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A β -carboline derivative-based nickel(II) complex as a potential antitumor agent: synthesis, characterization, and cytotoxicity†‡

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A novel nickel(II) complex of 6-methoxy-1-pyridine- β -carboline (**4a**) was synthesized and characterized. The cytotoxicities of the complex towards six cancer cell lines, including MGC-803, Hep G2, T24, OS-RC-2, NCI-H460, and SK-OV-3, and human normal liver cell line HL-7702 were investigated. The IC₅₀ values for MGC-803, Hep G2, T24, OS-RC-2, NCI-H460 and SK-OV-3 were generally in the micromolar range (3.77–15.10 μ M), lower than those of ligand **4** and cisplatin. Furthermore, **4a** (6 μ M) significantly induced cell cycle arrest at the S phase, and caused the down-regulation of p-AKT, cyclin E, cyclin A and CDK2 and the up-regulation of p27. Various experiments showed that **4a** induced apoptosis, activated caspase-3, increased the levels of reactive oxygen species (ROS) and enhanced the intracellular [Ca²⁺]_c levels in MGC-803. In addition, the expression of intrinsic apoptotic proteins, including cytochrome c and apaf-1, increased. Further intrinsic apoptosis was triggered via executive molecular caspase-9 and caspase-3. In short, **4a** exerted its cytotoxic activity primarily through inducing cell cycle arrest at the S phase and intrinsic apoptosis.

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1. Introduction

Cisplatin, oxaliplatin and carboplatin are widely used as anti-cancer agents in clinical oncology.¹ Cisplatin mainly crosslinks the pyrimidine and purine bases in DNA to trigger DNA damage responses,² and the anti-tumor effect of cisplatin primarily results from the apoptosis induced by DNA damage.³ However, platinum-based anticancer chemotherapy is exposed to severe adverse effects.⁴ For instance, systemic toxicities of cisplatin like nephrotoxicity, neurotoxicity, ototoxicity, and emetogenesis inflict serious injuries to patients during treatment, which severely restrict its efficacy.^{5,6} Moreover,

the intrinsic and acquired resistance hinders the efficacy of cisplatin in numerous cancers.^{7–9} Therefore, much effort has been devoted to developing novel antitumor drugs, which would be more efficient, less toxic, and more specific.

As we know, nickel plays a key role in the biological system. In 1975, the enzyme urease containing nickel was discovered.¹⁰ Since then, many nickel-containing or nickel-dependent enzymes have also been discovered.^{10,11} A number of nickel(II) complexes were reported to possess notable anti-HIV,¹² anticonvulsant,¹³ antibacterial,^{14,15} antifungal,^{16,17} anti-inflammatory,¹⁸ anti-leishmania,¹⁹ and antioxidant^{20–22} activities, as well as antiproliferative activity towards diverse cell lines.^{23–25}

Harmine, a natural β -carboline, is widely distributed in fungi,²⁶ plants,^{27–29} and mammalian and marine invertebrates.^{30,31} The biological and pharmacological activities of β -carbolines include antimicrobial,³² antithrombotic,³³ parasiticidal,³⁴ anti-HIV,³⁵ anti-Alzheimer,³⁶ and antitumor activities.³⁷ The antitumor activity of β -carbolines is mediated through various mechanisms, such as intercalation into DNA,³⁸ inhibition of topoisomerases I and II^{39,40} and kinase Eg5,⁴¹ blocking cell mitosis via inhibition of cyclin-dependent kinases (CDKs),^{42,43} and targeting specific cancer signaling pathways.

The antitumor activity of β -carboline complexes has caught the interest of medicinal chemists. The effect of an

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iridium(III) β -carboline complex on autophagy was studied⁴⁴ and a ruthenium(II) β -carboline complex was found to induce p53-mediated apoptosis.^{45,46} Here, a novel nickel and β -carboline complex **4a** was synthesized and characterized. Its cytotoxicity towards tumor cells and the underlying mechanisms were studied.

2. Results

2.1. Synthesis and characterization

2.1.1. Synthesis. The synthetic route to the novel nickel(II) complex (**4a**) is outlined in Scheme 1. The intermediate (**3**) was obtained from commercially available 5-methoxytryptamine (**1**) and pyridine-2-carbaldehyde (**2**) by Pictet-Spengler cyclization. 6-Methoxy-1-pyridine- β -carboline (**4**) was acquired from intermediate **3** by dehydrogenation which was catalyzed by Pd/C. **4a** was prepared through hydrothermal synthesis in 12:1 methanol/DMSO from 6-methoxy-1-pyridine- β -carboline (**4**) and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$.

2.1.2. Characterization. The obtained **4a** was characterized by UV-vis absorption spectroscopy, elemental analysis, ESI-MS, and single crystal X-ray diffraction analysis, the results of which are presented in Fig. 1, S1, S4 and S5.† X-ray diffraction analysis revealed that the Ni(II) atom rests in the center of a distorted octahedron and is coordinated by four nitrogen atoms from two 6-methoxy-1-pyridine- β -carboline ligands, one oxygen atom from the nitrate anion, and one oxygen atom from methanol. Selected bond lengths and angles are listed in Tables S3 and S4.† Fig. 1 shows the crystal structure of **4a**, which has a similar structure to $[\text{MnCl}_2(\text{phen})]\cdot\text{saox}$.⁴⁷

2.2. In vitro cytotoxicity test by MTT assay

The inhibition rates and IC_{50} values of **4a** against seven cell lines (MGC-803, Hep G2, T24, OS-RC-2, NCI-H460, SK-OV-3 and HL-7702) were determined by the MTT method, and they were compared with those of **4** and cisplatin. The IC_{50} (MGC-803, Hep G2, T24, OS-RC-2, NCI-H460 and SK-OV-3) values

Fig. 1 X-ray crystal structures of ligand **4** and complex **4a**.

(Fig. 2 and Table S5†) of **4a** were lower than those of **4** and cisplatin. Specifically, the IC_{50} value of **4a** for MGC-803 was $3.77 \pm 0.06 \mu\text{M}$, which is 4.6 times lower than that of cisplatin ($17.57 \pm 0.03 \mu\text{M}$).

2.3. Cell cycle assay

To further study the mechanism underlying the inhibitory activity of **4a** on MGC-803 cells, we investigated its effect on cell cycle progression. Hence, we monitored the cell cycle phase distribution of MGC-803 cells treated with 2.0 μM , 4.0 μM , and 6.0 μM concentrations of **4a** for 48 h. The cell cycle distribution was examined by measuring the DNA content of nuclei labeled with propidium iodide (PI) using flow cytometry. The cell cycle analysis in Fig. 3 shows that after treating the MGC-803 cells with **4a** for 48 h, the proportion of cells in the S phase increased to 33.34% and 53.74% for the 4.0 μM and 6.0 μM groups, respectively. At the same time, the proportions of cells in the G2 phase in the treatment groups were lower than that in the control. This result indicated that the MGC-803 cells were blocked in the S phase after treatment with **4a** for 48 h.

Scheme 1 Synthesis route to **4a**.

Fig. 2 IC₅₀ (μM) values of **4a**, **4** and cisplatin for seven human cell lines.

2.4. Study on the mechanism of S phase arrest

To investigate the mechanism of cell cycle arrest induced by **4a**, we examined the changes of the cyclin E1–CDK2 complex and cyclin A2–CDK2 complex in the cell cycle regulation system.⁴⁸ Fig. 4 indicates that **4a** down-regulated cyclin E1, cyclin A2 and CDK2 in MGC-803, resulting in cell cycle S phase arrest. Suppression of Akt phosphorylation resulted in a distinct increase of p27 expression. The inhibitory protein p27 acts on two subtypes of cyclin and cycle-dependent kinase complexes to inhibit CDK2 activity.⁴⁹ P27 was negatively regulated by oncogene p-AKT and blocked the cell cycle in the S phase through the Akt–p27–CDK2 pathway.

2.5. Cell apoptosis assay

The interaction of MGC-803 cells with **4a** was further investigated by PE-Annexin V/7-AAD staining to assess apoptosis induction (Fig. 5). Because phosphatidylserine (PS) exposure usually precedes the loss of cell membrane integrity in apoptosis, PE-Annexin V can be combined with PS. FITC-labeled Annexin-V was used as a probe to detect the occurrence of apoptosis by flow cytometry. PE-Annexin V (+)/7-AAD (–) (Q4) staining is considered as an indicator of early apoptosis. PE-Annexin V (+)/7-AAD (+) (Q2) staining indicated necrosis and the later period of apoptosis. The apoptosis ratios (sum of ratios in Q2 and Q4) caused by **4a** at different concentrations were found to be 12.7%, 17.43%, and 62.0% for the control, 4.0 μM, and 6.0 μM groups, respectively. The results in Fig. 5 suggested that apoptosis was induced after the MGC-803 cells were treated with **4a**.

2.6. Assessment of caspase-3 activation in apoptosis induction

The intrinsic pathway of apoptosis is regulated by the caspase family members. It is of obvious interest to determine which of the caspase family members actively participated in the signaling after the cells were treated with **4a**. Hence, flow cytometry was adopted to examine the abundance of candidate caspase members. The experiment took the advantage of FITC-DEVD-FMK (for caspase-3) probes. Fig. 6 shows that the proportion of active caspase-3 cells was 3.91%, 7.83%, 16.46%, and 57.75% for the control, 2.0 μM, 4.0 μM, and 6.0 μM

Fig. 3 FACS analysis of the cell cycle of MGC-803 cells by PI staining after treatment with **4a** (2.0 μM, 4.0 μM, and 6.0 μM) for 48 h.

Fig. 4 Detection of S phase-related proteins of MGC-803 cells by western blot after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h. (A) Western blot was used to determine the expression of p-AKT, p27, cyclin E1, cyclin A2, CDK2 and actin in MGC-803 cells treated with 4a (2 μ M, 4 μ M, and 6 μ M) for 48 h. (B) Densitometric analysis of p-AKT, p27, cyclin E1, cyclin A2 and CDK2 proteins normalized with actin. The relative expression of each protein is represented by the density of the protein band/density of the actin band. The mean SD was from three independent measurements. *** $P < 0.001$, compared to the control.

Fig. 5 FACS analysis of the apoptosis of MGC-803 cells by Annexin V-PE/7-AAD staining after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h.

μ M groups, respectively. The results indicated that 4a induced cell apoptosis by triggering caspase-3 activation in the MGC-803 cells.

2.7. Reactive oxygen species (ROS) generation in cells treated with 4a

Under normal circumstances, cells maintain balanced reactive oxygen species (ROS) generation and cellular antioxidant

system activity. When the balance is broken, collective generation of ROS leads to oxidative stress. Oxidative stress plays a vital role in influencing tumor cell behavior. Research found that ROS not only regulate subcellular functions but also induce cell death *via* the apoptotic pathway.⁵⁰ Moreover, accumulating evidence suggests that oxidative stress leads to mitochondrial apoptosis *via* regulating the activity of the Bcl-2 family of proteins.^{51,52} Flow cytometry with DCFH-DA staining was carried out to examine ROS generation in MGC-

Fig. 6 Activation of caspase-3 in MGC-803 cells after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h.

803 cells after they were treated with 4a. The DCFH-DA probe could be converted to the fluorescent DCF when bound with ROS. Fig. 7 shows that compared with the control, the level of ROS generation in the MGC-803 cells increased significantly after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h. Therefore, the cell apoptosis induced by 4a was closely related to the ROS-mediated caspase-initiated apoptosis pathway.

2.8. Ca^{2+} release in cell apoptosis

It is known that a close relationship exists between ROS generation and Ca^{2+} release at the intracellular level.⁵³ Increased

intracellular $[\text{Ca}^{2+}]_c$ is regarded as one of the major messenger molecules in apoptosis induction.⁵⁴ The intracellular $[\text{Ca}^{2+}]_c$ of the MGC-803 cells treated with 4a was evaluated by flow cytometry using the fluorescent probe Fluo-3/AM. Fig. 7 shows that after treatment with 4a (6.0 μ M), the $[\text{Ca}^{2+}]_c$ in the MGC-803 cells markedly increased by 28.4% compared with the control. This observation, combined with the previous results, clearly shows that ROS generation is a precursor for enhancing intracellular $[\text{Ca}^{2+}]_c$ levels. The findings here suggest that the apoptotic pathway induced by 4a occurred through ROS generation, as indicated by the enhanced intracellular $[\text{Ca}^{2+}]_c$ levels.

Fig. 7 (a) Increased generation of reactive oxygen species in the MGC-803 cells after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h. (b) Intracellular levels of $[\text{Ca}^{2+}]_c$ in the MGC-803 cells after treatment with 4a (2.0 μ M, 4.0 μ M, and 6.0 μ M) for 48 h. * P < 0.05, *** P < 0.001, compared to the control.

2.9. Effect of 4a on the apoptotic pathway

The mitochondrial pathway is one of the most important apoptosis pathways. Mitochondrial membrane permeability is modulated by the Bcl-2 family of proteins.⁵⁵ Bax is a pro-apoptotic protein that localizes principally in the cytoplasm and redistributes to mitochondria in response to apoptotic stimuli, where it induces cytochrome c release. Bax is a component of the mitochondrial pore, which allows some small substances (ions and molecules) to pass through the mitochondrial membrane into the cytoplasm.⁵⁶ Caspase activation also correlates with the Bax activity. Bcl-2 is an anti-apoptotic protein that localizes with apaf-1 on the mitochondrial membrane⁵⁷ and protects against collapse of the mitochondrial membrane, which would lead to release of mitochondrial substances such as cytochrome c and apoptotic protease activating factor-1 (apaf-1).⁵⁸ As shown by the western blot

Fig. 8 (A) Western blot was used to determine the expression of Bax, Bcl-2, cytochrome c and apaf-1 in MGC-803 cells treated with **4a** (2 μ M, 4 μ M, and 6 μ M) for 48 h. (B) Densitometric analysis of Bax, Bcl-2, cytochrome c and apaf-1 proteins normalized with GAPDH. The relative expression of each protein is represented by the density of the protein band/density of the GAPDH band. The mean SD was from three independent measurements. ** P < 0.01, *** P < 0.001, compared to the control.

results in Fig. 8, treatment of MGC-803 cells with **4a** for 48 h led to the down-regulation of Bcl-2 and up-regulation of Bax, cytochrome c and apaf-1. These results suggested that **4a** (2 μ M, 4 μ M, and 6 μ M) induced apoptosis by regulating the levels of Bax, Bcl-2, cytochrome c and apaf-1.

Apoptosis signals may activate caspase and the caspase cascade. Cytochrome c binds to apaf-1, which may trigger the activation of caspase-3/9 (caspase-3 as shown in Fig. 6 and 8), leading to apoptosis.^{59–61} It is well known that the activation of caspase-3 during apoptosis can cause the cleavage of PARP, a major indicator of the mitochondrial pathway. Our results showed that **4a** dose-dependently increased the expression of caspase-3/9 and PARP, which supports the theory that **4a** induced mitochondrial apoptosis which led to caspase cascade activation in the MGC-803 cells.

3. Discussion and conclusion

In this study, a new nickel(II) complex of 6-methoxy-1-pyridine- β -carboline (**4a**) was synthesized and fully characterized. It was present as a deformed octahedral mono-nuclear nickel(II) complex both in the crystal state and in the aqueous solution state, suggesting the high stability of the complex to maintain the coordinated state of 6-methoxy-1-pyridine- β -carboline (**4**) with nickel(II). The MGC-803 cell line was most sensitive to **4a**. In particular, **4a** showed higher selectivity against MGC-803 cells than ligand **4** and cisplatin. **4a** promoted cell apoptosis in MGC-803 cancer cells. The tightly regulated apoptosis extrinsic pathway and the mitochondrial-dependent intrinsic pathway are important in maintaining cellular homeostasis.⁵⁹ High concentrations of ROS can cause damage in DNA, proteins and lipids, which results in cell death. Moreover, growing evidence suggests that oxidative stress causes mitochondrial dysfunction.⁶² We found that treatment of MGC-803

with **4a** led to enhanced apoptosis which can be caused by reactive oxygen species (ROS)-mediated mitochondrial dysfunction. Additionally, treatment with **4a** resulted in enhanced PARP cleavage and caspase activation.⁵⁰

Furthermore, **4a** could induce cell cycle arrest at the S phase through the Akt-p27-CDK2 pathway, suggesting its different antitumor mechanism compared with cisplatin. **4a** caused the down-regulation of cyclin E1, cyclin A2, CDK2, and p-AKT and the up-regulation of p27.

The relationship between the Akt-p27-CDK2 pathway and mitochondrial apoptosis can be explained by AKT. Bax is regulated by phosphorylation of Ser184 in an Akt-dependent manner and that phosphorylation inhibits Bax effects on the mitochondria by maintaining the protein in the cytoplasm, heterodimerized with anti-apoptotic Bcl-2 family members.⁶³

Previous reports indicate that the apoptosis mechanism involved Akt inactivation, which directly correlated with Bax translocation to mitochondria, cytochrome c release to the cytosol, and the associated activation of caspase-9 and caspase-3, culminating in prominent apoptosis.^{63,64}

We also observed that **4a** decreased the expression of p-AKT and markedly suppressed serine/threonine kinase AKT/PKB (AKT) phosphorylation and kinase activity in cultured MGC-803 cells. Because p-AKT negatively regulates the expression of p27 and Bax, treatment with **4a** increased the expression of p27 and Bax.

Taken together, the results demonstrated that **4a** could induce apoptosis and render cell cycle arrest at the S phase in cancer cells. It should be further evaluated as a potential chemotherapeutic agent for human cancers.

Conflicts of interest

The authors declare no competing interests.

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Notes and references

- B. Rosenberg, F. Charles and P. Kettring, *Cancer*, 1985, **55**, 2303–2316.
- P. U. Maheswari, D. S. M. Van, S. Smulders, S. Barends, G. P. van Wezel, C. Massera, S. Roy, D. H. Den, P. Gamez and J. Reedijk, *Inorg. Chem.*, 2008, **47**, 3719–3727.
- A. R. Banerjee, J. A. Jaeger and D. H. Turner, *Biochemistry*, 1993, **32**, 153–163.
- X. Wang and Z. Guo, *Chem. Soc. Rev.*, 2013, **42**, 202.
- M. W. English, R. Skinner, A. D. J. Pearson, L. Price, R. Wyllie and A. W. Craft, *Br. J. Cancer*, 1999, **81**, 336–341.
- R. Skinner, A. D. Pearson, M. W. English, L. Price, R. A. Wyllie, M. G. Coulthard and A. W. Craft, *Br. J. Cancer*, 1998, **77**, 1677–1682.
- E. Wong and C. M. Giandomenico, *ChemInform*, 1999, **30**, 2451–2466.
- R. D. Baird and S. B. Kaye, *Eur. J. Cancer*, 2003, **39**, 2450–2461.
- N. E. Dixon, C. Gazzola, R. L. Blakeley and B. Zerner, *J. Am. Chem. Soc.*, 1975, **97**, 4131–4133.
- F. Meyer and H. Kozłowski, *Compr. Coord. Chem. II*, 2003, 247–554.
- P. Zimmermann and C. Limberg, *J. Am. Chem. Soc.*, 2017, **139**, 4233–4242.
- C. W. Hsu, C. F. Kuo, S. M. Chuang and M. H. Hou, *BioMetals*, 2013, **26**, 1–12.
- G. Morgant, N. Bouhaida, L. Balde, N. E. Ghermani and J. D'Angelo, *Polyhedron*, 2006, **25**, 2229–2235.
- K. Skyrianou, E. Efthimiadou, V. Psycharis, A. Terzis, D. Kessissoglou and G. Psomas, *J. Inorg. Biochem.*, 2009, **103**, 1617–1625.
- R. Kurtaran, L. T. Yildirim, A. D. Azaz, H. Namli and O. Atakol, *J. Inorg. Biochem.*, 2005, **99**, 1937–1944.
- B. Xu, P. Shi, Q. Guan, X. Shi and G. Zhao, *J. Coord. Chem.*, 2013, **66**, 2605–2614.
- K. Alomar, A. Landreau, M. Allain, G. Bouet and G. Larcher, *J. Inorg. Biochem.*, 2013, **126**, 76–83.
- H. B. Shawish, Y. W. Wan, L. W. Yi, W. L. Sheng, C. Y. Looi, P. Hassandarvish, A. Y. L. Phan, W. F. Wong, H. Wang and I. C. Paterson, *PLoS One*, 2014, **9**, e100933.
- I. Ramírezmacías, C. R. Maldonado, C. Marín, F. Olmo, R. Gutiérrez-sánchez, M. J. Rosales, M. Quirós, J. M. Salas and M. Sánchezmoreno, *J. Inorg. Biochem.*, 2012, **112**, 1–9.
- K. C. Skyrianou, F. Perdih, A. N. Papadopoulos, I. Turel, D. P. Kessissoglou and G. Psomas, *J. Inorg. Biochem.*, 2011, **105**, 1273–1285.
- P. Sathyadevi, P. Krishnamoorthy, E. Jayanthi, R. R. Butorac, A. H. Cowley and N. Dharmaraj, *Inorg. Chim. Acta*, 2012, **384**, 83–96.
- Y. Li, Z. Y. Yang and J. C. Wu, *Eur. J. Med. Chem.*, 2010, **45**, 5692–5701.
- A. Buschini, S. Pinelli, C. Pellacani, F. Giordani, M. B. Ferrari, F. Bisceglie, M. Giannetto, G. Pelosi and P. Tarasconi, *J. Inorg. Biochem.*, 2009, **103**, 666–677.
- F. Bisceglie, S. Pinelli, R. Alinovi, M. Goldoni, A. Mutti, A. Camerini, L. Piola, P. Tarasconi and G. Pelosi, *J. Inorg. Biochem.*, 2014, **140**, 111–125.
- S. Betanzos-Lara, C. Gómez-Ruiz, L. R. Barrón-Sosa, I. Gracia-Mora, M. Flores-Álamo and N. Barba-Behrens, *J. Inorg. Biochem.*, 2012, **114**, 82–93.
- A. Teichert, J. Schmidt, A. Porzel, N. Arnold and L. Wessjohann, *J. Nat. Prod.*, 2007, **70**, 1529–1531.
- Y. Chen, P. Kuo, H. Chan, I. Kuo, F. Lin, C. Su, M. Yang, D. Li and T. Wu, *J. Nat. Prod.*, 2010, **73**, 1993–1998.
- D. T. Youssef, *J. Nat. Prod.*, 2001, **64**, 839–841.
- S. L. Wong, H. S. Chang, G. J. Wang, M. Y. Chiang, H. Y. Huang, C. H. Chen, S. C. Tsai, C. H. Lin and I. S. Chen, *J. Nat. Prod.*, 2011, **74**, 2489–2496.
- P. S. Kearns and J. A. Rideout, *J. Nat. Prod.*, 2008, **71**, 1280–1282.
- D. Inman, W. M. Bray, N. C. Gassner, R. S. Lokey, K. Tenney, Y. Y. Shen, K. Tendyke, T. Suh and P. Crews, *J. Nat. Prod.*, 2010, **73**, 255–257.
- D. J. McKenna, G. H. Towers and F. Abbott, *J. Ethnopharmacol.*, 1984, **12**, 179–211.
- N. Sunderplassmann, V. Sarli, M. Gartner, M. Utz, J. Seiler, S. Huemmer, T. U. Mayer, T. Surrey and A. Giannis, *Bioorg. Med. Chem.*, 2005, **13**, 6094–6111.
- T. Takeuchi, S. Oishi, T. Watanabe, H. Ohno, J. Sawada, K. Matsuno, A. Asai, N. Asada, K. Kitaura and N. Fujii, *J. Med. Chem.*, 2011, **54**, 4839–4846.
- Y. Song, J. Wang, S. F. Teng, D. Kesuma, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi and M. M. Sim, *ChemInform*, 2002, **12**, 1129–1132.
- A. C. Castro, L. C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella and J. Adams, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2419–2422.
- Y. Chen, M. Y. Qin, L. Wang, H. Chao, L. N. Ji and A. L. Xu, *Biochimie*, 2013, **95**, 2050–2059.
- Z. Taira, S. Kanzawas, C. Dohara, S. Ishida, M. Matsumoto and Y. Sakiya, *Jpn. J. Toxicol. Environ. Health*, 1997, **43**, 83–91.
- R. Cao, W. Peng, H. Chen, Y. Ma, X. Liu, X. Hou, H. Guan and A. Xu, *Biochem. Biophys. Res. Commun.*, 2005, **338**, 1557–1563.
- Y. Song, J. Wang, S. F. Teng, D. Kesuma, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi and M. M. Sim, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1129–1132.

- 41 P. A. Barsanti, W. Wang, Z. Ni, D. Duhl, N. Brammeier and E. Martin, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 157–160.
- 42 Y. Song, J. Wang, S. F. Teng, D. Kesuma, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi and M. M. Sim, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1129–1132.
- 43 Y. Song, D. Kesuma, J. Wang, Y. Deng, J. Duan, J. H. Wang and R. Z. Qi, *Biochem. Biophys. Res. Commun.*, 2004, **317**, 128–132.
- 44 L. He, S. Y. Liao, C. P. Tan, Y. Y. Lu, C. X. Xu, L. N. Ji and Z. W. Mao, *Chem. Commun.*, 2014, **50**, 5611–5614.
- 45 C. Tan, S. Lai, S. Wu, S. Hu, L. Zhou, Y. Chen, M. Wang, Y. Zhu, W. Lian, W. Peng, L. Ji and A. Xu, *J. Med. Chem.*, 2010, **53**, 7613–7624.
- 46 J. S. Lan, S. S. Xie, S. Y. Li, L. F. Pan, X. B. Wang and L. Y. Kong, *Bioorg. Med. Chem.*, 2014, **22**, 6089–6104.
- 47 E. Yang, J. Zhang, Y. B. Chen, Y. Kang, Y. Y. Qin, Z. Li, J. K. Cheng, R. F. Hu, Y. H. Wen and Y. G. Yao, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2004, **60**, 390–391.
- 48 D. O. Morgan, *Annu. Rev. Cell Dev. Biol.*, 1997, **13**, 261–291.
- 49 N. Horie, T. Mori and H. Asada, et al. Implication of CDK inhibitors p21 and p27 in the differentiation of HL-60 cells, *Biol. Pharm. Bull.*, 2004, **7**, 992–997.
- 50 F. Salehi, H. Behboudi and G. Kavosi, *RSC Adv.*, 2017, **68**, 43141–43150.
- 51 D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery*, 2009, **8**, 579–591.
- 52 H. U. Simon, A. Hajyehia and F. Levischaffer, *Apoptosis*, 2000, **5**, 415–418.
- 53 G. I. Giles, *Curr. Pharm. Des.*, 2006, **12**, 4427–4443.
- 54 V. Pachauri, A. Mehta, D. Mishra and S. J. Flora, *Neurotoxicology*, 2013, **35**, 137–145.
- 55 J. C. Sharpe, D. Arnoult and R. J. Youle, *Biochim. Biophys. Acta*, 2004, **1644**, 107.
- 56 E. Er, L. Oliver, P. F. Cartron, P. Juin, S. Manon and F. M. Vallette, *Biochim. Biophys. Acta*, 2006, **1757**, 1301.
- 57 Y. Yun, M. Zong and W. Xu, *Chem.-Biol. Interact.*, 2017, **262**, 38–45.
- 58 D. R. Green and J. C. Reed, *Science*, 1998, **281**, 1309.
- 59 H. Zou, W. J. Henzel, X. Liu, A. Lutschg and X. Wang, *Cell*, 1997, **90**, 405–413.
- 60 N. Inohara, T. Koseki, P. L. Del, Y. Hu, C. Yee, S. Chen, R. Carrio, J. Merino, D. Liu and J. Ni, *J. Biol. Chem.*, 1999, **274**, 14560–14567.
- 61 M. Zhou, Y. Li, Q. Hu, X. C. Bai, W. Huang, C. Yan, S. H. Scheres and Y. Shi, *Genes Dev.*, 2015, **29**, 2349–2361.
- 62 A. K. Mukherjee, A. J. Saviola, P. D. Burns and S. P. Mackessy, *Apoptosis*, 2015, **20**, 1358–1372.
- 63 Z. Zhang, G. H. Lai and A. E. Sirica, *Hepatology*, 2004, **39**, 1028.
- 64 F. Tsuruta, N. Masuyama and Y. Gotoh, *J. Biol. Chem.*, 2002, **277**, 14040–14047.