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Platinum(II) complexes with rutaecarpine and tryptanthrin derivatives induce apoptosis by inhibiting telomerase activity and disrupting mitochondrial function†

 Qi-Pin Qin, ^{‡ad} Bi-Qun Zou, ^{‡acd} Fei-Long Hu, ^b Guo-Bao Huang,^a
 Shu-Long Wang,^{ad} Yun-Qiong Gu^{ad} and Ming-Xiong Tan ^{*a}

Four new platinum(II) complexes, [Pt(Rut)(DMSO)Cl₂] (**Rut-Pt**), [Pt(Try)(DMSO)Cl₂] (**Try-Pt**), [Pt(ITry)(DMSO)Cl₂] (**ITry-Pt**) and [Pt(BrTry)(DMSO)Cl₂] (**BrTry-Pt**), with rutaecarpine (Rut), tryptanthrin (Try), 8-iodine-tryptanthrin (ITry) and 8-bromo-tryptanthrin (BrTry) as ligands were synthesized and fully characterized. In these complexes, the platinum(II) adopts a four-coordinated square planar geometry. The inhibitory activity evaluated by the MTT assay showed that **BrTry-Pt** (IC₅₀ = of 0.21 ± 0.25 μM) could inhibit the growth of T-24 tumor cells (human bladder cancer cell line) more so than the other three complexes. In addition, all of these Pt complexes exhibited low toxicity against non-cancerous HL-7702 cells. **BrTry-Pt** induced cell cycle arrest in the S phase, leading to the down-regulation of cyclin A and CDK2 proteins. **BrTry-Pt** acts as a telomerase inhibitor targeting the c-myc promoter. In addition, **BrTry-Pt** also caused mitochondrial dysfunction. Importantly, the *in vitro* anticancer activity of **BrTry-Pt** was higher than those of **Rut-Pt**, **Try-Pt** and **ITry-Pt**, and it was more selective for T-24 cells than for non-cancerous HL-7702 cells.

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Introduction

In recent years, G-quadruplex DNA (G4 DNA) has been found not only in telomeres but also in promoter regions of c-myc, k-RAS, c-kit, Bcl-2, HIF-1α, VEGF, PDGF-A, and other genes^{1–8} that are related to HIV, diabetes, and cancer.^{1,9} Consequently, G4 DNA is now seen as a promising drug target.^{8,10,11} An important proto-oncogene, c-myc, is associated with cellular proliferation, cell cycle arrest, cell growth, and apoptosis.^{1,8,12–17}

In addition, most studies have focused on down-stream mechanisms of hTERT inhibition, such as cell cycle arrest and cell apoptosis,^{18,19} which directly inhibit telomerase activity in human tumor cells.²⁰ Since then, porphyrin analogs,² Cu(II) complexes,^{21,22} Ir(III) complexes,²³ berberine derivatives,²⁴ Pt(II) complexes,^{1,5,9} quindoline derivatives,²⁵ Ru(II) complexes,^{10,15} and hydrazone derivatives²⁶ have been reported to interact with c-myc G4 DNA. Therefore, the design of drugs specifically interacting with the c-myc promoter or G4 DNA could pave the way for the discovery of novel anti-cancer agents.^{1,7}

Previous studies found that rutaecarpine and tryptanthrin derivatives exhibited diverse biological effects, such as anti-tumor, antimicrobial, and anti-inflammatory activities.^{27–35} Recently, our group has reported that tryptanthrin derivatives and their Fe^{II} and Co^{II} complexes can specifically stabilize telomeric G4 DNA. However, there are only a few reported metal complexes containing rutaecarpine and tryptanthrin derivatives as c-myc G4 DNA ligands. Here we report the synthesis and characterization of four new rutaecarpine and tryptanthrin based platinum(II) complexes, [Pt(Rut)(DMSO)Cl₂] (**Rut-Pt**), [Pt(Try)(DMSO)Cl₂] (**Try-Pt**), [Pt(ITry)(DMSO)Cl₂] (**ITry-Pt**) and [Pt(BrTry)(DMSO)Cl₂] (**BrTry-Pt**), with rutaecarpine (Rut), tryptanthrin (Try), 8-iodine-tryptanthrin (ITry) and 8-bromo-tryptanthrin (BrTry) as ligands. **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** were selective for T-24 cancer cells over normal

^a Guangxi Key Lab of Agricultural Resources Chemistry and Biotechnology, School of Chemistry and Food Science, Yulin Normal University, 1303 Jiaoyudong Road, Yulin 537000, P. R. China. E-mail: mxtan2018@126.com; Fax: +86 775 2623650; Tel: +86 775 2623650

^b Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, Guangxi University for Nationalities, Nanning, 530006, P. R. China

^c Department of Chemistry, Guilin Normal College, 21 Xinyi Road, Guilin 541001, P. R. China

^d State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmacy, Guangxi Normal University, 15 Yucai Road, Guilin 541004, P. R. China

† Electronic supplementary information (ESI) available: Inhibitory rates (%) of **Rut-Pt**, **Try-Pt**, **ITry-Pt**, **BrTry-Pt**, Rut, Try, ITry and BrTry against the selected six human cells for 48 h, and ESI-MS, UV-vis, IR, NMR and crystal data. CCDC 1819739, 1819740, 1819742 and 1819743 for **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** contain the supplementary crystallographic data for this study. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8md00247a

‡ These authors contributed equally to this work.

HL-7702 cells. In addition, we demonstrated for the first time that **BrTry-Pt** was a telomerase inhibitor targeting the c-myc promoter and triggering T-24 cell apoptosis, which also caused mitochondrial dysfunction and S phase arrest. Therefore, **BrTry-Pt** may have the potential to be further developed as a safe and effective anticancer agent.

Results and discussion

Synthesis

Tryptanthrin (Try), 8-iodine-tryptanthrin (ITry) and 8-bromo-tryptanthrin (BrTry) were synthesized and characterized using procedures similar to those published by Tan and Sharma.^{33,34} Rutaecarpine (Rut) is a quinazolinocarboline alkaloid isolated from *E. rutaecarpa*.³⁵ The reaction of Rut, Try, ITry and BrTry with *cis*-Pt(DMSO)₂Cl₂ in CH₃OH and CHCl₃ (v:v = 5 mL:5 mL) at 55 °C afforded **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** in

44.58–62.23% yield, respectively. In addition, the structure of the synthesized Pt(II) complexes was determined by spectrometry (IR, ¹H NMR, ESI-MS, UV-Vis and ¹³C NMR), elemental analysis, and X-ray crystallography (Fig. S1–S17†).

Crystal structures of platinum(II) complexes

As shown in Fig. 1, the crystal structure showed that in **Rut-Pt**, the Pt(II) was four-fold coordinated by Rut *via* a nitrogen atom, one DMSO ligand *via* the sulfur atom, and two chlorine atoms to form a square-planar geometry. The bond angles at the platinum atom (N1–Pt1–Cl1 and N1–Pt1–S1) were 89.2(3) and 174.5(3)°, respectively. The average Pt1–N1, Pt1–Cl1 and Pt1–S1 distances were 2.066(12), 2.288(4) and 2.200(4) Å, respectively, which were within the expected range.

Try-Pt, **ITry-Pt** and **BrTry-Pt** were similar to **Rut-Pt** in structure and the coordination geometry of Pt(II) could also be

Fig. 1 The ORTEP drawings of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** showing atom labeling.

described as approximately four-coordinated square planar (Fig. 1). In addition, selected bond angles (deg) and bond lengths (Å) are reported, and the structure refinement parameters and crystal data are included in Tables S1–S12.†

Stability of Pt complexes in TBS buffer

The solubility and stability of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** in TBS buffer (10 mM Tris-HCl buffer containing 1% DMSO, pH = 7.35) were determined by ESI-MS and UV-vis spectroscopy.^{36,37} The UV-vis absorption peaks and spectra of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** (2.0×10^{-5} M) showed no obvious change at 48 h, which suggested that **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** were stable for 48 h under these conditions (Fig. S17†). In addition, their stabilities were further confirmed by ESI-MS spectrometry. As shown in Fig. S4, S8, S12 and S16,† the ESI-MS characterization of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** (2.0×10^{-5} M) revealed the maximum abundance at $m/z = 551.4$ for $[M - H - DMSO]^+$, $m/z = 762.4$ for $[M + Cl + 2CH_3OH + DMSO]^+$, $m/z = 745.4$ for $[M + Cl + 4H_2O - DMSO]^+$, and $m/z = 712.7$ for $[M - Cl + DMSO]^+$, respectively (Tables S2 and S9†). The maximum abundance of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** (2.0×10^{-5} M) incubated in the TBS buffer for 48 h was observed at $m/z = 673.1$ for $[M - Cl + DMSO]^+$, $m/z = 762.9$ for $[M + Cl + 2CH_3OH + DMSO]^+$, $m/z = 747.0$ for $[M + Cl + 4H_2O - DMSO]^+$, and $m/z = 762.4$ for $[M + Cl + 3H_2O]^+$, respectively. All of these results indicated that **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** were stable in TBS for 48 h, which ensured adequate cellular uptake of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** during the time course of the MTT assay.

Anticancer activity

The *in vitro* inhibitory activities of cisplatin, **Try**, **ITry**, **BrTry**, **cis-Pt(DMSO)₂Cl₂**, **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** against Hep-G2, T-24, A549, SK-OV-3, HeLa and HL-7702 cells were evaluated by the MTT assay after 48 h of treatment, and the results are listed in Tables 1 and S13.† As shown in Table 1, the **BrTry** ligand showed a relatively higher cytotoxicity than **Rut-Pt**, **Try-Pt** and **ITry-Pt** against T-24 cells. Against the tested tumor cells, **BrTry-Pt** showed the highest inhibitory effects on T-24 and SK-OV-3 tumor cells with low IC_{50} values (Table 1) of 0.21 ± 0.25 μ M and 1.80 ± 0.45 μ M, respectively.

Particularly, **BrTry-Pt** was more sensitive to T-24 cells, and exhibited lower IC_{50} values (0.21 ± 0.25 μ M) in T-24 cells, which were approximately 72.3 times and 386.0 times higher than those of cisplatin (15.19 ± 1.23 μ M) and the free ligand **BrTry** (81.06 ± 0.84 μ M), respectively, suggesting a synergistic effect upon the combination of Pt(II) with the corresponding **BrTry** ligand. Interestingly, **BrTry-Pt** displayed low inhibitory rates against a non-cancerous cell line (HL-7702), suggesting that it has a better therapeutic profile than cisplatin (17.69 ± 1.26 μ M). All of these data suggested that **BrTry-Pt** may have potential utility in the clinic with acceptable inhibitory activity against T-24 tumor cells.^{38,39}

Cellular uptake of Pt(II) complexes.

The cellular uptake characteristics of a small molecule are critical to its application as a therapeutic or diagnostic agent.⁴⁰ Thus, the cellular uptake of cisplatin (15 μ M), **Rut-Pt** (82 μ M), **Try-Pt** (68 μ M), **ITry-Pt** (19 μ M) and **BrTry-Pt** (0.2 μ M) was examined *in vitro* with an ICP-MS assay. As shown in Fig. 2A, **BrTry-Pt** (6.84 ± 0.11 nmol of Pt) per 10^6 cells) was taken up by these T-24 cells approximately 2.44 times and 1.5 times more efficiently than cisplatin (3.98 ± 0.21 nmol of Pt) per 10^6 cells), **Rut-Pt** (0.86 ± 0.15 nmol of Pt) per 10^6 cells), **Try-Pt** (1.45 ± 0.09 nmol of Pt) per 10^6 cells) and **ITry-Pt** (3.05 ± 0.05 nmol of Pt) per 10^6 cells).

Moreover, the distribution of cisplatin (15 μ M), **Rut-Pt** (82 μ M), **Try-Pt** (68 μ M), **ITry-Pt** (19 μ M) and **BrTry-Pt** (0.2 μ M) in T-24 cells was further studied using the previously reported method.^{36,37,41,42} Interestingly, the percentage of Pt from **BrTry-Pt** (0.2 μ M) in the nuclear fraction and mitochondrial fraction was slightly higher than those from cisplatin (15 μ M), **Rut-Pt** (82 μ M), **Try-Pt** (68 μ M) and **ITry-Pt** (19 μ M), as shown in Fig. 2B, suggesting that they may act differentially in T-24 cell apoptotic pathways.⁴³

Telomerase inhibition (TRAP assay)

Because telomerase is a potential drug target for anticancer therapy,^{44–47} we also examined the efficiency of the synthesized compounds **Rut-Pt** (82 μ M), **Try-Pt** (68 μ M), **ITry-Pt** (19 μ M) and **BrTry-Pt** (0.2 μ M) toward telomerase inhibition in T-24 tumor cells by the TRAP assay. As shown in Fig. 2C, the

Table 1 IC_{50} ^a (μ M) values of cisplatin, **Rut**, **Try**, **ITry**, **BrTry**, **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** in the six human cells for 48 h

Compounds	T-24	A549	Hep-G2	SK-OV-3	HeLa	HL-7702
Rut	>150	>150	>150	>150	>150	112.58 $\pm$ 1.94
Rut-Pt	81.61 $\pm$ 0.45	>150	>150	57.29 $\pm$ 2.06	>150	105.36 $\pm$ 0.69
Try	>150	>150	90.14 $\pm$ 0.47	85.09 $\pm$ 0.74	>150	90.12 $\pm$ 1.12
Try-Pt	67.77 $\pm$ 1.57	>150	93.33 $\pm$ 1.86	51.79 $\pm$ 0.33	>150	100.20 $\pm$ 0.55
ITry	85.23 $\pm$ 1.08	>150	88.56 $\pm$ 0.72	79.06 $\pm$ 1.05	>150	94.06 $\pm$ 1.19
ITry-Pt	18.51 $\pm$ 1.07	112.10 $\pm$ 1.22	52.40 $\pm$ 0.36	19.94 $\pm$ 0.29	135.98 $\pm$ 0.44	90.52 $\pm$ 0.53
BrTry	15.07 $\pm$ 0.88	89.64 $\pm$ 1.20	99.56 $\pm$ 0.69	75.03 $\pm$ 1.98	>150	50.11 $\pm$ 0.82
BrTry-Pt	0.21 $\pm$ 0.25	31.41 $\pm$ 0.74	27.54 $\pm$ 0.85	1.80 $\pm$ 0.45	100.70 $\pm$ 1.85	92.06 $\pm$ 1.17
Cisplatin ^b	15.19 $\pm$ 1.23	14.85 $\pm$ 1.97	18.02 $\pm$ 0.55	14.25 $\pm$ 1.57	13.02 $\pm$ 1.03	17.69 $\pm$ 1.26

^a IC_{50} values are presented as the mean $\pm$ SD (standard error of the mean) from five independent experiments. ^b Cisplatin was dissolved at a concentration of 1.0 mM in 0.154 M NaCl.²¹

Fig. 2 The Pt(II) complexes inhibited the telomerase activity through directly regulating the level of the c-myc promoter. (A and B) ICP-MS data for T-24 tumor cells incubated with cisplatin (15 μ M), Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M), and BrTry-Pt (0.2 μ M), respectively. (*) $P < 0.05$, treatment group vs. the control group. (C) TRAP assay performed in the presence of Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M), respectively. (D) The expression percentage of hTERT and c-myc genes after 24 h of incubation in T-24 tumor cells with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) as determined by RT-qPCR. (*) $P < 0.05$, treatment group vs. the control group. (E and F) Western blot of hTERT and c-myc expression in the T-24 tumor cells treated with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M). The whole cell extracts were prepared and analyzed by western blot analysis using antibodies against hTERT and c-myc proteins. The same blots were stripped and reprobed with a β -actin antibody to show equal protein loading. Western blot bands shown in (F) from three independent measurements were quantified with ImageJ. (*) $P < 0.05$, treatment group vs. the control group. (G and H) Transfection of 2.0 μ g EGFP (enhanced green fluorescent protein) plasmid and 2.0 μ g c-myc plasmid vectors in the T-24 cancer cells treated with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24.0 h, respectively, and these results were assessed by fluorescence microscopy or using a multimodel plate reader with a luciferase reporter gene assay kit. (*) $P < 0.05$, treatment group vs. the control group.

inhibition of telomerase by Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) was 15.02%, 42.93%, 3.08% and 56.44% in T-24 cells, respectively. Importantly, BrTry-Pt (0.21 μ M) exhibited stronger telomerase inhibition than Rut-Pt (82 μ M), Try-Pt (68 μ M) and ITry-Pt (19 μ M).

Pt(II) complexes downregulate c-myc and hTERT expression

It has been shown that the human telomerase activity is regulated by the transcriptional control of hTERT and c-myc.^{48,49} Therefore, to determine whether Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) were able to regulate the expression of c-myc and hTERT genes, we have performed RT-qPCR and western blotting in T-24 tumor cells.

The T-24 cells were incubated for 24 h in the presence of 5% DMSO (control), Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M). As shown in Fig. 2D–F, BrTry-Pt (0.2 μ M) showed the highest ability to down-regulate the expression of c-myc and hTERT compared to Rut-Pt (82 μ M), Try-Pt (68 μ M), and ITry-Pt (19 μ M).

Transfection

To evaluate whether Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) can directly regulate the c-myc promoter (including Pu27 G4 DNA) and consequently inhibit hTERT expression and telomerase function, a transfection assay was carried out.^{50,51} Fig. 2G shows that the T-24 cancer

cells displayed green fluorescence, suggesting that the EGFP plasmid transfection was successful. In addition, the fluorescence emission of the T-24 cells treated with 0.2 μM **BrTry-Pt** for 24 h was reduced by 81.1%, whereas for **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM), the fluorescence emission was only reduced by 14.6%, 19.8% and 8.8% under the same conditions, respectively. All of these results confirmed the much greater inhibitory effect of **BrTry-Pt** (0.2 μM) on telomerase activity by directly down-regulating the c-myc promoter in T-24 cells (Fig. 2H), as compared with **Rut-Pt** (82 μM), **Try-Pt** (68 μM), and **ITry-Pt** (19 μM).

Cell cycle analysis by flow cytometry

It was recently reported that T-24 cell cycle arrest is highly related to telomerase activity.⁵² Herein, the cell cycle of T-24 tumor cells was determined by flow cytometry after treatment with **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) for 24 h. In the case of **BrTry-Pt** (0.2 μM), the percentage of T-24 cancer cells in the S phase showed an increase from 43.79% to 69.77%. Consistent with our previous results, **BrTry-Pt** (0.2 μM) led to more pronounced changes in the cell cycle than **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM) (Fig. 3A).

Expressions of S phase-related proteins

To further confirm the cell cycle arrest in the S phase upon treatment with the four Pt(II) complexes, the T-24 tumor cells were treated with **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM), and the levels of the S phase-related proteins (including cyclin A and CDK2 proteins) were analyzed using western blotting. As shown in Fig. 3B and C, western blotting analysis revealed that the cyclin A and CDK2 levels decreased more after 24 h of **BrTry-Pt** (0.2 μM) treatment than after treatment with **Rut-Pt** (82 μM), **Try-Pt** (68 μM), and **ITry-Pt** (19 μM), due to the S phase arrest and the changes of the cyclin A-CDK2 complex,^{43,53} finally inducing cell apoptosis.

Induction of ROS and Ca^{2+} fluctuation in T-24 cells

Several reports proposed that increases in Ca^{2+} levels and ROS generation are related to cell apoptosis.^{54,55} In order to study whether the cell death induced by **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) was dependent on ROS levels, the T-24 cancer cells were exposed to each Pt complex for 24 h, and then the ROS levels were determined using DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) as the fluorescent probe.^{54,55} As shown in Fig. 4A, ROS overproduction was found in the T-24 tumor cells exposed to **BrTry-Pt** (0.2 μM). The results also showed that **BrTry-Pt** (0.2 μM) produced higher levels of ROS than **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM).

In addition, upon addition of **BrTry-Pt** (0.2 μM) to the T-24 cells, the level of intracellular Ca^{2+} increased steadily (Fig. 4B), and a lesser effect was seen with **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM). All of these results

suggested that the increase of intracellular Ca^{2+} was related to **BrTry-Pt** (0.2 μM)-induced T-24 cell apoptosis more so than that in **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM) treatment.

$\Delta\psi_{\text{m}}$ loss

Previous studies have demonstrated that mitochondria play a vital role in T-24 cancer cell apoptosis triggered by chemical reagents.^{55,56} In addition, loss of the mitochondrial membrane potential ($\Delta\psi_{\text{m}}$) is a crucial step in apoptosis. Thus, to compare the effects of **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) on the changes in mitochondrial membrane potential ($\Delta\psi_{\text{m}}$), the fluorescent probe JC-1 was used in T-24 tumor cells, and the ratio of green/red fluorescence intensity was determined by flow cytometry (Fig. 4C). After the treatment of T-24 cells with **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) for 24 h, the percentages of green fluorescence were 5.79%, 6.70%, 13.70% and 57.50%, respectively, and the results follow the order of **BrTry-Pt** > **ITry-Pt** > **Try-Pt** > **Rut-Pt**. Taken together, these results suggested that **BrTry-Pt** can induce a decrease in mitochondrial membrane potential.^{55,56}

Expressions of apoptosis-related proteins

To demonstrate whether the release of cytochrome c, bcl-2, apaf-1, caspase-3, and caspase-9 occurred in **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) treated T-24 tumor cells, flow cytometry and western blotting were carried out.⁵⁷ As shown in Fig. 4D and E, after the T-24 cancer cells were treated with **BrTry-Pt** (0.2 μM) for 24 h, a significant decrease in bcl-2 levels and increase in cytochrome c, bax and apaf-1 levels were observed, as compared with those of the control cells (5% DMSO). In addition, with the **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) treatment of T-24 cells, the protein levels of caspase-3 and caspase-9 expressions were 2.21% and 1.72%, 7.39% and 8.47%, 11.80% and 12.50%, and 40.80% and 52.20%, respectively (Fig. S18 and S19[†]), suggesting that **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) induced apoptosis by triggering caspase-3/9 expression.⁵⁷ The observations also suggested that **BrTry-Pt** (0.2 μM) triggered the upregulation of apoptosis-related proteins more than **Rut-Pt** (82 μM), **Try-Pt** (68 μM) and **ITry-Pt** (19 μM), which is in agreement with the results mentioned above.

Cell apoptosis

Various experiments showed that **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM), especially **BrTry-Pt** (0.2 μM), caused mitochondrial dysfunction and inhibited telomerase activity by directly interacting with c-myc promoter elements, consequently probably triggering cell apoptosis.^{44–57} The ability of **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) to promote cell apoptosis was determined by flow cytometry after staining with propidium iodide (PI) and Annexin-V-FITC.^{58–61} As shown in Fig. 4F, apoptotic cells were detected at **BrTry-Pt**

Fig. 3 Effect of Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) on the levels of cell cycle and its regulators in the T-24 cancer cells. (A) Cell cycle distribution of T-24 tumor cells exposure to Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h. (B) T-24 cells were treated with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h and the expression levels of the S phase-related proteins were examined by western blotting. (C) The whole cell extracts were prepared and analyzed by western blot analysis using antibodies against the S phase-related proteins. The same blots were stripped and reprobed with a β -actin antibody to show equal protein loading. Western blot bands from three independent measurements were quantified with ImageJ. (*) $P < 0.05$, treatment group vs. the control group.

concentrations as low as 0.2 μ M. At a dose of 0.2 μ M, 27.06% of the T-24 cancer cells were undergoing apoptosis, whereas only 5.56%, 5.68% and 7.36% of the T-24 cells were undergoing apoptosis after treatment with Rut-Pt (82 μ M), Try-Pt (68 μ M) and ITry-Pt (19 μ M) for 24 h, respectively.

Conclusions

Four new platinum(II) complexes, [Pt(Rut)(DMSO) Cl_2] (Rut-Pt), [Pt(Try)(DMSO) Cl_2] (Try-Pt), [Pt(ITry)(DMSO) Cl_2] (ITry-Pt) and

[Pt(BrTry)(DMSO) Cl_2] (BrTry-Pt), were synthesized and characterized. Rut-Pt, Try-Pt, ITry-Pt and BrTry-Pt displayed synergistic effects on cytotoxicity as compared with the free Pt(II) ions and the ligands Rut, Try, ITry or BrTry. In particular, BrTry-Pt displayed higher selectivity against T-24 tumor cells than against other human tumor cells, but lower toxicity in the non-cancerous human cell line HL-7702. This is the first report demonstrating that BrTry-Pt can be a telomerase inhibitor targeting the c-myc promoter. BrTry-Pt could cause the T-24 cell cycle arrest in the S phase, leading to the down-

Fig. 4 Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) caused the mitochondrial dysfunction pathway in T-24 cancer cells. (A) Intracellular ROS levels in T-24 tumor cells exposed to Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h and the DCF fluorescence intensity was determined by flow cytometric analysis, respectively. (B) Effects of Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) on the Ca²⁺ activation level in T-24 tumor cells. (C) Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) induce the depletion of mitochondrial membrane potential ($\Delta\psi_m$) in T-24 tumor cells. These cells were treated with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h and then analyzed by JC-1 flow cytometry. (D) T-24 cells were treated with Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h and the expression levels of the apoptosis-related proteins were examined by western blotting. (E) The whole cell extracts were prepared and analyzed by Western blot analysis using antibodies against the apoptosis-related proteins. The same blots were stripped and reprobed with a β -actin antibody to show equal protein loading. Western blot bands from three independent measurements were quantified with ImageJ. (*) $P < 0.05$, treatment group vs. the control group. (F) Apoptosis of T-24 tumor cells induced by Rut-Pt (82 μ M), Try-Pt (68 μ M), ITry-Pt (19 μ M) and BrTry-Pt (0.2 μ M) for 24 h, respectively.

regulation of cyclin A and CDK2 proteins. Cytotoxicity studies indicated that BrTry-Pt significantly induced the loss of $\Delta\psi_m$,

the increase in ROS, cytochrome c, bax, apaf-1 and caspase-3/9 levels, and the decrease in bcl-2 levels in T-24 cells. Taken

together, **BrTry-Pt** was a telomerase inhibitor targeting the c-myc promoter and triggered T-24 cell apoptosis, which also caused mitochondrial dysfunction and S phase arrest to a greater extent than **ITry-Pt**, **Try-Pt** and **Rut-Pt**.

Experimental methods

Synthesis

Synthesis of ligands. Tryptanthrin (**Try**), 8-iodine-tryptanthrin (**ITry**) and 8-bromo-tryptanthrin (**BrTry**) were synthesized and characterized using similar procedures as published by Tan and Sharma.^{33,34} Rutaecarpine (**Rut**) is a major quinazolinocarboline alkaloid isolated from *E. rutaecarpa*.³⁵

Synthesis of Rut-Pt. The complex [Pt(**Rut**)(DMSO)Cl₂] (**Rut-Pt**) was prepared by treating **Rut** (0.029 g, 0.10 mmol) with *cis*-Pt(DMSO)₂Cl₂ (0.063 g, 0.15 mmol) in ethanol (5.0 mL) and CHCl₃ (5 mL) under solvothermal conditions. Red brown block crystals suitable for X-ray diffraction analysis were harvested. Yield (58.17%). ESI-MS *m/z*: 551.4 [M - H - DMSO]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.88 (s, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 7.94–7.72 (m, 1H), 7.67 (dd, *J* = 16.7, 8.0 Hz, 1H), 7.48 (t, *J* = 6.5 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 5.76 (s, 1H), 4.46 (t, *J* = 6.8 Hz, 1H), 2.54 (s, 2H), 2.50 (s, 2H), 2.45 (s, 3H), 2.38 (d, *J* = 19.5 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 160.70 (s), 147.45 (s), 145.39 (s), 138.74 (s), 134.53 (s), 127.18 (s), 126.68 (s), 126.53 (s), 126.08 (s), 124.98 (s), 124.83 (s), 120.79 (s), 120.05 (s), 119.83 (s), 117.97 (s), 112.65 (s), 99.60 (s), 40.91 (s), 40.51 (s), 19.02 (s). IR (KBr): 3782, 3318, 3010, 2919, 1681, 1588, 1555, 1518, 1491, 1468, 1410, 1333, 1240, 1150, 1024, 766, 744, 690, 489, 438 cm⁻¹. Elemental analysis calcd (%) for C₂₀H₁₉Cl₂N₃O₂PtS: C 38.04, H 3.03, N 6.65; found: C 38.00, H 3.09, N 6.62.

Synthesis of Try-Pt. The complex [Pt(**Try**)(DMSO)Cl₂] (**Try-Pt**) was prepared by treating **Try** (0.025 g, 0.10 mmol) with *cis*-Pt(DMSO)₂Cl₂ (0.063 g, 0.15 mmol) in ethanol (5.0 mL) and CHCl₃ (5 mL) under solvothermal conditions. Red brown block crystals suitable for X-ray diffraction analysis were harvested. Yield (62.23%). ESI-MS *m/z*: 762.4 [M + Cl + 2CH₃OH + DMSO]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.48 (d, *J* = 7.9 Hz, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 3.6 Hz, 1H), 7.90–7.85 (m, 1H), 7.80–7.60 (m, 1H), 7.48 (dd, *J* = 10.9, 4.1 Hz, 1H), 3.33 (s, 1H), 3.17 (s, 1H), 2.51 (d, *J* = 1.7 Hz, 3H), 2.49 (d, *J* = 1.7 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 182.55 (s), 157.80 (s), 146.53 (s), 146.07 (s), 145.13 (s), 137.83 (s), 135.24 (s), 129.99 (s), 129.93 (s), 127.02 (s), 126.97 (s), 124.80 (s), 123.39 (s), 122.33 (s), 117.10 (s), 40.51 (s), 40.03 (s). IR (KBr): 3781, 3440, 3018, 2922, 1731, 1704, 1597, 1462, 1330, 1194, 1154, 1026, 934, 769, 687, 584, 471, 441 cm⁻¹. Elemental analysis calcd (%) for C₁₇H₁₄Cl₂N₂O₃PtS: C 34.47, H 2.38, N 4.73; found: C 34.52, H 2.35, N 4.76.

Synthesis of ITry-Pt. The complex [Pt(**ITry**)(DMSO)Cl₂] (**ITry-Pt**) was prepared by treating **ITry** (0.037 g, 0.10 mmol) with *cis*-Pt(DMSO)₂Cl₂ (0.063 g, 0.15 mmol) in ethanol (5.0 mL) and CHCl₃ (5 mL) under solvothermal conditions. Red brown block crystals suitable for X-ray diffraction analysis were harvested. Yield (44.58%). ESI-MS *m/z*: 745.4 [M + Cl + 4H₂O -

DMSO]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.50 (s, 1H), 8.34–8.24 (m, 1H), 8.24–8.15 (m, 1H), 7.94 (t, *J* = 16.5 Hz, 1H), 7.76 (td, *J* = 8.4, 4.3 Hz, 1H), 3.36 (s, 1H), 2.55 (s, 1H), 2.51 (d, *J* = 1.7 Hz, 3H), 2.50 (d, *J* = 1.7 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 181.30 (s), 157.75 (s), 146.54 (s), 145.65 (s), 135.43 (s), 132.73 (s), 130.13 (s), 130.08 (s), 128.04 (s), 127.10 (s), 124.39 (s), 123.26 (s), 119.16 (s), 117.65 (s), 108.91 (s), 40.51 (s), 40.02 (s). IR (KBr): 3428, 3009, 2917, 1630, 1407, 1311, 1154, 1021, 725, 690, 431 cm⁻¹. Elemental analysis calcd (%) for C₁₇H₁₃Cl₂IN₂O₃PtS: C 28.43, H 1.82, N 3.90; found: C 28.40, H 1.88, N 3.95.

Synthesis of BrTry-Pt. The complex [Pt(**BrTry**)(DMSO)Cl₂] (**BrTry-Pt**) was prepared by treating **Try** (0.033 g, 0.10 mmol) with *cis*-Pt(DMSO)₂Cl₂ (0.063 g, 0.15 mmol) in ethanol (5.0 mL) and CHCl₃ (5 mL) under solvothermal conditions. Red brown block crystals suitable for X-ray diffraction analysis were harvested. Yield (45.38%). ESI-MS *m/z*: 712.7 [M - Cl + DMSO]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.42 (d, *J* = 8.3 Hz, 3H), 8.35–8.31 (m, 2H), 8.09–8.04 (m, 7H), 7.97 (d, *J* = 3.7 Hz, 4H), 7.84–7.69 (m, 7H), 3.34 (s, 134H), 3.17 (s, 2H), 2.51 (d, *J* = 1.8 Hz, 23H), 2.49 (d, *J* = 1.8 Hz, 21H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 179.95 (s), 157.61 (s), 139.67 (s), 135.31 (s), 133.12 (s), 132.29 (s), 130.74 (s), 129.94 (s), 127.06 (s), 126.97 (s), 124.23 (s), 123.10 (s), 121.81 (s), 119.08 (s), 118.91 (s), 40.43 (s), 40.08 (s). IR (KBr): 3453, 3064, 3006, 2913, 1737, 1709, 1594, 1459, 1324, 1188, 1133, 1047, 1017, 982, 944, 877, 836, 769, 722, 684, 469, 440, 409 cm⁻¹. Elemental analysis calcd (%) for C₁₇H₁₃BrCl₂N₂O₃PtS: C 30.42, H 1.95, N 4.17; found: C 30.46, H 2.00, N 4.11.

Materials, abbreviations, instrumentation and the experimental methods

The X-ray crystallography structures of **Rut-Pt**, **Try-Pt**, **ITry-Pt** and **BrTry-Pt** were solved by the Sheldrick method.⁶² The antitumor mechanisms of **Rut-Pt** (82 μM), **Try-Pt** (68 μM), **ITry-Pt** (19 μM) and **BrTry-Pt** (0.2 μM) were studied according to the reported procedures.^{36,43,60,61,63–66} In addition, all the materials, instrumentation and detailed procedures for the experimental methods are described in the ESI.†

Conflicts of interest

The authors declare no conflicts of interest.

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