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Synthesis and in vitro antitumor activity of novel iridium(III) complexes with enantiopure C_2-symmetrical vicinal diamine ligands

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ABSTRACT

Four novel iridium(III) complexes with enantiopure C_2-symmetrical vicinal diamine ligands were designed, synthesized, and characterized by FT-IR, NMR, and MS. The cytotoxicities of all of the complexes against the human solid tumor cell lines A2780, A549, KB, and MDA-MB-231 were evaluated. Both *R,R*-configured complexes (*R,R*)-**5a** and (*R,R*)-**5b** exhibited more potent or similar activity compared with oxaliplatin, whereas their corresponding (*S,S*)-isomers (*S,S*)-**5a** and (*S,S*)-**5b** were found to be mostly inactive. As indicated by the activation of caspase-3, the cleavage of PARP, and the upregulation of p53, the preliminary mechanism studies revealed that the mode of cell death initiated by (*R,R*)-**5a** in A2780 cells was predominantly p53-mediated apoptosis. In addition, the structure of (*R,R*)-**5a** was unambiguously confirmed through single crystal X-ray structure determination.

Metal-based anticancer drugs are administered in more than 50% of the treatment regimes used for patients suffering from cancer.[1] However, the number of these metal compounds is extremely limited and concerns platinum compounds exclusively. The only three Pt(II) compounds approved worldwide, that is, cisplatin, carboplatin, and oxaliplatin, are currently used singly or in combination with other anticancer drugs to treat testicular, ovarian, bladder, and lung cancers, as well as melanoma, lymphomas, myelomas, and colorectal cancers.[2] However, the clinical success of these drugs is compromised by severe side effects (e.g., nephrotoxicity, ototoxicity, and neurotoxicity) and spontaneous or acquired resistance.[3,4] To overcome these limitations of platinum drugs, many studies have attempted to develop other metal compounds, including ruthenium, osmium, gold, gallium, iron, and cobalt complexes.[5] A large number of these non-platinum metal compounds have shown some type of anticancer activity, although most have only exhibited in vitro antiproliferative activity against some cancer cell lines. The most promising candidates, namely the Ru(III) compounds NAMI-A and KP1019 and the Ga(III) compounds KP46 and gallium maltolate, are at best in clinical phase II trials.[6] In contrast, iridium(III) complexes have not been widely investigated in the search for novel metal-based anticancer drugs largely due to their chemical inertness.[7] In earlier studies, the iridium(III) complexes [ImH][*trans*-$IrCl_4(Im)_2$] (Im = imidazole), [ImH][*trans*-$IrCl_4$(Im)(DMSO)] and [$(DMSO)_2$H][*trans*-$IrCl_4(DMSO)_2$] (DMSO = dimethylsulfoxide) were all found to be biologically inactive. These findings are consistent with the general assumption that the extreme inertness of these iridium(III) complexes is correlated with an absence of cytotoxic effects on human tumor cell lines.[8–10] However, recent studies have demonstrated that some trichloridoiridium(III) polypyridyl complexes[11–13] and organometallic iridium(III) complexes containing negatively charged pentamethylcyclopentadienyl ligand[14–22] have shown promising anticancer activity. Additionally, a review has summarized recent work using iridium(III) complexes as anticancer drugs, focusing on the cytotoxic activity, cellular uptake efficiency, and mechanisms of these complexes.[23]

Although the mechanism of established platinum drugs is not fully understood, activation by hydrolysis and formation of intrastrand DNA cross-links appear to be crucial to their cytotoxicity. As the ligand in the oxaliplatin complex, it was believed that (1*R*,2*R*)-cyclohexane-1,2-diamine (*R,R*-DACH) plays an important role in the success of oxaliplatin against cisplatin resistance.[2] Thus, as a variation of this ligand, we have introduced a bulky hydrophobic chiral diamine to the metal as a bidentate ligand to prepare a series of new platinum(II) complexes.[24] Preliminary biological results have indicated that, similarly to oxaliplatin, several platinum(II) complexes with *R,R*- and *S,S*-configured C_2-symmetric vicinal diamine ligands exhibit potent cytotoxicity against some human cancer cell lines. Furthermore, bulk of investigative results have demonstrated that the stereochemistry of the diamine ligands of platinum compounds with chiral C-substituted ethylenediamines had an important influence on their interactions of DNA, anticancer activity, toxicity, and resistance.[25,26] Particularly, it has been reported that optically pure (1,2-diarylethane-1,2-diamine)dichloroplatinum(II) complexes induce significant inhibitory effects on the growth of leukemic and tumor cells, and a

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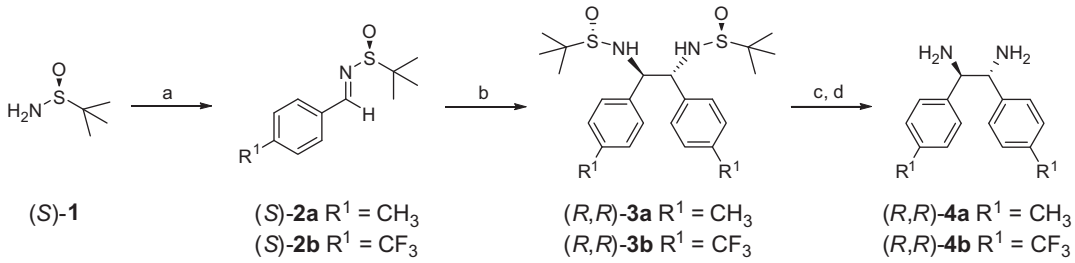
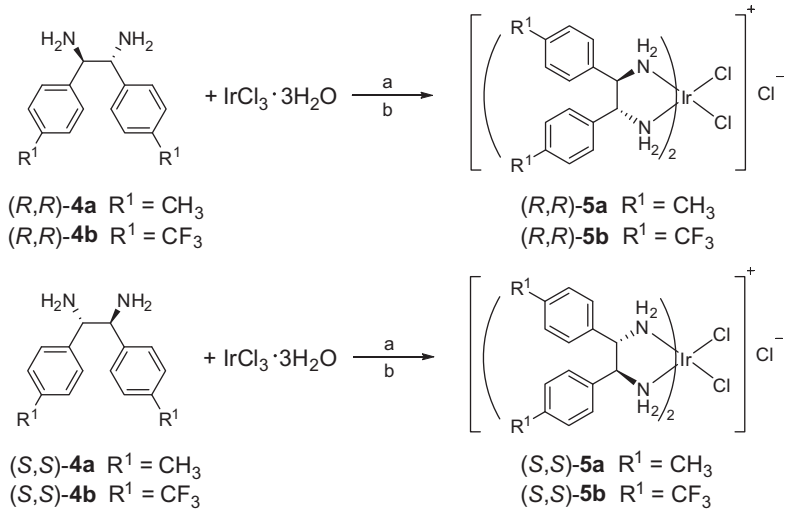


Scheme 1. Exemplified synthesis of (1*R*,2*R*)-configured 1,2-diarylethane-1,2-diamines. The following reagents and conditions were used: (a) corresponding R^1-ring substituted benzaldehyde (1.0 equiv), $Ti(OEt)_4$ (1.7 equiv), CH_2Cl_2, room temperature; (b) SmI_2 (2.5 equiv), HMPA (2.0 equiv), THF, −78 °C; (c) HCl, MeOH, room temperature; (d) KOH, H_2O, room temperature.



Scheme 2. Synthesis of dichlorobis[enantiopure 1,2-diarylethane-1,2-diamine]iridium(III) chlorides. The following reagents and conditions were used: (a) diamines (2.5 equiv), $IrCl_3 \cdot 3H_2O$ (1.0 equiv), CH_3COOH (1.0 equiv), 2-ethoxyethanol/H_2O (1:3, v/v), refluxing; (b) neat, 170 °C.

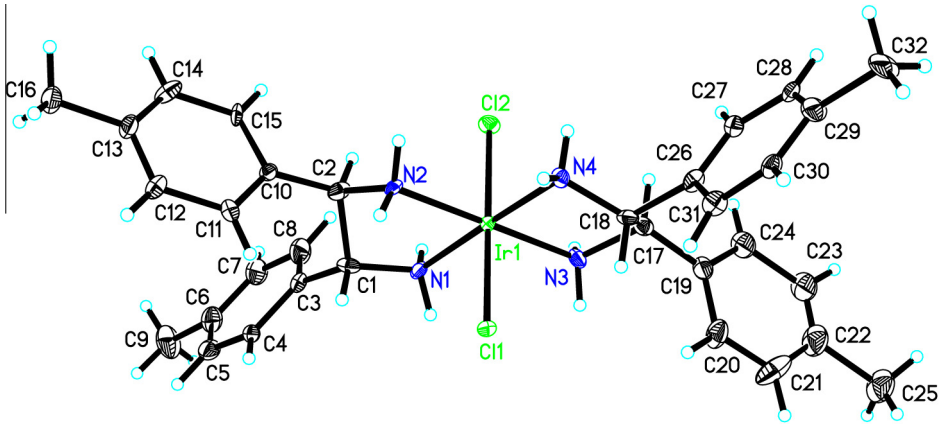


Figure 1. X-ray crystal structure of iridium(III) complex (*R*,*R*)-**5a**. The counterion has been omitted. Selected bond lengths [Å] and angles [deg] are the following: Ir1–Cl1 2.3367(15), Ir1–Cl2 2.3529(15), Ir1–N1 2.073(5), Ir1–N2 2.090(5), Ir1–N3 2.085(6), Ir1–N4 2.068(5), N4–Ir1–N1 177.7(2), N4–Ir1–N3 81.6(2), N1–Ir1–N3 98.6(2), N4–Ir1–N2 99.5(2), N1–Ir1–N2 80.3(2), N3–Ir1–N2 178.8(2), N4–Ir1–Cl1 90.50(16), N1–Ir1–Cl1 87.18(16), N3–Ir1–Cl1 87.92(16), N2–Ir1–Cl1 92.60(15), N4–Ir1–Cl2 90.01(15), N1–Ir1–Cl2 92.32(16), N3–Ir1–Cl2 91.46(16), N2–Ir1–Cl2 88.01(16), and Cl1–Ir–Cl2 179.13(7).

distinctly better effect was generally observed with the *R*,*R*/*S*,*S* configurations compared with the *R*,*S* counterparts.[27–30] These results motivated us to apply the findings from platinum(II) complexes to iridium(III) complexes. In this manuscript, we report the synthesis of novel dichlorobis[enantiopure 1,2-diarylethane-1,2-diamine]iridium(III) chlorides (general formula see Graphic Abstract) and their antiproliferative effects on a panel of representative human solid tumor cell lines. Cell apoptosis studies were also preformed in an attempt to investigate the mechanism of cell death elicited by the representative compound. This study appears to be the first time that these enantiopure C_2-symmetrical vicinal diamines are used as ligands in iridium(III) complexes.

Table 1
In vitro cytotoxicity of complexes **5a** and **5b**.

Compound	R^1	IC_{50}[a] (μM)			
		A2780	A549	KB	MDA-MB-231
(1*R*,2*R*)-**5a**	$R^1 = CH_3$	2.40 ± 0.60	7.57 ± 0.57	3.64 ± 2.43	8.99 ± 2.04
(1*R*,2*R*)-**5b**	$R^1 = CF_3$	3.61 ± 0.46	6.90 ± 1.14	10.97 ± 2.56	19.52 ± 5.37
(1*S*,2*S*)-**5a**	$R^1 = CH_3$	>50	40.84	>50	>50
(1*S*,2*S*)-**5b**	$R^1 = CF_3$	>50	>50	>50	>50
Oxaliplatin		3.85 ± 0.46	2.64 ± 1.82	5.80 ± 2.59	34.11 ± 5.67

[a] Inhibitory concentration (IC_{50}, μM) obtained from the MTT assay. The experiments were performed in triplicate, and the results represent the mean ± SD of three independent experiments. The cells were incubated for a period of 72 h.

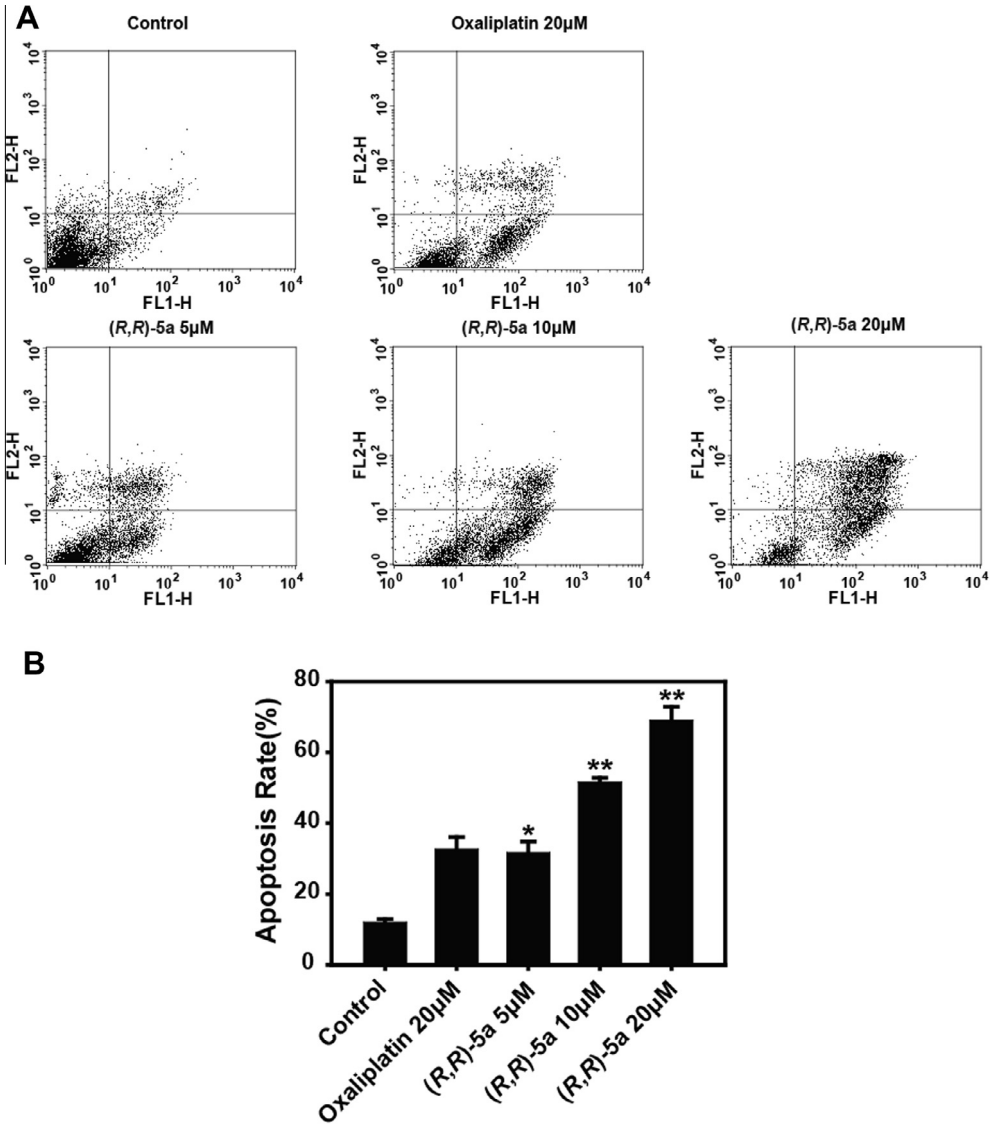


Figure 2. (A) Flow cytometric analysis of the distribution of A2780 cells after 72 h of exposure to various concentrations of (*R*,*R*)-**5a** (5, 10 and 20 μM) or 20 μM oxaliplatin. The cells were analyzed through Annexin V/PI staining. The four quadrants represent four different cell states: viable cells (lower left, Annexin-V- and PI−), early apoptotic cells (lower right, Annexin-V+ and PI−), late apoptotic cells (upper right, Annexin-V + and PI+), and necrotic cells (upper left, Annexin-V− and PI+). (B) The apoptosis rates of A2780 cells induced by complexes (*R*,*R*)-**5a** and oxaliplatin. The data represent the mean ± SD of three independent experiments.*P <0.05 compared with the control group, **P <0.01 compared with the control group.

Enantiomerically pure isomers of the 1,2-diarylethane-1,2-diamines **4** were prepared from the commercially available (*R*)- or (*S*)-2-methyl-2-propanesulfinamide (**1**) using a method reported by Zhong et al.[31] with one modification. As shown in Scheme 1, the exemplified syntheses of (*R*,*R*)-**4a** and (*R*,*R*)-**4b** are described here. Compound (*S*)-**1** was reacted with the corresponding R^1-ring substituted benzaldehydes at room temperature in the presence of $Ti(OEt)_4$ to obtain the (*S*)-*N*-*tert*-butanesulfinyl imines (*S*)-**2a** and (*S*)-**2b** at yields of 82% and 97%, respectively. Then, the coupling products (1*R*,2*R*)-**3a** and (1*R*,2*R*)-**3b** were prepared through the

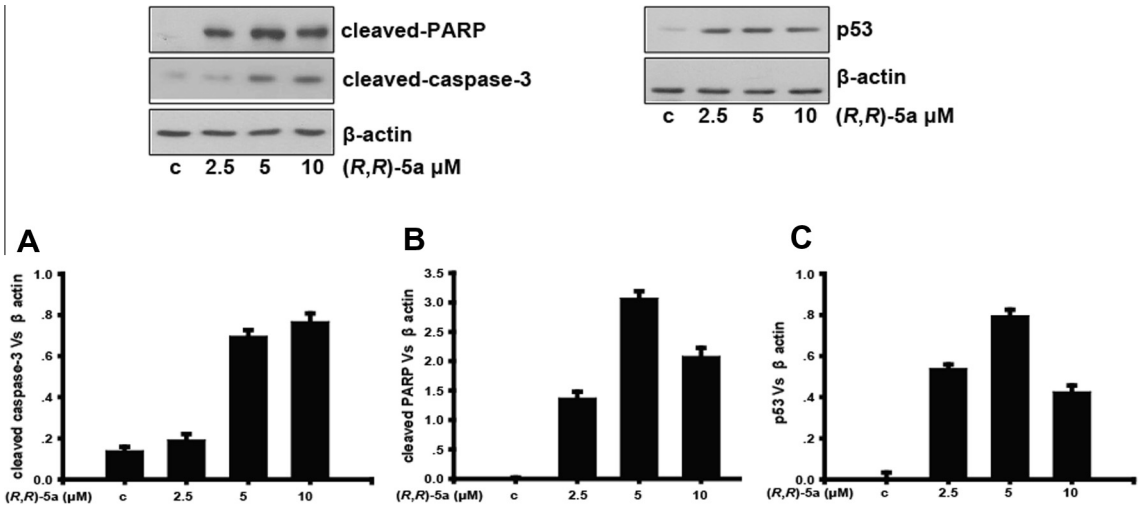


Figure 3. Western blots for the analysis of caspase-3 activation/cleavage, PARP cleavage, and p53 expression in A2780 ovarian cancer cells after 24 h treatment with (*R*,*R*)-**5a** at various concentrations (2.5, 5, and 10 μM). β-Actin was used as a loading control. The term 'c' refers to untreated control cells. (A) Relative quantity of cleaved caspase-3 relative to β-actin. (B) Relative quantity of cleaved-PARP relative to β-actin. (C) Relative quantity of p53 relative to β-actin. The data represent the mean ± SD of three independent experiments.

asymmetric reductive homocoupling of the corresponding (*S*)-**2a** and (*S*)-**2b** mediated by SmI_2 in THF at −78 °C in the presence of HMPA; these products were produced at yields of 66% and 74%, respectively. Finally, (1*R*,2*R*)-**3a** and (1*R*,2*R*)-**3b** were converted to their corresponding enantiopure free diamines (*R*,*R*)-**4a** and (*R*,*R*)-**4b** under acidic conditions at yields of 85% and 94%, respectively. Using (*R*)-2-methyl-2-propanesulfinamide [(*R*)-**1**] as the starting material, both (1*S*,2*S*)-**4a** and (1*S*,2*S*)-**4b** were synthesized in the same manner as (*R*,*R*)-**4**. The enantiomeric purity of the two pairs of diamines **4a** and **4b** were then ascertained by chiral HPLC analysis of the corresponding diacetate.[32] The diacetates of diamines (*R*,*R*)-**4a**, (*S*,*S*)-**4a**, (*R*,*R*)-**4b**, and (*S*,*S*)-**4b** each showed an extremely high ee of >99%.

With the enantiopure diamines (*R*,*R*)-**4** (**a**, **b**) and (*S*,*S*)-**4** (**a**, **b**), $IrCl_3{\cdot}3H_2O$ was used for the coordination to form the desired complexes (Scheme 2). Due to the chemical inertness of $IrCl_3{\cdot}3H_2O$, the coordination reaction was conducted under rather harsh conditions, i.e., high temperatures and prolonged reaction times, according to the modified method described by Galsbol et al.[33] These reactions resulted in the formation of the final iridium(III) complexes (*R*,*R*)-**5** (**a**, **b**) and (*S*,*S*)-**5** (**a**, **b**) at yields in the range of 28–39%.[34] All of the synthesized iridium(III) complexes were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectra (ESI and HRES-I). The spectroscopic and analytical data of these iridium(III) complexes were in complete agreement with their assigned structures. In the IR spectra, the bands of the N–H stretching vibrations of the complexes **5** were redshifted relative to the single amino group of the corresponding ligands due to the coordination of the amino group with iridium. The positive ESI mass spectra of all of the iridium(III) complexes (*R*,*R*)-**5** (**a**, **b**) and (*S*,*S*)-**5** (**a**, **b**) showed the expected $[M-Cl]^+$ peaks, each of which had six ion peaks due to the presence of iridium and chlorine isotopes. Since the stable five membered chelate ring at the iridium blocks rotation around the C–N axis, both N-bound protons become diastereotopic owing to the neighborhood of the asymmetric C-atoms. This led to the appearance of separate signals for the axially and for the equatorially orientated N–H atoms in the ^{1}H NMR spectra of these iridium(III) complexes. Furthermore, the structure of compound (*R*,*R*)-**5a** was unambiguously confirmed by single crystal X-ray structure determination (Fig. 1).[35] The two chloride ligands of (*R*,*R*)-**5a** were found to be situated oppositely, and the two asymmetric C-atoms of the 1,2-di(4-methylphenyl)ethane-1,2-diamine ligands possess the (*R*,*R*)-configuration.

To evaluate their anticancer activity, the human solid tumor cell lines A2780 (human ovarian carcinoma), A549 (human non-small cell lung cancer), KB (human oral epithelial carcinoma), and MDA-MB-231 (human breast cancer) were used. The in vitro cytotoxicity was evaluated after the cells were exposed to the iridium(III) complexes **5** for 72 h using the MTT assay.[36] Oxaliplatin was used as the positive control. The results, which are expressed as IC_{50} values (drug concentration giving 50% survival),[37] are shown in Table 1.

For the *p*-methyl-substituted complexes **5a** and the *p*-trifluoromethyl-substituted complexes **5b**, a pronounced difference in the activities of the (*R*,*R*)- and the (*S*,*S*)-isomers was observed on all tested tumor cell lines. Both (*R*,*R*)-configured complexes exhibited significantly higher activity than their corresponding (*S*,*S*)-enantiomers. The activities of (*R*,*R*)-**5a** and (*R*,*R*)-**5b** were higher than or at least comparable to that of oxaliplatin, whereas (*S*,*S*)-**5a** and (*S*,*S*)-**5b** were almost inactive. Complex (*R*,*R*)-**5a** had lower IC_{50} values than oxaliplatin toward A2780, KB, and MDA-MB-231 cells, and complex (*R*,*R*)-**5b** exhibited better activity than oxaliplatin against A2780 and MDA-MB-231 cells. The antitumor activity of these complexes toward A2780 and MDA-MB-231 cells decreased in the following order: (*R*,*R*)-**5a** > (*R*,*R*)-**5b** > oxaliplatin. For the KB cells, the order of cytotoxicity was (*R*,*R*)-**5a** > oxaliplatin > (*R*,*R*)-**5b**. The analysis of the A549 cells revealed that neither (*R*,*R*)-**5a** nor (*R*,*R*)-**5b** exhibited higher activity than oxaliplatin (∼two-fold less potent). The above results indicate that the antitumor activity of these iridium(III) complexes may depend, in addition to the critical configuration of the 1,2- diarylethane-1,2-diamine moiety, on the type of tumor cells and the nature of the substituents on the phenyl rings.

Due to their promising potent cytotoxicity, it was of interest to further explore the molecular mechanism underlying the (*R*,*R*)-**5**-induced cell death. To investigate whether the (*R*,*R*)-**5**-initiated cell death is mediated by apoptosis, A2780 cells were treated with (*R*,*R*)-**5a** or oxaliplatin for 72 h and then analyzed by flow cytometry through Annexin V/PI staining. The apoptotic rates, including early and late apoptosis, for A2780 cells treated with 5, 10, and 20 μM (*R*,*R*)-**5a** were 31.8%, 52.1%, and 69.1%, respectively, whereas the apoptosis rates for A2780 cells untreated or treated with 20 μM

oxaliplatin were 11.0% and 32.9%, respectively (Fig 2). These results indicate that complex (*R*,*R*)-**5a** is able to induce the apoptosis of A2780 cells in a concentration-dependent manner and that this complex exhibits a better ability of inducing cell apoptosis than oxaliplatin.

To verify that apoptosis is induced as a result of treatment with complex (*R*,*R*)-**5a**, we performed Western blotting assays for caspase-3, which is a key enzyme in the apoptotic pathway, and PARP [poly(ADP-ribose) polymerase] protein, which plays a pivotal role in the nucleotide excision repair of DNA lesions and is cleaved by caspase-3 during apoptosis.[38] As shown in Figure 3A and B, complex (*R*,*R*)-**5a** caused a significant increase in the activated/cleaved form of caspase-3 and the cleaved form of PARP compared with the untreated control. This result is typical of apoptotic cell death. Thus, it was concluded that (*R*,*R*)-**5a** induced the death of A2780 cells through apoptosis. To clarify the apoptotic mechanism triggered by (*R*,*R*)-**5a** in A2780 cells, the regulation of p53 was also assessed through Western blotting analyses (Fig. 3C). The p53 protein is a tumor suppressor and one of the most common mediators of apoptosis.[39] The results showed a marked accumulation of p53 in A2780 cells exposed to (*R*,*R*)-**5a** for 24 h. Thus, the apoptosis triggered by (*R*,*R*)-**5a** correlated with the upregulation of p53.

In conclusion, four novel iridium(III) complexes with enantiopure C_2-symmetrical vicinal diamine ligands were synthesized, characterized, and evaluated against human solid tumor cell lines. The results of the in vitro antiproliferative activity assays indicated that the *R*,*R*-configured complexes (*R*,*R*)-**5a** and (*R*,*R*)-**5b** were significantly more active than their corresponding (*S*,*S*)-isomers (*S*,*S*)-**5a** and (*S*,*S*)-**5b** toward all of the tested human tumor cell lines. The most potent complex (*R*,*R*)-**5a** exhibited better cytotoxicity than oxaliplatin against the A2780, KB, and MDA-MB-231 cell lines and similar cytotoxicity to that of oxaliplatin against the A549 cell line. The mode of cell death initiated by (*R*,*R*)-**5a** in A2780 cells was p53-mediated apoptosis, as indicated by the activation of caspase-3, the cleavage of PARP, and the upregulation of p53. Further studies are underway to elucidate the molecular mechanism of (*R*,*R*)-**5a** and to evaluate its efficacy in vivo.

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32. *Determination of the enantiomeric excess of diacetates of (R,R)-**4a** and (S,S)-**4a***: both showed ee >99%. HPLC: Daicel Chiralpak AD-H column, detected at 214 nm, eluent: *n*-hexane/*iso*-propanol 9:1, 0.7 mL/min, Retention time: $T_{(R,R)}$ = 10.3 min, $T_{(S,S)}$ = 7.8 min; ^{1}H NMR (400 MHz, CD_3OD) δ 7.00 (d, J = 8.2 Hz, 4H), 6.97 (d, J = 8.2 Hz, 4H), 5.16 (s, 2H), 2.22 (s, 6H), 1.94 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 172.81, 138.16, 137.63, 129.84, 128.65, 59.07, 22.65, 21.05; ESI-MS *m*/*z*: 324.2 $[M+H]^+$.
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34. *Typical procedures for the synthesis of iridium(III) complexes: Preparation of (1R,2R)-2-di(4-methylphenyl)ethane-1,2-diamine hydrochloride [(R,R)-**4a**·2HCl]*: First, 4-methylbenzaldehyde (3.18 mL, 27.0 mmol) and (*S*)-2-methyl-2-propanesulfinamide [(*S*)-1] (3.64 g, 30.0 mmoL) were dissolved in DCM (60 mL). $Ti(OEt)_4$ (9.40 mL, 45.0 mmol) was then added, and the mixture was stirred for 24 h at room temperature. Brine (10 mL) was then added to the reaction mixture under vigorous stirring. The resulting suspension was filtered through a plug of Celite, and the filter cake was washed with DCM. The filtrate was transferred to a separatory funnel, where the organic layer was washed with brine. The brine layer was extracted once with a small volume of DCM, and the combined organic portions were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified through silica-gel column chromatography to obtain the compound (*S*)-**2a** (4.91 g, 82%) as a white solid. Under nitrogen, HMPA (1.54 mL, 8.83 mmol) in THF (8 mL) was added to a freshly prepared SmI_2 (8.83 mmol) solution in THF (45 mL) at −78 °C. After approximately 30 min, (*S*)-**2a** (788 mg, 3.53 mmol) in THF (11 mL) was added dropwise. The mixture was stirred at −78 °C for a period of 4 h. The reaction was monitored by TLC and quenched with saturated aqueous $Na_2S_2O_3$ solution (13 mL) after completion. After being extracted with ethyl acetate, the solvent was evaporated, and the residue was purified through flash column chromatography to obtain the desired homocoupling product (1*R*,2*R*)-**3a** (519 mg, 66%) as a pale yellow solid. Then, (1*R*,2*R*)-**3a** (515 mg, 1.15 mmol) was dissolved in MeOH (12 mL), and an HCl saturated methanol solution (1.15 mL) was then added to the mixture. The mixture was stirred for 3 h at room temperature and then concentrated. The resulting solid was recrystallized using a mixture of MeOH and ethyl ether to obtain the hydrochloride salt of diamine [(*R*,*R*)-**4a**·2HCl] (304 mg) as a white solid. Yield: 46%. $[\alpha]_D^{20}$ = 29.5° (c 0.48, CH_3OH); ^{1}H NMR (400 MHz, D_2O) δ 7.08 (d, J = 7.6 Hz, 4H), 6.93 (d, J = 7.6 Hz, 4H), 4.81 (s, 2H), 2.16 (s, 6H); ESI-MS *m*/*z*: 241.1 $[M+H]^+$. *Preparation of dichlorobis[(1R,2R)-2-di(4-methylphenyl)ethane-1,2-diamine]iridium(III) chloride [(R,R)-**5a**]*: (*R*,*R*)-**4a**·2HCl (179 mg, 0.58 mmol) was dissolved in distilled water (10 mL). Then, aqueous KOH solution (3N) was added to basify the mixture to a pH value of ~10. The resulting suspension was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, and concentrated in vacuum to obtain the free diamine (*R*,*R*)-**4a** (119 mg, 0.50 mmol) as a white solid. Under nitrogen, $IrCl_3 \cdot 3H_2O$ (70 mg, 0.20 mmol) was dissolved in a mixture of acetic acid (11.5 μL, 0.20 mmol) and distilled water (3 mL) by heating to boiling in a 25-mL two-neck flask fitted with a condenser. Then, (*R*,*R*)-**4a** (0.50 mmol) in 2-ethoxyethanol (1 mL) was added dropwise (1 mL/h) with reflux. After the addition of 2-ethoxyethanol, the solution was refluxed for an additional 5 h and then evaporated to dryness in vacuo. The solid yellowish brown residue was heated to 170 °C for 24 h under nitrogen and then cooled to room temperature. The resulting dark brown residue was isolated through column chromatography (eluting with *n*-hexane/DCM 35:1 to 20:1) to obtain the crude

product. Further HPLC purification [gradient elution: CH_3CN–water 20–75% (0.1% TFA)] provided the pure iridium(III) complex (*R*,*R*)-**5a** (54 mg) as a yellow solid. Yield: 35%. Mp: decomposed at approximately 260 °C before melting; IR (cm^{-1}): 3211, 2923, 2854, 1586, 1516, 1456, 1377, 1181, 1046, 959, 815, 720, 563, 510; ^1H NMR (400 MHz, CD_3OD) δ 7.15 (d, J = 8.1 Hz, 8H), 7.09 (d, J = 8.1 Hz, 8H), 6.98 (d, J = 9.8 Hz, 4H), 5.45 (br s, 4H), 4.19–4.17 (m, 4H), 2.24 (s, 12H); ^{13}C NMR (100 MHz, CD_3OD) δ 139.89, 135.62, 130.67, 128.68, 66.82, 21.14; ESI-MS *m*/*z* (%): 741.2 (45), 743.2 (100), 744.2 (38), 745.3 (57), 746.3 (18), 747.2 (10) $[M-Cl]^+$; HRESI-MS: calcd for $C_{32}H_{40}IrCl_2N_4$ 741.22305 $[M-Cl]^+$, found 741.22124.

35. Crystallographic data for complex (*R*,*R*)-**5a** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 931594. Copies of the data can be obtained, free of charge, upon application to the CCDC (email: deposit@ccdc.cam.ac.uk).
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37. The cytotoxicity in vitro was evaluated by MTT assay. Briefly, A2780, A549, KB, and MDA-MB-231 cells in their exponential growth phase were seeded in the 96-well flat-bottomed culture plates at the density of 5×10^3 cells/well. After incubation for 24 h, various concentrations of the iridium(III) complex were added into each well and each concentration was repeated in more than 3 wells. After incubation with the complex for another 72 h, the medium was removed and MTT solution (0.5 mg/ml) was added into each well and the plates were incubated for an additional 4 h at 37 °C. The medium was then aspirated and 150 μL of DMSO was added into each well. The absorbance at 490 nm was recorded using a plate recorder (none of the complexes tested absorbs at this wavelength). The reported IC_{50} data were calculated with the Probit analysis (GW. Basic 3.22) and are averages of three independent experiments.
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