European Journal of Medicinal Chemistry 44 (2009) 2736–2740

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

The preparation and *in vitro* antiproliferative activity of phthalimide based ketones on MDAMB-231 and SKHep-1 human carcinoma cell lines

Sau Hing Chan [a], Kim Hung Lam [a,*], Chung Hin Chui [a,b], Roberto Gambari [c], Marcus Chun Wah Yuen [a], Raymond Siu Ming Wong [b], Gregory Yin Ming Cheng [b], Fung Yi Lau [b], Yiu Kwok Au [a], Chor Hing Cheng [a], Paul Bo Shan Lai [d], Chi Wai Kan [a], Stanton Hon Lung Kok [a], Johnny Cheuk On Tang [a,**], Albert Sun Chi Chan [a,***]

[a] *Lo Ka Chung Centre for Natural Anti-cancer Drug Development (Laboratory B), State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Departement of Applied Biology and Chemical Technology, Institute of Textiles and Clothing, Hong Kong Polytechnic University, 852 Hong Kong, China*
[b] *Department of Medicine and Therapeutics, Li Ka Shing Medical Sciences Building, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China*
[c] *BioPharmaNet, Department of Biochemistry and Molecular Biology, The University of Ferrara, Ferrara, Italy*
[d] *Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong*

ARTICLE INFO

Article history:
Received 18 August 2008
Received in revised form
21 October 2008
Accepted 23 October 2008
Available online 5 November 2008

Keywords:
Anti-tumor activity
Breast carcinoma
Hepatoma
α-Phthalimide ketones

ABSTRACT

The 'one pot' condensation reaction for the synthesis and potent antiproliferative inhibition of α-phthalimide based ketones is reported here. 2-Phthalimide-1-(4-fluoro-phenyl)ethanone (**5**) showed the best growth inhibition on human MDAMB-231 breast carcinoma and SKHep-1 hepatoma cell lines. Preliminary studies showed that the reported bioactivity may be due to the presence of strong electronegative fluorine group at the *para*-position of the aryl ring.

© 2008 Elsevier Masson SAS. All rights reserved.

1. Introduction

Phthalimide-containing compounds have attracted attention for scientists [1–8], and reports showed that they possess anti-inflammatory [2,3], histone deacetylase inhibitory [4], liver X receptor (LXR) antagonistic [5], leukotriene D_4 receptor antagonistic [6] and their angiogenesis inhibitor [7] activities. Ketones, such as nitrogen-containing heterocyclyl ketones, are effective for prophylaxis and treatment of HGF mediated diseases [9]. Zheng and his coworkers reported that phthalimide ketones can potentially be employed as substrates for producing chiral primary amino alcohols [10–12]; however, no biological activity was available for this type of compounds. Our group previously reported that the phthalimide substituted benzothiazole **1** (Fig. 1) [13,14] showed important activity against CA46, K-562 and SKHep-1 human cancer cells. Based on the design rationale of compound **1**, we turn our focus in searching for more leads using phthalimide as a structural basis for further studies. As part of our on-going work at the synthesis and activity evaluation of anti-tumor candidates, we herein firstly reported the biological activities of several easily available phthalimide ketones, which have not been reported so far.

2. Chemistry

α-Phthalimide ketone **2** was synthesized by the condensation of α-bromoketone and potassium phthalimide, where further purification can be easily achieved by recrystallization in ethanol. All reagents, such as α-bromoketone, potassium phthalimide, DMF and ethanol were used directly without any purification or predrying, and the reaction can proceed at room temperature. To confirm the chemical structures of the α-phthalimide ketones, we have performed the ^{1}H NMR spectrum, ^{13}C NMR spectrum and mass spectrometry analysis.

3. Pharmacology

The human breast cancer cell line MDAMB-231 and the hepatoma SKHep-1 were initially selected for the purpose of preliminary

* Corresponding author. Tel.: +852 3400 8705; fax: +852 2364 9932.
** Corresponding author. Tel.: +852 3400 8727; fax: +852 2364 9932.
*** Corresponding author. Tel.: +852 2766 5222; fax: +852 2364 9932.
E-mail addresses: bcjoelam@inet.polyu.edu.hk (K.H. Lam), bccotang@inet.polyu.edu.hk (J.C.O. Tang), bcachan@inet.polyu.edu.hk (A.S.C. Chan).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.10.024

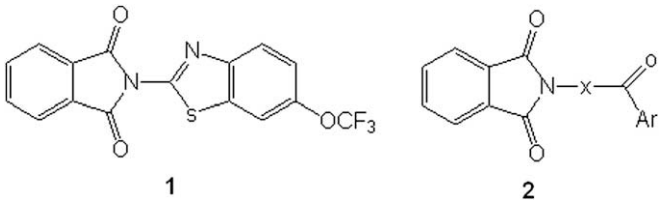


Fig. 1. Phthalimide derivatives: (a) naphthalimide benzothiazole **1**; (b) α-phthalimide ketone **2**.

anticancer screening of these compounds. As shown in Fig. 2, all of our five α-phthalimide ketones at an initial testing concentration from 176 μM to 179 μM showed growth inhibitory activity towards the two human cancer cell lines tested.

Of those compounds tested, both compounds **4** (179 μM) and **5** (177 μM) showed a significant growth inhibitory and cytotoxicity on MDAMB-231 and SKHep-1 human carcinoma cells. The percentage of growth inhibition of both compounds on these cancer cell lines was more than a mean value of 50%. When considering MDAMB-231 breast cancer cells, their degree of growth inhibition was comparable to cisplatinum (167 μM) while for SKHep-1 hepatoma cells, cisplatinum showed a better cytotoxic activity. Compound **3** (179 μM) showed a better cyototoxic potential than compound **6** (176 μM). Compound **7** (179 μM) was the weakest among these series (Fig. 2). We also demonstrated that compounds **4** and **5** exhibited a dose dependent antiproliferative effect on both MDAMB-231 and SKHep-1 cancer cells (Figs. 3 and 4).

Further morphological investigations showed that both compounds **4** and **5** could induce cell rounding and cell shrinkage on both MDAMB-231 breast cancer cells and SKHep-1 hepatoma cells (Fig. 5). These indicated that both compounds could readily induce cancer cell death on these two cancer cell lines. The significant inhibitory activity of compound **5** may due to the presence of strong electronegative fluorine group at the *para*-position of the aryl ring. The steric effect appears not significant to account for the activity when we compared the activity of compound **6** or **4** against the activity of compound **3**. Further investigation revealed a significant decrease in biological activity when the "X" residue changed from a methylene (CH_2) to –$CH(CH_3)$– group (compound **7**).

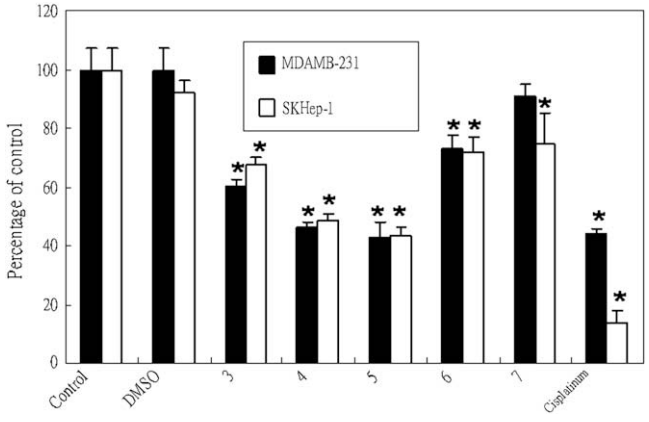


Fig. 2. Growth inhibitory activity of α-phthalimide ketones on MDAMB-231 and SKHep-1 human cancer cells after 48 h as investigated by quantification of the cellular ATP levels. The concentrations tested were 179 μM (compound **3**), 179 μM (compound **4**), 177 μM (compound **5**), 176 μM (compound **6**), 179 μM (compound **7**) and 167 μM (cisplatinum). The control received complete cell culture medium. DMSO was equal to 0.1% by volume. The reported results represent the mean ± standard deviation from triplicate tests. Student *t*-test was used in data analysis where results were considered as statistically significant when *p* value was smaller than 0.05 if compared with untreated control and indicated with an asterisk (*). The figure shows a representative experiment taken from three independent experiments giving similar results.

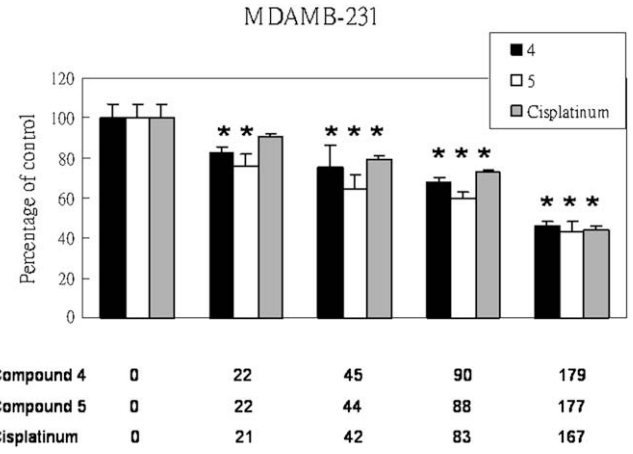


Fig. 3. Dose response inhibitory activity of α-phthalimide ketones on MDAMB-231 human breast cancer cells after 48 h as investigated by quantification of the cellular ATP levels. Concentrations of the compounds are expressed as μM. The results represent the mean ± standard deviation from triplicate tests. Student *t*-test was used in data analysis where results were considered as statistically significant when *p* value was smaller than 0.05 if compared with untreated control and indicated with an asterisk (*). The figure shows a representative experiment taken from three independent experiments giving similar results.

4. Conclusion

In conclusion, we have here described the simple preparation of a group of α-phthalimide ketones which showed significant growth inhibitory effect towards MDAMB-231 breast carcinoma and SKHep-1 hepatoma human cancer cell lines tested. Two of these compounds (compounds **4** and **5**) showed biological activities comparable with that exhibited by cisplatinum on MDAMB-231 breast cancer cells. The relative cytotoxic activity against SK-Hep-1 hepatoma cells is concluded as cisplatinum > compound **1** > compound **5** > compound **4**. Further experimental work is

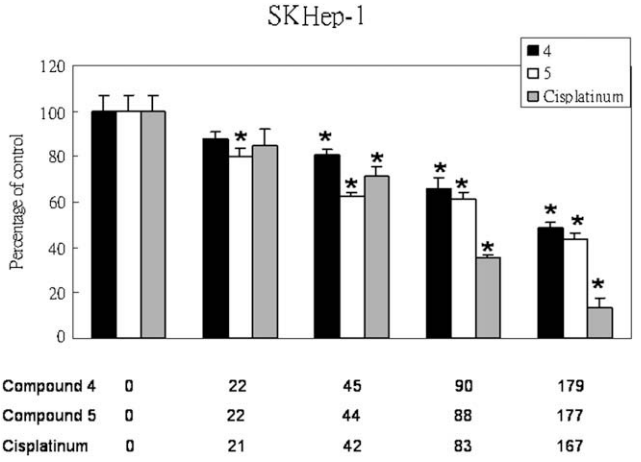


Fig. 4. Dose response inhibitory activity of α-phthalimide ketones on SKHep-1 human hepatoma cancer cells after 48 h as investigated by quantification of the cellular ATP levels. Concentrations of the compounds are expressed as μM. The results represent the mean ± standard deviation from triplicate tests. Student *t*-test was used in data analysis where results were considered as statistically significant when *p* value was smaller than 0.05 if compared with untreated control and indicated with an asterisk (*). The figure shows a representative experiment taken from three independent experiments which gave similar results.

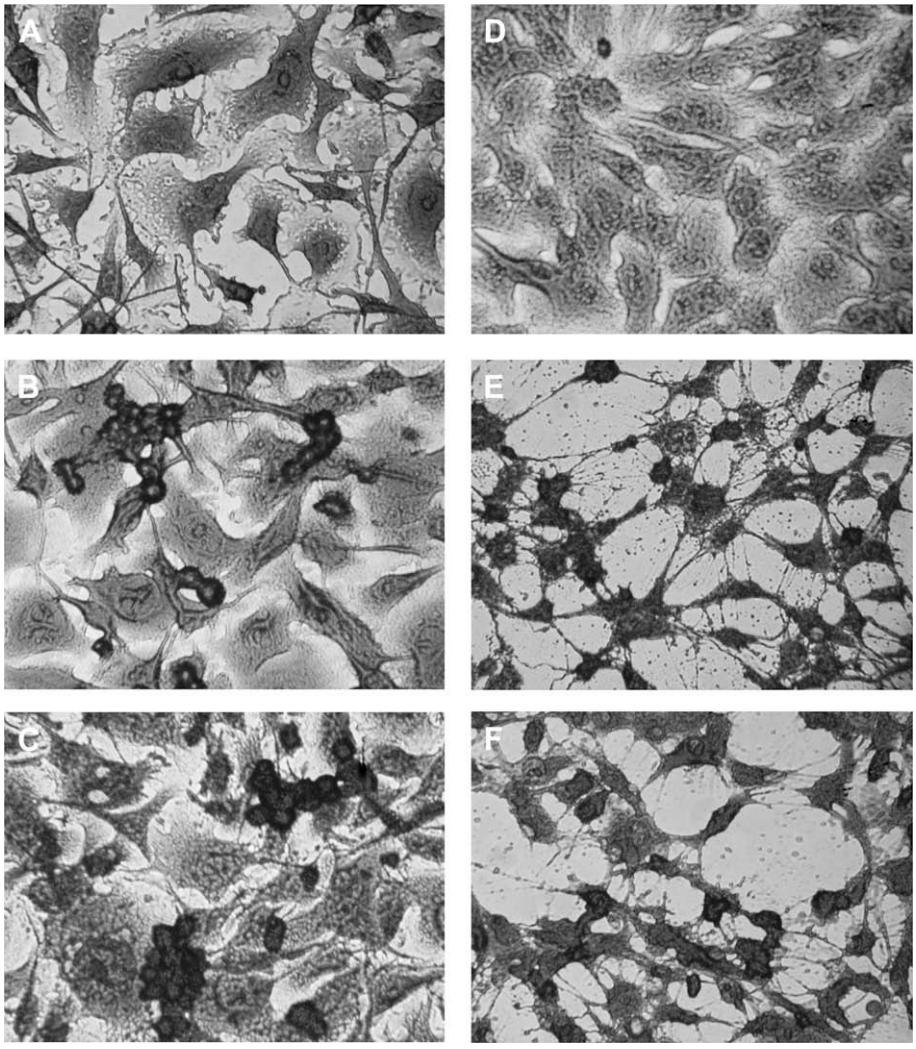


Fig. 5. Morphological investigation of the effect of α-phthalimide ketones on MDAMB-231 breast cancer cells (A–C) and SKHep-1 hepatoma cells (D–F). Cells in A and D were incubated with 0.1% of DMSO. Cells in B and E were treated with compound **4** (179 μM) while cells in C and F were treated with compound **5** (177 μM) and Sulforhodamine B stained. Details are reported in Section 5.

on-going to test their anti-tumour activity on athymic nude mice xenograft model systems.

5. Experimental protocols

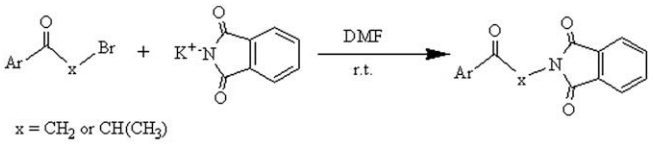
5.1. Preparation of phthalimide ketones [10–12]

All reactions were monitored by thin layer chromatography (TLC) on Merck aluminum-coated plates of silica gel 60 F 254 and visualized under ultra-violet light (254 nm). NMR spectra were recorded on a Varian Oxford AS 500 MHz Fourier transform spectrometer using $CDCl_3$ as solvent. As internal reference for ^1H NMR and ^{13}C NMR spectra, residual protic solvents in $CDCl_3$ (δ_H 7.26 ppm, s; δ_C 77.7 ppm, t) was used; a positive value of the chemical shift denotes a resonance downfield from TMS. Coupling constants were recorded in hertz (Hz). Multiplicities were recorded as the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Melting points were determined using a BUCHI Melting Point B-545 machine in capillaries sealed under atmosphere and the data were uncorrected. Mass analyses were performed utilizing a Micromass Q-Tof-2 spectrometer.

In a dried flask, a solution of α-bromoketone (6 mmol) in DMF (10 mL) was added with potassium phthalimide (6 mmol) in one portion with stirring (Scheme 1). The reaction was run at room temperature and monitored by TLC. After the reaction was complete, the reaction mixture was poured into water (100 mL). The desired products were collected by filtration and recrystallized in ethanol to give the pure products.

5.1.1. 2-Phthalimide-1-phenylethanone **3**

Prepared according to the general procedure described above from 2-bromoacetophenone (6 mmol, 2.0 g) and potassium phthalimide (6 mmol, 1.11 g) and isolated as a white solid, ^1H NMR



3 Ar = Ph; x = CH_2
4 Ar = 4-Me-Ph; x = CH_2
5 Ar = 4-F-Ph; x = CH_2
6 Ar = 4-Ph-Ph; x = CH_2
7 Ar = Ph; x = $CH(CH_3)$

Scheme 1. Condensation of α-bromoketone with potassium phthalimide.

(500 MHz, $CDCl_3$): δ 5.13 (s, 2H), 7.51 (t, 2H, J = 8.0 Hz), 7.63 (t, 1H, J = 7.5 Hz), 7.73–7.75 (m, 2H), 7.88–7.90 (m, 2H), 8.00 (d, 2H, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.85, 124.20, 128.79, 129.55, 132.87, 134.59, 134.70, 134.79, 135.04, 168.55, 191.62; HRMS (ESI): Calcd. for $C_{16}H_{11}NO_3Na$ $[M + Na]^+$, 288.0637; found 288.0641; Melting Point = 168–169 °C; Yield = 68% (4.1 mmol., 1.1 g).

*5.1.2. 2-Phthalimide-1-*p*-tolylethanone* ***4***

Prepared according to the general procedure described above from 2-bromo-4′-methylacetophenone (6 mmol, 1.28 g) and potassium phthalimide (6 mmol, 1.11 g) and isolated as a white solid, ^1H NMR (500 MHz, $CDCl_3$): δ 2.44 (s, 3H), 5.11 (s, 2H), 7.31 (d, 2H, J = 8.5 Hz), 7.74–7.76 (m, 2H), 7.89–7.92 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.48, 44.79, 124.22, 128.93, 130.25, 132.64, 132.96, 134.78, 145.71, 168.64, 191.20; HRMS (ESI): Calcd. for $C_{17}H_{13}NO_3Na$ $[M + Na]^+$, 302.0793; found 302.0779; Melting Point = 177–177.5 °C; Yield = 66% (4.0 mmol., 1.1 g).

5.1.3. 2-Phthalimide-1-(4-fluoro-phenyl)ethanone ***5***

Prepared according to the general procedure described above from 2-bromo-4′-fluoroacetophenone (6 mmol, 1.30 g) and potassium phthalimide (6 mmol, 1.11 g) and isolated as a white solid, ^1H NMR (500 MHz, $CDCl_3$): δ 5.09 (s, 2H), 7.15–7.19 (m, 2H), 7.73–7.75 (m, 2H), 7.87–7.88 (m, 2H), 8.01–8.04 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.67, 116.69, 116.87, 124.20, 131.50, 132.79, 134.83, 165.84, 167.88, 168.48, 190.14; HRMS (ESI): Calcd. for $C_{16}H_{10}NO_3FNa$ $[M + Na]^+$, 306.0542; found 306.0540; Melting Point = 158–158.9 °C; Yield = 45% (2.7 mmol., 0.77 g).

5.1.4. 2-Phthalimide-1-biphenyl-4-yl-ethanone ***6***

Prepared according to the general procedure described above from 2-bromo-4′-phenylacetophenone (6 mmol, 1.65 g) and potassium phthalimide (6 mmol, 1.11 g) and isolated as a white solid, ^1H NMR (500 MHz, $CDCl_3$): δ 5.17 (s, 2H), 7.43–7.44 (m, 1H), 7.49–7.51 (m, 2H), 7.64–7.66 (m, 2H), 7.74–7.78 (m, 4H), 7.91–7.93 (m, 2H), 8.08–8.10 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.90, 124.25, 127.99, 128.19, 129.14, 129.42, 129.70, 132.95, 133.78, 134.81, 140.30, 147.40, 168.60, 191.24; HRMS (ESI): Calcd. for $C_{22}H_{15}NO_3Na$ $[M + Na]^+$, 364.0950; found 364.0964; Melting Point = 198–199 °C; Yield = 73% (4.4 mmol., 1.5 g).

5.1.5. 2-Phthalimide-1-phenylpropanone ***7***

Prepared according to the general procedure described above from 2-bromopropiophenone (6 mmol, 896 μl) and potassium phthalimide (6 mmol, 1.11 g) and isolated as a white solid, ^1H NMR (500 MHz, $CDCl_3$): δ 1.73 (d, 3H, J = 7.5 Hz), 5.66 (q, 1H, J = 7.0 Hz), 7.39 (t, 2H, J = 7.5 Hz), 7.48 (t, 1H, J = 7.5 Hz), 7.68–7.71 (m, 2H), 7.79–7.83 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.59, 51.62, 124.18, 128.70, 129.38, 132.47, 133.71, 134.84, 135.94, 168.13, 196.79; HRMS (ESI): Calcd. for $C_{17}H_{13}NO_3Na$ $[M + Na]^+$, 302.0793; found 302.0801; Melting Point = 85.7–86.4 °C; Yield = 65% (3.9 mmol, 1.1 g).

5.2. Cell lines and cell culture

Human breast cancer MDAMB-231 and hepatoma SKHep-1 cell lines were used for preliminary anti-cancer screening of these compounds. Both cancer cell lines were routinely maintained in RPMI cell culture medium supplemented with 5% heat inactivated fetal bovine serum and penicillin/streptomycin antibiotics (complete cell culture medium). The cells were incubated in a humidified cell culture incubator at 37 °C with 5% CO_2.

5.3. Antiproliferative assay of α-phthalimide ketones

Cancer cells were removed from 75 cm^3 sterile cell culture flasks with 1 fold trypsin and neutralized with fetal bovine serum. After washing with phosphate buffered saline and centrifugation, cancer cells were re-suspended in complete cell culture medium at a concentration of approximately 1×10^5/mL and counted manually using a haematocytometer under an inverted microscope. Cancer cells seeded in the 96 wells microtitre plates for 24 h were prepared for the α-phthalimide ketones screening. The α-phthalimide ketones were dissolved in molecular biology grade dimethylsulfoxide (DMSO). Cisplatinum was used as the positive reference and added at a starting concentration of 167 μM. Compounds were added at a starting concentration of 176 μM–179 μM and followed by a serial of two-fold dilutions and incubated for a further 48 h. The effective concentration of DMSO in each case was approximately 0.1% by volume. Untreated control received either total complete cell culture medium or 0.1% DMSO. Afterwards, the evaluation of possible antiproliferative potential of our synthesized compounds was evaluated by the One Step ATP lite assay cell proliferation kit purchased from PerkinElmer (Netherlands). The one step ATP lite assay is a homogeneous experiment of determining the number of viable cells in culture based on quantitation of the ATP present, which is an indicator of metabolically active cells. The homogeneous assay procedure involves adding the single reagent directly to cells cultured in serum-supplemented medium. This results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture.

5.4. Morphological investigation of cancer cells

MDAMB-231 and SKHep-1 human carcinoma cells were seeded at a concentration of 1×10^5/mL. After 24 h, α-phthalimide ketones were added (compound **4** at 179 μM and compound **5** at 177 μM) and incubated for 48 h. Cells were then fixed with trichloroacetic acids, washed and Sulforhodamine B stained. Any morphological changes were compared with the vehicle control under the inverted microscope.

Acknowledgments

We acknowledge a donation fund from Mr. KC Lo to Dr. JCO Tang for the establishment of Lo Ka Chung Centre for Natural Anti-cancer Drug Development (Laboratory B), the matching fund provided by the Hong Kong Polytechnic University (BB65) and a Niche area grant offered by the Hong Kong Polytechnic University to Dr. KH Lam and Dr. JCO Tang (BB8T). Drs. CH Chui and FY Lau are the honorary tutors kindly offered by Professor J Sung and Professor GYM Cheng from Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong. Dr. CH Chui is supported by the post of "Senior Research Fellow" kindly offered by Dr. JCO Tang (Niche area grant: BB8P to Dr. JCO Tang). Special thank is due to Professor R Gambari, Drs. CH Chui and SHL Kok who gave us valuable advice on the anticancer drug screening experiments and their constructive criticism on this paper. Professor R Gambari is sponsored by AIRC (Italian Association for Cancer Research).

Appendix A. Supplemental material

Supplementary information for this manuscript can be downloaded at doi: 10.1016/j.ejmech.2008.10.024.

References

[1] H. Zhu, M. Huang, F. Yang, Y. Chen, Z.-H. Miao, X.-H. Qian, Y.-F. Xu, Y.-X. Qin, H.-B. Luo, X. Shen, M.-Y. Geng, Y.-J. Cai, J. Ding, Mol. Cancer Ther. 6 (2007) 484–495.
[2] L.M. Lima, P. Castro, A.L. Machado, C.A.M. Fraga, C. Lugnier, V.L.G. de Moraes, E.J. Barreiro, Bioorg. Med. Chem. 10 (2002) 3067–3073.

[3] A.L. Machado, L.M. Lima, J.X. Araujo, C.A.M. Fraga, V.L.G. Koatz, E.J. Barreiro, Bioorg. Med. Chem. Lett. 15 (2005) 1169–1172.
[4] C. Shinji, T. Nakamura, S. Maeda, M. Yoshida, Y. Hashimoto, H. Miyachi, Bioorg. Med. Chem. Lett. 15 (2005) 4427–4431.
[5] T. Noguchi-Yachide, A. Aoyama, M. Makishima, H. Miyachi, Y. Hashimoto, Bioorg. Med. Chem. Lett. 17 (2007) 3957–3961.
[6] M.L. Lima, F.C.F. de Brito, S.D. de Souza, A.L.P. Miranda, C.R. Rodrigues, C.A.M. Fraga, E.J. Barreiro, Bioorg. Med. Chem. Lett. 12 (2002) 1533–1535.
[7] T. Noguchi, H. Fujimoto, H. Sano, A. Miyajima, H. Miyachi, Y. Hashimoto, Bioorg. Med. Chem. Lett. 15 (2005) 5509–5513.
[8] N.J. Lee, I.C. Jeong, M.Y. Cho, C.W. Jeon, B.C. Yun, Y.O. Kim, S.H. Kim, I. Chung, Eur. Polym. J 42 (2006) 3352–3359.
[9] B.K. Albrecht, S. Bellon, S. Booker, A.C. Cheng, D. D'Amico, N. D'Angelo, JC. Harmange, TS. Kim, L. Liu, MH. Norman, AC. Siegmund, M. Stec, N. Xi, K. Yang, PCT Int Appl. WO 2008103277, 2008.
[10] A.W. Lei, S.L. Wu, M.S. He, X.M. Zhang, J. Am. Chem. Soc. 126 (2004) 1626–1627.
[11] W.B. Lin, A.G. Hu, Org. Lett. 7 (2005) 455–458.
[12] Y.Q. Wang, S.M. Lu, Y.G. Zhou, Org. Lett. 7 (2005) 3235–3238.
[13] S.H.L. Kok, C.H. Chui, W.S. Lam, J. Chen, F.Y. Lau, R.S.M. Wong, G.Y.M. Cheng, P.B.S. Lai, T.W.T. Leung, M.W.Y. Yu, J.C.O. Tang, A.S.C. Chan, Bioorg. Med. Chem. Lett. 17 (2007) 1155–1159.
[14] S.H.L. Kok, R. Gambari, C.H. Chui, M.C.W. Yuen, E. Lin, R.S.M. Wong, F.Y. Lau, G.Y.M. Cheng, W.S. Lam, S.H. Chan, K.H. Lam, C.H. Cheng, P.B.S. Lai, M.W.Y. Yu, F. Cheung, J.C.O. Tang, A.S.C. Chan, Bioorg. Med. Chem. 16 (2008) 3626–3631.