oxaliplatin. This indicated that (*R*,*R*)-**11a** and (*S*,*S*)-**11a** may selectively inhibit the leukemia cell lines. Further

mechanism study showed that (*R*,*R*)-**11a** increased the apoptotic K562 cells in a concentration-dependent manner and arrested K562 cells in G2/M phases in the flow cytometry assay.

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