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Synthesis and biological evaluation of 3-nitro-4-chromanone derivatives as potential antiproliferative agents for castration-resistant prostate cancer†

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A series of novel 3-nitro-4-chromanones were synthesized and their *in vitro* cytotoxicity was evaluated on castration-resistant prostate cancer cell (CRPC) lines using the sulforhodamine B (SRB) assay. The amide derivatives showed more potent antitumor activity than their corresponding ester derivatives. Most of the tested compounds showed less toxicity towards human fibroblasts (HAF) compared with the tumor cell lines. The optimal compound **36** possessed much more potent antiproliferative activity than the positive compound cisplatin. The colony formation, cell cycle distribution, apoptosis, transwell migration and wound healing assays of **36** were performed on CRPC cell lines.

Introduction

Prostate cancer (PC) is a type of malignancy that arises in the prostate gland and it tends to develop in older men. Globally, prostate cancer is the second most common cancer among men. The International Agency for Research on Cancer (IARC) estimated 1.27 million new PC cases and 359 000 deaths in 2018 worldwide.[1] Although various anti-cancer agents are used solely or in combination with radiotherapy to treat advanced diseases, none of the conventional therapies have been proven to be highly successful for PC.[2] It often finally develops into fatal castration-resistant prostate cancer (CRPC) with the ability to grow in the absence of androgens.[3] CRPC is not responsive to hormonal therapy, readily re-emerges and is highly metastatic, resulting in most of the deaths in PC patients.[4] The presence of androgen receptor (AR)-negative cell populations in CRPC has been identified and new therapeutic strategies targeting AR-negative PC cells would provide a potential approach for treatment of CRPC.[5] The AR-negative metastatic DU145 and PC3 cell lines[6] are often studied as *in vitro* models for CRPC.[7] Studies disclosed that 3-nitro-4-chromanones possessed anticancer activity against murine leukemia L1210 (Fig. 1, **A** and **B**),[8,9] human colon HT-29 (Fig. 1, **B**)[9] and human acute myeloid leukemia U937 cell line (Fig. 1, **C**).[10] Our previous research discovered that 3-nitro-4-chromanone derivatives (Fig. 1, **D**), especially compound **1** (Table 1) exhibited as potent anti-proliferative activity as cisplatin against CRPC-like DU145 and PC3 cell lines.[11] Herein, we have evaluated the antiproliferative activity of these newly synthesized 3-nitro-4-chromanones in DU145, PC3 and its more metastatic derivative PC3M cell lines[12] and disclosed the preliminary structure–activity relationships (SAR) of these compounds. Further investigations including colony formation, cell cycle distribution, apoptosis and migration have also been performed.

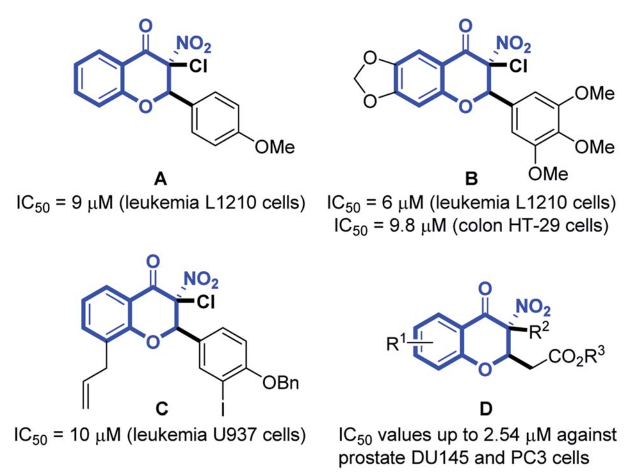

Fig. 1 Structures of reported antitumor 3-nitro-4-chromanones.

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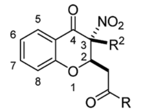
‡ These authors contributed equally to this work.

Table 1 IC_{50} values of 3-nitro-4-chromanones against the growth of prostate cancer cell lines[a]


1-32 (R^2 = Me)
33 and **34** (R^2 = Et)

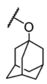
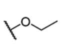
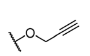
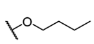
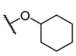
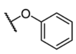
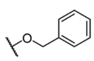
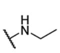
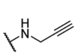
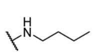
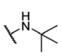
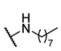
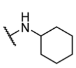
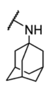
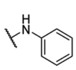
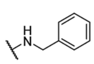
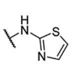
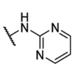
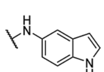
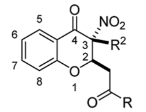
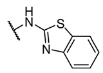
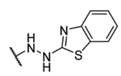
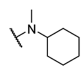
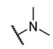
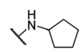
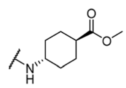
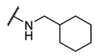
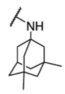
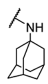
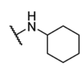
No.	R^2	R^2	DU145 (IC_{50}[b] (μM))	PC3 (IC_{50}[b] (μM))	HAF (IC_{50}[b] (μM))
1[c]		Me	2.54 ± 0.27	10.60 ± 0.68	>100
3a	OH	Me	>50	>50	>100
4[c]		Me	>50	>50	>100
5[b]		Me	>50	>50	>100
6		Me	>50	26.21 ± 1.42	>100
7[c]		Me	21.08 ± 1.26	>50	>100
8[c]		Me	20.91 ± 1.36	32.93 ± 2.96	>100
9[c]		Me	>50	>50	>100
10		Me	32.26 ± 1.51	20.13 ± 1.30	>100
11	NH_2	Me	46.23 ± 1.67	23.24 ± 1.37	>100
12		Me	24.66 ± 1.39	>50	>100
13		Me	18.69 ± 1.27	9.35 ± 0.97	>100
14		Me	38.17 ± 1.58	12.97 ± 1.11	>100
15		Me	15.67 ± 1.20	9.97 ± 1.00	>100
16		Me	5.01 ± 0.70	10.03 ± 1.00	>100
17		Me	6.81 ± 0.83	4.83 ± 0.68	>100
18		Me	5.09 ± 0.71	3.35 ± 0.52	>100
19		Me	1.73 ± 0.24	3.09 ± 0.79	>100
20		Me	20.32 ± 1.31	6.44 ± 0.81	>100
21		Me	19.52 ± 1.29	9.91 ± 1.00	>100
22		Me	49.24 ± 3.04	36.90 ± 0.98	>100
23		Me	>50	>50	>100
24		Me	23.94 ± 1.89	15.82 ± 0.33	>100

Table 1 (*Contd.*)


1-32 (R^2 = Me)
33 and **34** (R^2 = Et)

No.	R^2	R^2	DU145 (IC_{50}[b] (μM))	PC3 (IC_{50}[b] (μM))	HAF (IC_{50}[b] (μM))
25		Me	>50	>50	>100
26		Me	29.7 ± 2.21	>50	>100
27		Me	10.58 ± 1.02	>50	>100
28		Me	>50	>50	>100
29		Me	8.00 ± 0.90	>50	>100
30		Me	14.36 ± 1.16	>50	>100
31		Me	4.22 ± 0.63	43.90 ± 1.64	>100
32		Me	5.41 ± 0.73	11.43 ± 1.06	>100
33		Et	5.16 ± 0.71	6.81 ± 0.83	>100
34		Et	11.08 ± 1.04	>50	>100
Cisplatin			2.80 ± 0.45	18.20 ± 1.26	32.63 ± 1.51

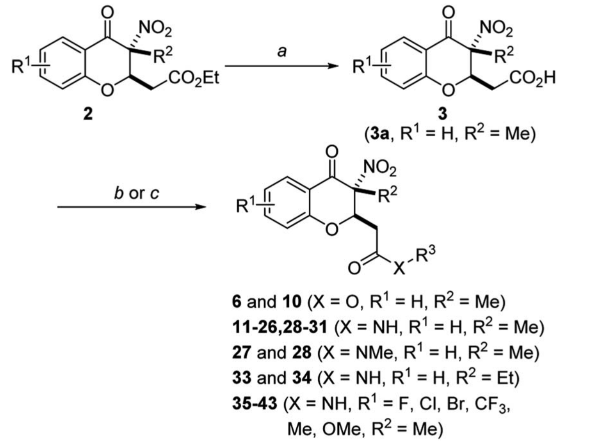
[a] From SRB assay after 96 h of treatment. [b] IC_{50} data are an average of at least 3 independent experiments. [c] These compounds were reported previously.[11]

Results and discussion

Chemistry

The general strategies developed for compounds synthesis is described as follows (Scheme 1). The esters **2**, previously synthesized in our group, were used as starting compounds. Hydrolysis of the esters **2** with 6 N HCl in H_2O/1,4-dioxane to provide the corresponding acids **3**. Afterwards, ester compounds **6** and **10** were obtained by condensation of **3** with corresponding alcohols under thionyl chloride. Amide compounds **11–43** were obtained by condensation of **3** with corresponding amines under 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), 1-

hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA) in CH_2Cl_2. Compound **44** was obtained by reduction of the nitro group of compound **36**. Structures were well characterized with ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrum.



Scheme 1 General synthetic route of compound **3–43**. Reagent and conditions: (a) 6 N HCl/1,4-dioxane, 100 °C, 95% for **3a**; (b) alcohols, $SOCl_2$, 1,4-dioxane, 70 °C, 68–81%; (c) amines, HOBt, EDCI, DIPEA, DCM, rt, 64–88%.

Biological activities

Antiproliferative activity. To evaluate the antiproliferative activity of the first-round synthetic 3-nitro-4-chromanones, compounds **1–34** (Table 1) were screened using the SRB assay in AR-negative PC cell lines (DU145 and PC3).

For the carboxylic acid (**3a**) and its ester compounds (**4–10**), the results showed that most compounds exhibiting moderate to weak antitumor activity. The carboxylic acid and ester compounds bearing small substituents (**4** and **5**) possessed almost no activity. The antiproliferative activity was increased to moderate as the small ester group was replaced by relatively large ester substituents (**8** and **10**), except phenyl ester group (**9**).

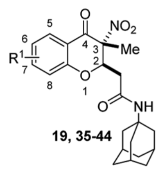
For C-2 amide compounds, they showed obviously improved IC_{50} values than their corresponding ester compounds (**19**, **12**, **13**, **14**, **15**, **18**, **20** and **21** *vs.* **1**, **4**, **5**, **6**, **7**, **8**, **9** and **10**). For alkyl amides, the antiproliferative activity was improved as the substituents increased from small amino (**11**, IC_{50} = 46.23 and 23.24 μM) and aminomethyl (**12**, IC_{50} = 24.66 and >50 μM) to large *n*-octylamine (**17**, IC_{50} = 6.81 and 4.83 μM) and especially cyclohexane (**18**, IC_{50} = 5.09 and 3.35 μM) and adamantanamine (**19**, IC_{50} = 1.73 and 3.09 μM) groups. For aryl amides, the phenyl amide (**20**) and benzyl amide (**21**) showed moderate antitumor activity, and most aromatic heterocyclic amides (**22–26**) showed moderate to weak or even no activity. Therefore, the aryl amide substituents were unfavorable substituted groups for improving antitumor activity. The cyclohexyl amide (**18**) and adamantane amide (**19**) were key structures for maintaining the potent antitumor activity. So, we further synthesized the derivatives (**27**, **29–34**) based on the compounds **18** and **19**. For the derivatives **27**, **29–31** and **34** (the C-3 methyl group was replaced by ethyl group), their antitumor activity, especially in PC3 cells, decreased significantly compared with **18**. The trialkylamines **27** and **28** showed the decreased activity, which means the acidic proton for amide derivative can be essential for the activity. For the derivatives **32** and **33** (the C-3 methyl group was replaced by ethyl group), the antitumor activity also decreased obviously compared with **19**. In Table 1, the most potent compound **19**, which bearing an adamantane amide substituent, possessed 1.6-fold (in DU145 cells) and 5.9-fold (in PC3 cells) more potent antiproliferative activity than the positive compound cisplatin. These results illustrated that the adamantane amide group showed more potent activity than other amide groups.

The second-round synthetic compounds **35–44** were obtained by modification of the benzene ring of compound **19** and their antiproliferative activity was screened using the SRB assay in DU145, PC3 and PC3M cell lines (Table 2). Results discovered that most compounds possessed moderate to potent antitumor activity, except **44**. Most compounds displayed almost no toxicity on human fibroblasts (HAF), except **38**. We introduced groups $-CF_3$, –F, –Cl, –Br, –Me and –OMe into the C-6 position of the benzene ring of **19** firstly. The electron withdrawing $-CF_3$ (**35**), –F (**36**), –Cl (**37**) and –Br (**38**) groups were favorable substituted groups for improving antitumor activity, except the compound **35** on PC3M cells. The electron donating –Me (**39**) and –OMe (**40**) groups decreased the antitumor activity obviously compared with **19**. Compound **38** possessed the most potent antiproliferative activity (IC_{50} values were about 0.5 μM) in DU145, PC3 and PC3M cells, while it also displayed potent toxicity on HAF cells (IC_{50} = 3.67 μM). The second-best active compound **36** (bearing 6-F group), which IC_{50} on HAF was more than 100 μM. So, **36** and its –F group was regarded as the optimal compound and substituent. The –F group was also introduced into the C-5 (**41**), C-7 (**42**) and C-8 (**43**) positions of the benzene ring of **19** and their antitumor activity was decreased slightly compared with **36**. If the nitro group was reduction to amino group at C-3 position of **36**, the antitumor activity was significantly decreased (IC_{50} > 50 μM). We have also tested the activity of compound **36** on AR-positive prostate cancer cell lines LNCaP (IC_{50} = 4.4 μM) and 22RV1 (IC_{50} = 7.43 μM), which were much weaker than on AR-negative prostate cancer cell lines DU145 (IC_{50} = 1.21 μM) and PC3 (IC_{50} = 0.94 μM).

Accordingly, although the antitumor activity of compound **36** was much weaker than the positive compound docetaxel, it was 2.3–19.4 times more potent than the positive compound cisplatin and displayed almost no toxicity on normal cells, thus it was selected for further evaluation.

Selective cytotoxicity towards cancer cells. The major challenge in the development of novel anticancer compounds is their selectivity towards cancer cells. All these synthetic compounds were chosen for selectivity test in human skin fibroblast (HAF) cells. The results (Tables 1 and 2) revealed that most of the tested compounds were almost no toxicity on human fibroblasts. The most potent compound **38** also possessed potent toxicity on HAF cells, which showed poor

Table 2 IC_{50} values of 3-nitro-4-chromanones against the growth of prostate cancer cell lines[a]



19, 35-44

		IC_{50}[b] (μM)			
No.	R^1	DU145	PC3	PC3M	HAF
19	H	1.73 ± 0.24	3.09 ± 0.79	7.31 ± 1.17	>100
35	6-CF_3	1.63 ± 0.21	1.69 ± 0.53	12.88 ± 1.11	>100
36	6-F	1.21 ± 0.08	0.94 ± 0.03	5.66 ± 1.05	>100
37	6-Cl	1.34 ± 0.13	3.07 ± 0.79	6.34 ± 0.80	>100
38	6-Br	0.47 ± 0.02	0.51 ± 0.03	0.45 ± 0.24	3.67 ± 0.56
39	6-Me	1.75 ± 0.54	6.39 ± 0.81	19.20 ± 1.58	>100
40	6-MeO	2.62 ± 0.42	6.77 ± 0.83	13.06 ± 1.42	>100
41	5-F	2.27 ± 0.66	2.91 ± 0.70	9.75 ± 0.99	>100
42	7-F	2.24 ± 0.65	3.03 ± 0.78	12.46 ± 1.40	>100
43	8-F	2.35 ± 0.67	3.02 ± 0.78	9.16 ± 1.26	>100
44[c]	6-F	>50	>50	>50	>100
Docetaxel		0.0076 ± 0.0005	0.013 ± 0.003	0.043 ± 0.005	0.157 ± 0.031
Cisplatin		2.80 ± 0.45	18.20 ± 1.26	12.86 ± 1.11	32.63 ± 1.51

[a] From SRB assay after 96 h of treatment. [b] IC_{50} data are an average of at least 3 independent experiments. [c] Replacement of nitro group with amine group at 3-position.

selectivity towards cancer cells. The optimal compound **36** showed up to 106.4 times more selective towards PC3 cells than human fibroblasts which was much better than docetaxel and cisplatin.

Effect on the inhibition of cell colony formation. Colony formation assay not only reveals the proliferation potential of a single cancer cell but also evaluate adaptability of cancer cells to the culture environment.[13] To further determine the anti-proliferation effect of **36**, colony formation assay was conducted. Colonies were enumerated after 7 days (Fig. 2). Results discovered that **36** could concentration-dependently inhibit the colony formation in DU145, PC3 and PC3M cell lines. The ability in reduction of colony formation of **36** was much more potent than the positive compound cisplatin.

Effect on cell cycle distribution of DU145 cells. In order to examine whether the antiproliferative effect of **36** is associated with cell cycle arrest,[14] we tested the effect of **36** on cell cycle distribution using flow cytometry. As shown in Fig. 3, compound **36** concentration-dependently caused the sustained arrest at S phase. After 24 h of incubation with 10 μM of **36**, the percentage of S phase cells increased from 40.57% to 57.27%,

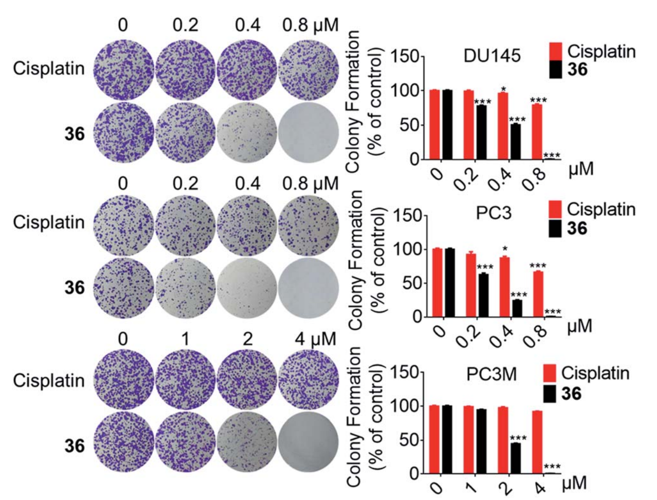


Fig. 2 The colony formation ability of DU145, PC3 and PC3M cells was inhibited by **36**. *p < 0.05, ***p < 0.001.

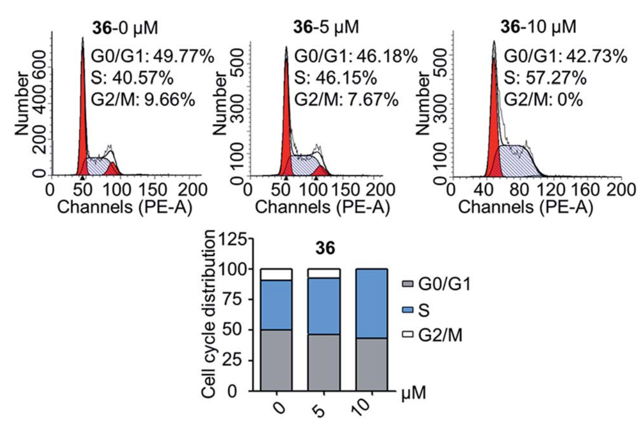


Fig. 3 Compound **36** disrupted the cell cycle distribution of DU145 cells.

while the cell population in the G2/M phase decreased from 9.66% to 0%.

Induction of cell apoptosis. To understand whether compound **36** affected cell viability through inducing cell apoptosis,[15] apoptosis assay was explored in DU145 cells using flow cytometry. As shown in Fig. 4A, the percentage of apoptotic cells significantly increased from 2.98% to 25.01% with doses of **36** increased from 0 to 10 μM after 48 h treatment. The results suggested that this compound inhibited cell proliferation through inducing apoptosis in the cell line mainly.

Western blot analysis. PARP play a key role in cell apoptosis,[16] thus we also detected the expression of poly ADP-ribose polymerase (PARP) and Cleaved PARP (CL.PARP) in DU145 cells after **36** treatment. The results showed that **36** dcould promote the PARP to CL. PARP (Fig. 4B) and its induction effect is superior to the apoptosis rate of cisplatin.

Effect on the inhibition of DU145 cell migration. Migration is a key step during the metastasis of cancer.[17] To determine anti-migration ability of compound **36**, transwell migration (Fig. 5A) and wound healing (Fig. 5B) assays were performed. The results showed that **36** notably prevented migration of DU145 cells in a concentration-dependent manner. The migration rate of 5 and 10 μM compound **36** treated DU145 cells was 52.78% and 23.94% respectively in comparison with control (0 μM).

In conclusion, a series of 3-nitro-4-chromanone derivatives were synthesized by a facile and convenient method. Their antiproliferative activity in CRPC cell lines were assessed *in vitro*. The amide derivatives showed more potent antitumor activity than their corresponding ester derivatives. Most of these compounds possessed more selectivity towards tumor cells than human fibroblasts. The C-2 adamantane amide and 6-F were favorable substituents for improving antitumor activity. The optimal compound **36**, which possessed 2.3–19.4 times more potent than the positive cisplatin in antitumor activity. **36** also displayed more potent than that of cisplatin in colony formation, apoptosis, transwell migration and wound healing assays. The primary mechanism studies disclosed that **36** led to S phase cell cycle arrest and promoted the PARP to CL. PARP in tumor cells. Collectively, we reported 3-nitro-4-chromanone derivatives as a series of new chemical entities for the first time. Especially **36**, which displayed potent antitumor activity in CRPC cell lines *in vitro*, could be used as a promising lead for further development.

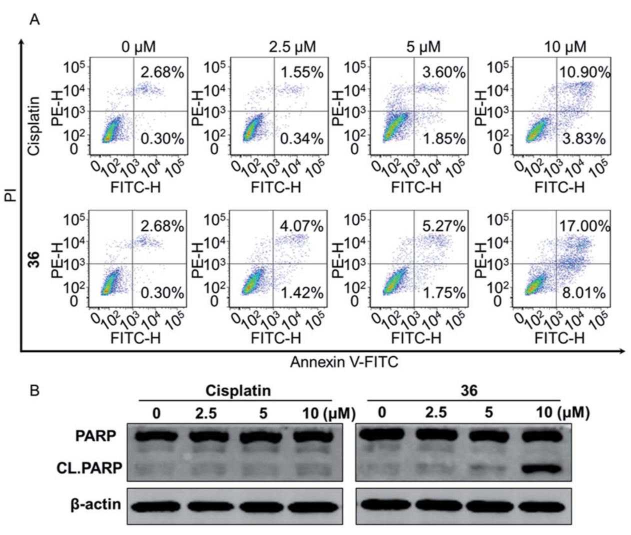


Fig. 4 (A) Compound **36** induced cell apoptosis significantly. (B) The expression of PARP and CL.PARP upon **36** treatment.

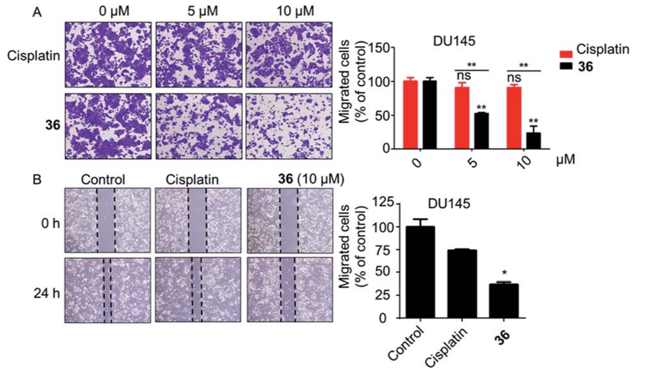


Fig. 5 The cell migration ability of DU145 cells was notably inhibited by **36**. (A) Transwell migration assay. (B) Wound healing migration assay (10 μM of **36**). *$p < 0.05$, **$p < 0.01$.

Conflicts of interest

The authors declare no conflicts of interest.

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References

1 (*a*) F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre and A. Jemal, *Ca-Cancer J. Clin.*, 2018, **68**, 394; (*b*) C.-C. Weng, P.-Y. Ding, Y.-H. Liu, J. R. Hawse, M. Subramaniam, C.-C. Wu, Y.-C. Lin, C.-Y. Chen, W.-C. Hung and K.-H. Cheng, *Oncogene*, 2019, **38**, 2005; (*c*) J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D. M. Parkin, M. Piñeros, A. Znaor and F. Bray, *Int. J. Cancer*, 2019, **144**, 1941.

2 (*a*) G. Attard, C. Parker, R. A. Eeles, F. Schröder, S. A Tomlins, I. Tannock, C. G. Drake and J. S. de Bono, *Lancet*, 2016, **387**, 70; (*b*) C. Parker, S. Gillessen, A. Heidenreich and A. Horwich, *Ann. Oncol.*, 2015, **26**(suppl. 5), v69; (*c*) T. Wüstemann, U. Haberkorn, J. Babich and W. Mier, *Med. Res. Rev.*, 2019, **39**, 40.

3 (*a*) P. A. Watson, V. K. Arora and C. L. Sawyers, *Nat. Rev. Cancer*, 2015, **15**, 701; (*b*) Y. Yamamoto, Y. Loriot, E. Beraldi, F. Zhang, A. W. Wyatt, N. Al Nakouzi, F. Mo, T. Y. Zhou, Y. Kim, B. P. Monia, A. R. MacLeod, L. Fazli,

Y. Z. Wang, C. C. Collins, A. Zoubeidi and M. Gleave, *Clin. Cancer Res.*, 2015, **21**, 1675; (*c*) J. W. Russo, C. Gao, S. S. Bhasin, O. S. Voznesensky, C. Calagua, S. Arai, P. S. Nelson, B. Montgomery, E. A. Mostaghel, E. Corey, M.-E. Taplin, H. H. Ye, M. Bhasin and S. P. Balk, *Cancer Res.*, 2018, **78**, 6354.

4 (*a*) T. A. Yap, A. D. Smith, R. Ferraldeschi, B. Al-Lazikani, P. Workman and J. S. de Bono, *Nat. Rev. Drug Discovery*, 2016, **5**, 699; (*b*) Y. Zong and A. S. Goldstein, *Nat. Rev. Urol.*, 2013, **10**, 90; (*c*) A. Davies, V. Conteduca, A. Zoubeidi and H. Beltran, *European Urology Focus*, 2019, **5**, 147.

5 C. Tran, S. Ouk, N. J. Clegg, Y. Chen, P. A. Watson, V. Arora, J. Wongvipat, P. M. Smith-Jones, D. Yoo, A. Kwon, T. Wasielewska, D. Welsbie, C. Degui Chen, C. S. Higano, T. M. Beer, D. T. Hung, H. I. Scher, M. E. Jung and C. L. Sawyers, *Science*, 2009, **324**, 787.

6 A. van Bokhoven, M. Varella-Garcia, C. Korch, W. U. Johannes, E. E. Smith, H. L. Miller, S. K. Nordeen, G. J. Miller and M. S. Lucia, *Prostate*, 2005, **57**, 205.

7 (*a*) T.-C. Shih, R. W. Liu, C.-T. Wu, X. C. Li, W. W. Xiao, X. J. Deng, S. Kiss, T. Wang, X.-J. Chen, R. Carney, H.-J. Kung, Y. Duan, P. M. Ghosh and K. S. Lam, *Clin. Cancer Res.*, 2015, **21**, 1675; (*b*) M. Recagni, M. L. Greco, A. Milelli, A. Minarini, N. Zaffaroni, M. Folini and C. Sissi, *Eur. J. Med. Chem.*, 2019, **177**, 401.

8 D. Dauzonne, B. Folléas, L. Martinez and G. G. Chabot, *Eur. J. Med. Chem.*, 1997, **32**, 71.

9 A. G. de Peredo, S. Léonce, C. Monneret and D. Dauzonne, *Chem. Pharm. Bull.*, 1998, **46**, 79.

10 S. Bouchet, M. Piedfer, S. Susin, D. Dauzonne and B. Bauvois, *AIMS Mol. Sci.*, 2016, **3**, 368.

11 H. Q. Chen, J. Xie, D. Xing, J. P. Wang, J. Tang, Z. F. Yi, F. Xia, W.-W. Qiu and F. Yang, *Org. Biomol. Chem.*, 2019, **17**, 1062.

12 Z. Y. Xu, Y. L. Wang, Z. G. Xiao, C. Zou, X. Zhang, Z. Wang, D. L. Wu, S. Yu and F. L. Chan, *Oncogene*, 2018, **37**, 6259.

13 (*a*) Y.-Y. Wang, Y. He, L.-F. Yang, S.-H. Peng, X.-L. He, J.-H. Wang, F. Lv, Y. Hao, M.-Y. Liu, Z. F. Yi and W.-W. Qiu, *Eur. J. Med. Chem.*, 2016, **120**, 13; (*b*) B. Yu, H. Y. Liu, X. Y. Kong, X. L. Chen and C. L. Wu, *Eur. J. Med. Chem.*, 2019, **163**, 500.

14 Y. Ai, Y. Hu, F. H. Kang, Y. S. Lai, Y. J. Jia, Z. J. Huang, S. X. Peng, H. Ji, J. D. Tian and Y. H. Zhang, *J. Med. Chem.*, 2015, **58**, 4506.

15 A. Valentini, F. Conforti, A. Crispini, A. De Martino, R. Condello, C. Stellitano, G. Rotilio, M. Ghedini, G. Federici, S. Bernardini and D. Pucci, *J. Med. Chem.*, 2009, **52**, 484.

16 (*a*) L. Virág, A. Robaszkiewicz, J. M. Rodriguez-Vargas and F. J. Oliver, *Mol. Asp. Med.*, 2013, **34**, 1153; (*b*) F. J. Oliver, G. de la Rubia, V. Rolli, M. C. Ruiz-Ruiz, G. de Murcia and J. M. -de Murcia, *J. Biol. Chem.*, 1998, **273**, 33533; (*c*) A. Nagarsenkar, L. Guntuku, S. D. Guggilapu, D. Bai K., S. Gannoju, V. G. M. Naidu and N. B. Bathini, *Eur. J. Med. Chem.*, 2016, **124**, 782.

17 (*a*) N. P. Kumar, P. Sharma, T. S. Reddy, S. Nekkanti, N. Shankaraiah, G. Lalita, S. Sujanakumari, S. K. Bhargava, V. G. M. Naidu and A. Kamal, *Eur. J. Med. Chem.*, 2017, **127**, 305; (*b*) D. J. Fu, L. Zhang, J. Song, R. W. Mao, R. H. Zhao, Y. C. Liu, Y. H. Hou, J. H. Li, J. J. Yang, C. Y. Jin, P. Li, X. L. Zi, H. M. Liu, S. Y. Zhang and Y. B. Zhang, *Eur. J. Med. Chem.*, 2017, **127**, 87.