Bioorganic & Medicinal Chemistry Letters 19 (2009) 5091–5094

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Atovaquone derivatives as potent cytotoxic and apoptosis inducing agents

Jing Zhou [a,b,†], Lei Duan [a,b,†], Huaming Chen [c], Xiaomei Ren [a,b], Zhang Zhang [a,b], Fengtao Zhou [a,b,d], Jinsong Liu [b], Duanqing Pei [a], Ke Ding [a,b,*]

[a] Key Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, International Business Incubator A-3, Guangzhou Science Park, Guangzhou 510663, China
[b] Institute of Chemical Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, International Business Incubator A-3, Guangzhou Science Park, Guangzhou 510663, China
[c] China Pharmaceutical University, # 24 Tongjiaxiang, Nanjing 210009, PR China
[d] Graduate School of the Chinese Academy of Sciences, 19 Yuquan Rd, Beijing 100049, China

ARTICLE INFO

Article history:
Received 28 April 2009
Revised 30 June 2009
Accepted 2 July 2009
Available online 10 July 2009

Keywords:
Atovaquone
Apoptosis
Cytotoxic

ABSTRACT

2-Piperazinyl naphthoquinones (**2**) and 2-piperidinyl naphthoquinones (**3**) were designed and synthesized as new cytotoxic and apoptosis inducing agents by utilizing the anti-parasite drug atovaquone as lead compound. Several compounds displayed significantly improved cytotoxic activities against a panel of cancer cell lines than that of atovaquone. These compounds also induced apoptosis through activating pro-apoptotic caspases 9 and 3.

Given the extraordinarily high costs and poor successful rates of drug development, repositioning (or repurposing) of existed drugs, in which to find new indications of old drugs, has become an attractive approach to accelerate drug development process.[1] As part of our chemical biology program, we aimed to identify small molecules with potent anti-tumor activity. By using cell growth inhibitory assay, a number of FDA approved anti-infectious drugs and antibiotics were found to display promising anticancer activity in vitro, among which atovaquone had moderate anti-proliferation activity against different cancer cells with IC_{50} values of 15–30 μM. Herein, we report the structural optimization and in vitro biological evaluation of atovaquone derivatives as new cytotoxic and apoptosis inducing agents.

Atovaquone is an anti-parasite drug selectively targeting the mitochondrial respiratory chain of malaria parasite.[2] This drug has poor water solubility and oral bioavailability because of its structurally hydrophobic nature. The chemical structure of atovaquone comprised a naphthoquinonyl head, a cyclohexanyl linker and a hydrophobic 4-chlorophenyl tail (Fig. 1). Many compounds consisted of naphthoquinonyl moiety have been reported to be cytotoxic,[3,4] which indicated that the naphthoquinonyl head might be the crucial part for atovaquone to display anti-tumor activity. The cyclohexanyl linker and/or the hydrophobic tail would provide potential sites for structural optimization. Based on these considerations, 2-piperazinyl naphthoquinones (**2**) and 2-piperidinyl naphthoquinones (**3**) were designed as new atovaquone derivatives. Of note, the 2-hydroxyl group in atovaquone was erased for the synthetic feasibility and chemical stability of the designed derivatives (Fig. 1).

The designed compounds were readily prepared by a condensation of naphthoquinones **4** with different arylpiperazines or arylpiperidines (Scheme 1). For the coupling reaction of 5-hydroxyl naphthoquinone **6** with arylpiperazine or arylpiperidine **5**, both 5-hydroxyl and 8-hydroxyl substituted products were obtained. The major products were identified as 8-hydroxyl analogues by using an X-ray crystallographic analysis of 8-hydroxy-2-(4-phenylpiperazin-1-yl)naphthalene-1,4-dione (**2q**)[5] (Fig. 2).

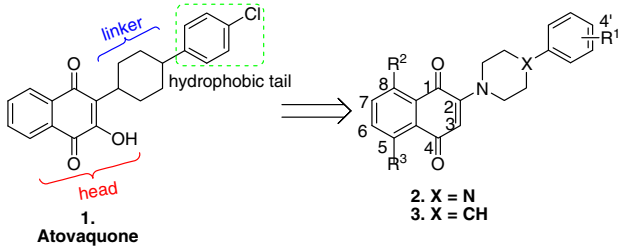
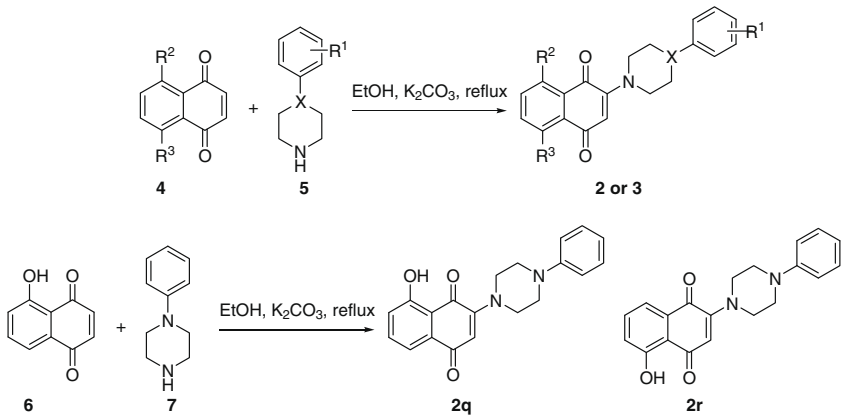


Figure 1. Chemical structures of atovaquone and designed derivatives.

* Corresponding author. Fax: +86 20 32290485.
E-mail address: ding_ke@gibh.ac.cn (K. Ding).
† These authors contributed equally.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.044



Scheme 1. Synthesis of 2-piperazinyl naphthoquinones (**2**) and 2-piperidinyl naphthoquinones (**3**).

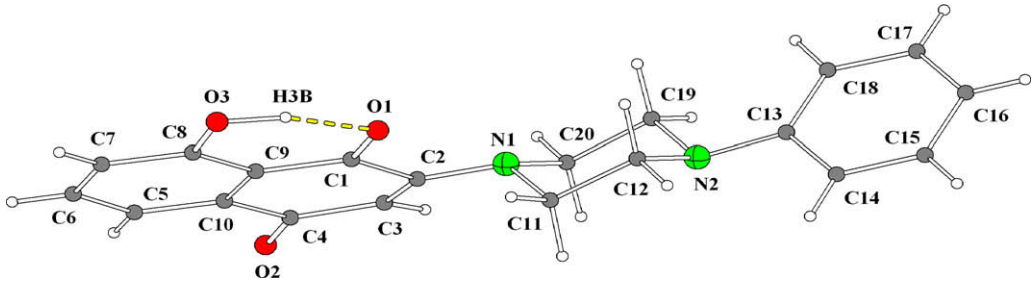


Figure 2. X-ray structure of compound **2q**.

The anti-tumor activity of the designed compounds was preliminarily screened by cell growth inhibitory assay (MTT assay) against Du145 human prostate carcinoma cells. From Table 1, it was clear that almost all the compounds designed display good or moderate anti-proliferation activity against Du145 cells. 2-(4-(4′-chlorophenyl)piperazin-1-yl)naphthalene-1,4-dione (**2a**), a structurally close derivative of atovaquone, display almost identical anti-tumor activity with that of atovaquone. When the 4′-Cl group was removed (**2f**) or replace with F, methyl groups (**2b**, **2e**), the activity was improved 3–4 folds. However, the Br or methoxyl group substituted analogues (**2c**, **2d**) were much less active. Further structure–activity relationship analysis indicated that *o*-position of the phenyl tail in compound **2** might be the optimal position for substitution. For examples, compound **2a** had an IC_{50} value of 29.5 μM, while the IC_{50} value for *m*-Cl (**2g**) and *o*-Cl (**2h**) analogues were 8.3 and 2.9 μM respectively. Compounds with multiple substitutions (**2m**, **2n**, **2o**, **2p**) were less potent.

5-Hydoxyl and 8-dydroxyl derivatives were also designed on the basis of **2f**. The results revealed that 8-dydroxyl compound **2q** is 3.5-fold more potent than **2f**, while 5-hydoxyl analogue **2r** was less active. Other 8-dydroxyl analogues **2s**, **2u**, **2v** and **2w** also displayed promising anti-tumor activity. The 5, 8-didydroxyl compound **2x** had an IC_{50} value of 1.3 μM.

The 2-piperidinyl naphthoquinones **3a**–**3f** also displayed good cell anti-tumor activity. Similar to that of 2-piperazinyl analogues (**2**), the introduction of 8-hydroxyl group obviously improved the anti-tumor activity (**3b**, **3d** and **3f**).

The anti-tumor activity of compounds **2q**, **2u**, **2v**, **2w**, **2x**, **3b**, **3d** and **3f** was further evaluated on various human cancer cell lines by using doxorubicin (adriamycin) as a positive control. As shown in Table 2, almost all the compounds displayed broad-spectrum anti-proliferation activity against solid tumor cells with low μM IC_{50} values. Although doxorubicin is highly cytotoxic to T-cell leukemia Molt-4 cells in our cell growth assay, most of the deigned

Table 1
Anti-proliferation activities of compounds **2** and **3** against prostate Du145 cells

Compds	R^1	R^2	R^3	X	Cell growth inhibitory activities[a] IC_{50} (μM)
Atovaquone					29.3 ± 1.2
2a	*p*-Cl	H	H	N	29.5 ± 2.7
2b	*p*-F	H	H	N	11.7 ± 1.8
2c	*p*-Br	H	H	N	>50
2d	*p*-MeO	H	H	N	46.1 ± 2.0
2e	*p*-Me	H	H	N	8.2 ± 1.6
2f	H	H	H	N	9.9 ± 1.3
2g	*m*-Cl	H	H	N	8.3 ± 1.7
2h	*o*-Cl	H	H	N	2.9 ± 0.8
2i	*m*-Me	H	H	N	7.4 ± 1.0
2j	*o*-Me	H	H	N	5.7 ± 0.8
2k	*m*-OMe	H	H	N	15.0 ± 0.7
2l	*o*-OMe	H	H	N	8.0 ± 1.9
2m	3,5-di Methoxyl	H	H	N	14.9 ± 2.6
2n	3,4-di Methoxyl	H	H	N	17.8 ± 2.4
2o	3,4,5-tri Methoxyl	H	H	N	11.6 ± 3.2
2p	4-F-3-Cl	H	H	N	32.2±4.9
2q	H	OH	H	N	2.8 ± 0.3
2r	H	H	OH	N	15.2 ± 1.2
2s	4-F	OH	H	N	8.4 ± 1.3
2t	4-F	H	OH	N	22.4 ± 3.5
2u	*o*-Cl	OH	H	N	2.3 ± 0.2
2v	*o*-OMe	OH	H	N	1.8 ± 0.2
2w	*o*-Me	OH	H	N	2.3 ± 0.3
2x	H	OH	OH	N	1.3 ± 0.3
3a	H	H	H	CH	6.0 ± 1.1
3b	H	OH	H	CH	3.0 ± 0.3
3c	*p*-Cl	H	H	CH	7.3 ± 1.6
3d	*p*-Cl	OH	H	CH	2.6 ± 0.4
3e	*o*-Me	H	H	CH	6.1 ± 0.9
3f	*o*-Me	OH	H	CH	2.5 ± 0.3

[a] Values are means of three experiments.

Table 2
Anti-proliferation activities of compounds **2q**, **2u**, **2v**, **2w**, **2x**, **3b**, **3d** and **3f** against 13 human cancer cell lines

Compds	IC_{50}[a] (μM)												
	IMR-90	A549	Hela	HepG2	HT-1080	HT-29	KB	L-78	MCF-7	MOLT-4	OS-RC-2	SGC-7901	U251[c]
Doxorubicin[b]	1.0	1.9 (7.9[7])	1.0 (0.7[7])	0.3 (1.2[8])	0.1 (0.2[9])	1.3 (1.1[10])	0.3 (0.2[11])	2.4	0.7 (0.7[10])	0.002 (0.06[12])	5.4	0.9 (3.7[13])	0.6
2q	>50	4.2	6.9	5.4	8.2	2.9	7.0	3.6	5.2	>50	1.5	3.7	6.1
2h	>50	10.3	11.3	11.1	15.4	18.9	3.7	28.8	10.3	>50	8.9	41.8	30.8
2u	16.9	4.5	7.2	7.7	5.8	1.4	4.4	5.2	23.8	27.6	2.5	8.2	8.0
2v	>50	3.6	6.5	3.9	5.1	2.8	2.8	4.6	5.6	25.8	1.9	6.8	4.4
2w	>50	5.3	7.2	10.3	11.0	2.8	6.6	15.1	9.7	>50	4.4	5.3	10.2
2x	>50	1.7	2.1	5.4	2.8	2.6	1.4	1.9	3.0	>50	1.5	2.5	6.1
3b	>50	>50	8.6	9.7	11.1	5.4	2.2	13.4	8.8	2.7	0.7	11.1	10.8
3d	>50	17.2	18.2	5.4	22.4	6.9	14.0	40.6	20.5	>50	5.2	>50	6.1
3f	>50	5.2	9.7	16.6	>50	6.7	4.4	4.4	8.9	>50	3.2	3.9	30.4

[a] Values are means of three independent experiments.
[b] Reported values are given in parentheses.
[c] A549: Lung adenocarcinoma cell line; hela: epithelial carcinoma cell line; HepG2: hepatocellular carcinoma cell line; HT1080: fibrosarcoma cell line; HT-29: colon adenocarcinoma cell line; KB: mouth epidermoid carcinoma cell line; L-78: lung squamous carcinoma cell line; MCF-7: breast adenocarcinom cell line; MOLT-4: acute lymphoblastic leukemia cell line; OS-RC-2: renal carcinoma cell line; SGC-7901: gastric carcinoma cell line; U251: human glioma cell line; IMR-90: a human lung fibroblast cell line.

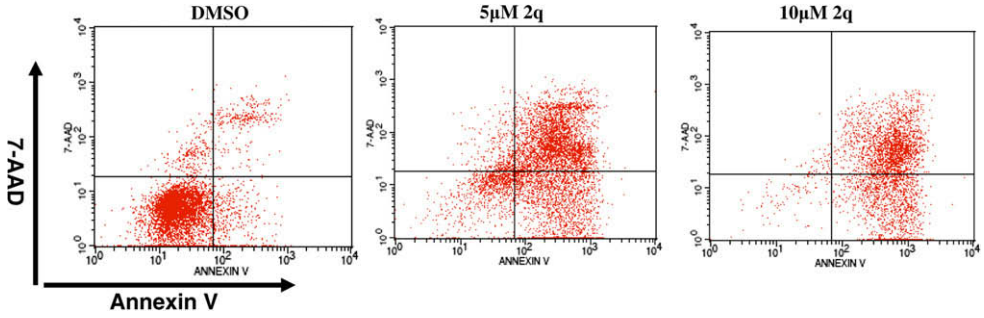


Figure 3. Induction of apoptosis by **2q** in the human prostate cancer Du145 cell line. Cells were treated for 72 h, and apoptosis was determined using AnnexinV conjugated 7-AAD staining by flow cytometry.

compounds did not showed obvious inhibition against Molt-4 cells. It was noteworthy that compound **2x** had comparable cell growth inhibitory activity with doxorubicin against several cancer cell lines such as A549 lung cancer cells, Hela epithelial carcinoma cells, HT-29 colon adenocarcinoma cells, L-78 lung squamous carcinoma cells,[6] and OS-RC-2 renal carcinoma cells in a parallel comparison. In order to monitor the potential toxicity of the compounds to non-cancerous cells, their inhibitory activities against IMR-90 human lung fibroblast cells were evaluated. Different from that of doxorubicin, almost all the compounds did not showed obvious cytotoxic activity to non-cancerous IMR-90 fibroblast cells under high concentrations (IC_{50} >50 μM), which indicated that the compounds might selectively target tumor cells.

The apoptosis inducing activity of the compounds was investigated by using flow cytometric analysis to detect the phosphatidylserine content (PS) on the outer leaflet of the plasma cell membrane (PS externalization) in Du145 cells. Annexin V is a PS-binding protein that can be conjugated to fluorescent groups and used in flow cytometric analysis to determine cell viability. 7-AAD (7-amino-actinomycin) is a fluorescent molecule that is impermeable to cells with intact membranes but permeable to dead cells. As shown in Figure 3, the majority of untreated cells appeared negative for staining with Annexin V and 7-AAD, indicating that the cells were healthy. Almost all the compounds dose-dependently induced the cells to be positive for both Annexin V and 7-AAD, which indicated these cells were dead either by necrosis or apoptosis (Fig. 3 and Supplementary data). In addition, the population of cells undergoing apoptosis, which were more sensitive to Annexin V than to 7-AAD, was also increased. For example, about 84% cells were in healthy condition before treatment, but the number was dramatically decreased to 2.8% after treatment with 10 μM compound **2q**. Over 58% cells were found to be dead, and 37.6% cells were under apoptosis.

Apoptosis can be characterized by morphological and biochemical changes in the cell nucleus, such as chromatin condensation and nuclear shrinking.[14] Morphological analysis of treated cells by using fluorescent micrograph with Hoechst 33342 staining clearly revealed compound **2q** significantly induced chromatin condensation after a 24-h treatment (Fig. 4). While western blot analysis indicated compound **2q** dose-dependently caused the activation of pro-apoptotic caspase 9 and 3 (Fig. 5). These data further verified the apoptosis inducing effect of compound **2q**.

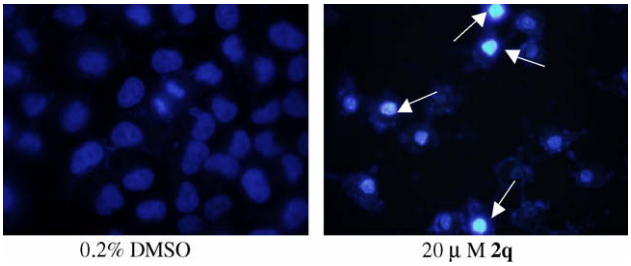


Figure 4. Fluorescent micrographs of Hoechst 33342 staining (100×). The arrows indicate apoptotic cells.

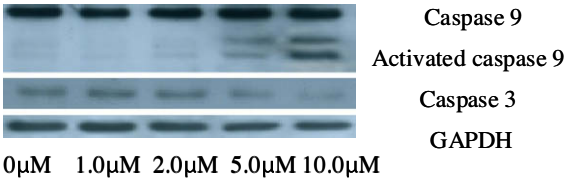


Figure 5. Compound **2q** induced the activation of caspase 9 and capase 3 after 24-h treatment.

In summary, atovaquone derivatives were designed and synthesized as new cytotoxic and apoptosis inducing agents. Both 2-piperazinyl analogues (**2**) and 2-piperidinyl analogues (**3**) displayed good cytotoxic activities against a panel of cancer cell lines. The study also indicated that the cell growth inhibitory activity might be related to apoptosis induced by the compounds. Further mechanism studies are under going and will be reported in due course.

Acknowledgments

This work was supported by 100-talent program of CAS, CAS grant (# KSCX2-YW-R-27), and 863 program (grant # 2007AA02Z490).

Supplementary data

Supplementary data (experimental procedures, characterization of final compounds and flow cytometric analysis results for compounds **2h**, **2n**, **2v**, **2w**, **2x**, **3h**, **3b** and **3f**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.044.

References and notes

1. (a) Chong, C. R.; Chen, X.; Shi, L.; Liu, J. O.; Sullivan, D. J. *Nat. Chem. Biol.* **2006**, *2*, 415; (b) Chong, C. R.; Sullivan, D. J. *Nature* **2007**, *448*, 645; (c) Ashburn, T. T.; Thor, K. B. *Nat. Rev. Drug Disc.* **2004**, *3*, 673.
2. Srivastava, I. K.; Rottenberg, H.; Vaidya, A. B. *J. Biol. Chem.* **1997**, *272*, 3961.
3. Papageorgiou, V. P.; Assimopoulou, A. N.; Couladouros, E. A.; Hepworth, D.; Nicolaou, K. C. *Angew. Chem., Int. Ed.* **1999**, *38*, 270.
4. Recent examples see: (a) Salaski, E. J.; Krishnamurthy, G.; Ding, W.; Yu, K.; Insaf, S. S.; Eid, C.; Shim, J.; Levin, J. I.; Tabei, K.; Toral-Barza, L.; Zhang, W.; McDonald, L. A.; Honores, E.; Hanna, C.; Yamashita, A.; Johnson, B.; Li, Z.; Laakso, L.; Powell, D.; Mansour, T. S. *J. Med. Chem.* **2009**, *52*, 2181; (b) Bolognese, A.; Correale, G.; Manfra, M.; Esposito, A.; Novellino, E.; Lavecchia, A. *J. Med. Chem.* **2008**, *51*, 8148; (c) Bolognesi, M. L.; Calonghi, N.; Mangano, C.; Masotti, L.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 5463; (d) He, J.; Roemer, E.; Lange, C.; Huang, X.; Maier, A.; Kelter, G.; Jiang, Y.; Xu, L.; Menzel, K.; Grabley, S.; Fiebig, H.; Jiang, C.; Sattler, I. *J. Med. Chem.* **2007**, *50*, 5168; (e) Wang, W.; Dai, M.; Zhu, C.; Zhang, J.; Lin, L.; Ding, J.; Duan, W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 735; (f) Eyong, K. O.; Kumar, P. S.; Kuete, V.; Folefoc, G. N.; Nkengfack, E. A.; Baskaran, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5387; (g) Yamashita, M.; Kaneko, M.; Iida, A.; Tokudab, H.; Nishimura, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6417; (h) Hadden, M. K.; Hill, S. A.; Davenport, J.; Matts, R. L.; Blagg, B. S. J. *Bioorg. Med. Chem.* **2009**, *17*, 634; (i) Bhasin, D.; Cisek, K.; Pandharkar, T.; Regan, N.; Li, C.; Pandit, B.; Lin, J.; Lia, P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 391; (j) Lee, E.; Lee, H.; Park, H. J.; Min, H.; Suh, M.; Chung, H.; Lee, S. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5175.
5. CCDC # 727371 contains the Supplementary data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/ retrieing.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44(1223)336033; E-mail: deposit@ccdc.cam.ac.uk].
6. L-78 Cells: kindly provided by Beijing Institute for Cancer Research, Peking University; OS-RC-2 cells and SGC-7901 cells: ordered from Shanghai Cellular Institute of the Chinese Academy of Sciences; Other cells: ordered from American Type Culture Collection (ATCC, USA).
7. Li, F.; Awale, S.; Tezuka, Y.; Kadota, S. *Bioorg. Med. Chem.* **2008**, *16*, 5434.
8. Hasegawa, T.; Bai, J.; Dai, J.; Bai, L.; Sakai, J.; Nishizawa, S.; Bai, Y.; Kikuchi, M.; Abe, M.; Yamori, T.; Tomida, A.; Tsuruo, T.; Hirose, K.; Ando, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3722.
9. De Groot, F. M.; Broxterman, H. J.; Adams, H. P.; van, Vliet A.; Tesser, G. I.; Elderkamp, Y. W.; Schraa, A. J.; Kok, R. J.; Molema, G.; Pinedo, H. M.; Scheeren, H. W. *Mol. Cancer Ther.* **2002**, *1*, 901.
10. Serova, M.; Galmarini, C. M.; Ghoul, A.; Benhadji, K.; Green, S. R.; Chiao, J.; Faivre, S.; Cvitkovic, E.; Le, T. C.; Calvo, F.; Raymond, E. *Br. J. Cancer* **2007**, *97*, 628.
11. Wu, J.; Lee, A.; Lu, Y.; Lee, R. J. *Int. J. Pharm.* **2007**, *337*, 329.
12. Svensson, S. P.; Lindgren, S.; Powell, W.; Green, H. *Pigment Cell Res.* **2003**, *16*, 351.
13. He, Q. C.; Zhang, G. Y.; Cao, W. J. *Ai Zheng (Chin. J. Cancer)* **2008**, *27*, 337.
14. Kerr, J. F.; Winterford, C. M.; Harmon, B. V. *Cancer* **1994**, *73*, 2013.