Synthetic Chalcone Derivatives as Inhibitors of Cathepsins K and B, and Their Cytotoxic Evaluation

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A series of chalcone derivatives, **1**–**15**, were prepared by *Claisen–Schmidt* condensation and evaluated for their cytotoxicities on tumor cell lines and also against proteolytic enzymes such as cathepsins B and K. Of the compounds synthesized, (*E*)-3-(3,4-dimethoxyphenyl)-1-phenylprop-2-en-1-one (**12**), (*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**13**), (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**14**), and (*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (**15**) showed significant cytotoxicities. The most effective compound was **15**, which showed high cytotoxic activity with an IC_{50} value lower than 1 μg/ml, and no selectivity on the tumor cells evaluated. Substituents at C(4) of ring *B* were found to be essential for cytotoxicity. In addition, it was also demonstrated that some of these chalcones are moderate inhibitors of cathepsin K and have no activity against cathepsin B.

Introduction. – Cancer is a leading cause of death worldwide. According to the World Health Organization (WHO), deaths from cancer are projected to continue rising to over 11 million in 2030. Cancer arises from a change in one single cell, and this change may be started by either external agents or inherited genetic factors [1]. Among the methods used to control the cancer are chemotherapeutic agents, which unfortunately possess high toxicity, multidrug resistance (MDR), and limited effectiveness [2–4]. So, the development of new drugs plays an important role in cancer control [5].

Most of the successful anticancer drugs acting as antimitotics originate in natural compounds [6]. Flavonoids are an extensive and diversified group of compounds found in edible plants with high abundance [7–9]. Among flavonoids, chalcones have been identified as interesting compounds, which are associated with a wide range of biological properties such as antimalarial [10], antileishmanial [11], antioxidative activities [12], antiviral [13], analgesic [14], anti-inflammatory activities [12], and cytotoxicity towards cancer cell lines [8][15]. They consist of open-chain flavonoids, and one of the most widely cited mechanism is that chalcones exert their cytotoxic activity by interfering with mitotic phase in the cell cycle [3][7][15][16]. In fact, it has been reported that chalcones act as tubulin-polymerization inhibitors. Tubulin occurs as a heterodimer of α- and β-subunits, and plays a vital role in various biochemical processes of cell survival and growth [17][18].

Structural requirements for cytotoxic activity vary depending upon the mechanisms of action. For antimitotic activity, the presence of MeO substituents is a favorable feature, and chalcone derivatives with larger number of MeO groups have been reported by *Pati et al.* [17] to be more potent than compounds with fewer similar substituents. Conformational restraint of the chalcone template generally leads to a decrease in cytotoxic activity [16][19].

In human diseases, cysteine proteases have been recognized as potential drug targets due to their excessive expression in many pathological conditions [20][21]. Two important members are the cathepsins B and K, which are also known as lysosomal proteases [22]. In cancer, cathepsin B degrades extracellular matrix proteins (ECMs) and thus facilitates local invasion, proliferation, and metastasis of tumor cells [23]. Cathepsin K is predominantly expressed in osteoclast and exhibits high collagenase activity towards collagens, being strongly involved in osteoporosis progression [24]. To the best of our knowledge, there are no reports in the literature on the inhibition of these synthetic chalcones against cathepsin B and cathepsin K.

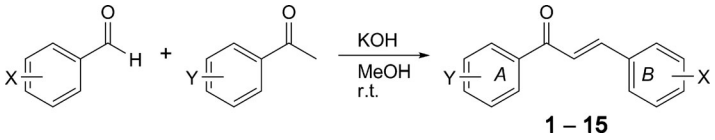
The aim of the current study was to evaluate the *in vitro* cytotoxicity of a series of synthetic chalcone derivatives against human cancer cell lines, namely, melanoma MDA-MB-435, colon cancer HCT-8, and central nervous system SF-295, using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. In addition, the compounds were also evaluated against cathepsins K and B, which are related with osteoporosis and tumor processes, respectively.

Results and Discussion. – *Synthesis.* Chalcones were synthesized, and various substitution patterns on the two aromatic rings were created to obtain a large number of potential analogs [10]. Most of the chalcones, *i.e.*, **3**–**15**, (*Table 1*) were prepared by a *Claisen–Schmidt* condensation of suitable aldehydes with acetophenone derivatives in the presence of methanolic KOH solution [25]. Alternatively, compounds **1** and **2**, involving hydroxybenzaldehyde were obtained under acidic conditions [26]. These products were recrystallized from MeOH and identified by comparison of their IR, MS and ^{1}H-NMR data with those in the literature [26–29]. A coupling constant of *ca.* 16 Hz for the vinyl H-atoms in the ^{1}H-NMR spectra confirmed the (*E*)-configuration. In *Table 1*, structures and reaction conditions for all chalcone derivatives synthesized are compiled.

Cytotoxicity. Fifteen synthetic chalcones were evaluated for their cytotoxic activities in MTT assay against human tumor cells line such as MDA-MB-435, HCT-8 and SF-295. It is already known that the cytotoxic activities of chalcones derivatives depend on the substituent on the aromatic rings [30]. Substituents at C(4) on ring *B* were found to be essential for activity. Hydroxylated chalcones were less active than the corresponding alkoxylated analogs.

In general, no cytotoxicity selectivity between the different cell lines was observed (*Table 2*). Four compounds, **12**–**15**, showed cell growth inhibition higher than 85% in at least two cell lines. Chalcones are soft electrophiles, and they would attract soft nucleophiles like thiols. The thiol reactivity of chalcones is likely to contribute to both cytotoxic and chemoprotective properties of these compound [16]. It has been reported that the compound **14** already suppressed the human PBMC blastogenesis and melanoma cell line A375 proliferation with IC_{50} values of 12.18 and 31.81 μM,

Table 1. *Structures, Reaction Conditions, and Yields of Chalcone Derivatives* **1**–**15**



1 – 15

Compound	X	Y	Time [h]	Temp. [°]	Yield [%]
1	4-OH	4-NO_2	24	64 (reflux)	45
2	4-OH	H	24	64 (reflux)	32
3	4-NO_2	4-NO_2	3	−4	87
4	3,4-O–CH_2–O	H	3	28 (r.t.)	48
5	4-Cl	4-NO_2	3	28 (r.t.)	54
6	4-F	H	4	28 (r.t.)	42
7	3,4-O–CH_2–O	4-NO_2	1	28 (r.t.)	75
8	2,4-$(MeO)_2$	4-NO_2	1	28 (r.t.)	46
9	3,4-$(MeO)_2$	4-NO_2	3	28 (r.t.)	75
10	4-F	4-NO_2	3	28 (r.t.)	65
11	4-MeO	4-NO_2	6	28 (r.t.)	68
12	3,4-$(MeO)_2$	H	12	28 (r.t.)	63
13	4-Cl	H	4	28 (r.t.)	42
14	4-MeO	H	10	64 (reflux)	48
15	4-NO_2	H	4	28 (r.t.)	78

Table 2. *Cytotoxicities of Chalcone Derivatives* **1**–**15** *against Three Tumor Cell Lines*[a]). Cell-growth-inhibition percentage (GI-%) at 125 μg/ml[b]).

Compound	MDA-MB-435	HCT-8	SF-295
1	21.5 ± 0.6	33.3 ± 5.3	26.2 ± 5.3
2	52.6 ± 2.3	53.9 ± 0.9	33.8 ± 6.3
3	14.4 ± 3.3	33.3 ± 1.3	16.3 ± 11.1
4	49.7 ± 17.6	48.9 ± 5.7	48.4 ± 3.5
5	19.9 ± 19.1	27.3 ± 8.6	19.8 ± 3.1
6	88.6 ± 13.4	72.5 ± 31.8	35.6 ± 5.9
7	6.7 ± 0.8	2.6 ± 2.7	21.6 ± 2.2
8	13.9 ± 0.9	20.3 ± 9.4	4.5 ± 6.9
9	24.1 ± 4.9	7.5 ± 0.1	25.1 ± 10.4
10	25.2 ± 5.8	39.8 ± 2.9	23.3 ± 9.3
11	31.5 ± 6.7	33.0 ± 4.5	12.7 ± 18.4
12	96.6 ± 3.1	50.5 ± 3.4	88.1 ± 5.8
13	96.3 ± 5.4	93.3 ± 1.	90.3 ± 2.8
14	96.7 ± 0.9	85.5 ± 2.2	93.1 ± 3.0
15	89.2 ± 0.1	98.2 ± 0.1	76.6 ± 9.9
Doxorubicin[c])	96.6 ± 0.1	97.1 ± 0.2	97.4 ± 0.1

[a]) Cancer cell lines: MDA-MB-435, melanoma; HCT-8, colon cancer; SF-295, central nervous system. [b]) GI-% Values shown were the average of three replicates. [c]) Positive control.

respectively [31]. All four selected chalcones except **12** were already evaluated against HSC-2 and HSC-4 human oral squamous cell carcinoma [23], kidney carcinoma cells TK-10, human mammary adenocarcinoma cells MCF-7, and human colon adenocarcinoma cells HT-29, and showed moderate cytotoxic potencies [3]. Nevertheless, *Juvale et al.* [4] reported that chalcone **12** exhibited no inhibitory effect on MCF-7 [4].

Compounds with cytotoxic activities higher than 85% were selected for IC_{50} determination (*Table 3*). Most of the chalcones, **12**–**15**, showed significant activities with IC_{50} values in the range of 4.1–0.28 μM. Compounds with electron-withdrawing substituents (Cl and NO_2) on the ring *B* and no substituent on the ring *A* (*i.e.*, **13** and **15**) were active; however, F-containing compound **6** exhibited no activity. The cytotoxicity progressively decreased for compounds with NO_2 group in the ring *A*. The presence of an electron-withdrawing group in the ring *B* (in particular in *para*-position) enhanced the electron deficiency of the β-C-atom, and thus its reactivity to words nucleophiles [16]. The presence of MeO group on the ring *B* of the chalcones **12** and **14** was found to contribute to cytotoxicity. Compounds **9** and **11**, which have NO_2 groups in the ring *A*, were less active than those without NO_2, *i.e.*, **12** and **14**.

Table 3. IC_{50} *Values of Chalcone Derivatives* **12**–**15** *against Four Tumor Cell Lines*[a])

Compound	MDA-MB-435	HCT-8	SF-295
12	3.3 (2.6–4.1)	0.6 (0.2–1.3)	4.1 (3.3–5.1)
13	2.6 (2.2–3.1)	2.5 (1.4–4.4)	1.9 (1.0–3.9)
14	1.6 (1.1–2.2)	0.3 (0.1–0.6)	1.5 (1.3–1.8)
15	0.5 (0.3–0.6)	0.8 (0.6–1.2)	0.3 (0.2–0.5)
Doxorubicin[c])	0.5 (0.4–0.7)	0.04 (0.03–0.05)	0.3 (0.2–0.4)

[a]) Cancer cell lines: MDA-MB-435, melanoma; HCT-8, colon cancer; SF-295, central nervous system. [b]) IC_{50} (CI 95%) [μg/ml]: 50% inhibitory concentration and 95% confidence interval in μg/ml. [c]) Positive control.

Compounds with IC_{50} values lower than 4 μg/ml were considered promising for the search of new anticancer agents. The chalcone **15** showed strong cytotoxicity with an IC_{50} value lower than 1 μg/ml and no selectivity in human tumor cells. Based on the IC_{50} values the promising results suggested that the structural features of compound **15** might be useful in designing new cytotoxic agents. Evaluating the selectivity, compound **12** and **14** turned out to be the most selective towards the cell line HCT-8. To investigate a possible correlation between the cytotoxic effects exhibited by selected chalcone derivatives, **12**, **13**, **14**, and **15**, and unspecific cell membrane damage, these compounds were submitted to the hemolytic assay as proposed by *Jimenez et al.* [32]. All the evaluated compounds showed no hemolytic activity at the concentration 50 μg/ml, indicating that cytotoxic activity does not occur by breaking the cell membrane.

Cathepsin. By inhibition assays of cathepsin B and cathepsin K, four compounds, **4**, **6**, **13**, and **14**, among the series of chalcones were identified to have moderate potencies as inhibitors cathepsin K. On the other hand, none of the chalcones showed significant activity against cathepsin B (*Table 4*).

Cathepsin B is known to be strongly involved in the metastasis process. High levels of this enzyme are found in different types of tumors [33–36]. Although both

Table 4. *Inhibitory Activities* [%] *of Chalcone Derivatives against Cathepsin B and Cathepsin K at 125* μM

Compound	Cathepsin B	Cathepsin K	Compound	Cathepsin B	Cathepsin K
1	63.6 ± 4.7	10.5 ± 0.6	**9**	0.0 ± 5.6	58.3 ± 7.8
2	59.2 ± 2.3	50.6 ± 4.5	**10**	0.0 ± 7.9	77.7 ± 0.5
3	63.0 ± 3.4	75.7 ± 2.1	**11**	0.0 ± 2.0	75.6 ± 1.1
4	29.6 ± 0.5	95.7 ± 1.2	**12**	0.0 ± 2.3	58.7 ± 14.0
5	0.0 ± 4.7	63.5 ± 7.7	**13**	0.0 ± 4.3	86.7 ± 1.6
6	0.0 ± 5.6	82.5 ± 8.1	**14**	0.0 ± 1.8	89.1 ± 0.2
7	15.6 ± 1.0	13.4 ± 21.8	**15**	0.0 ± 6.5	52.0 ± 3.6
8	0.0 ± 4.7	63.2 ± 6.2	E-64[c])	98.1 ± 0.2	97.4 ± 0.5

[c]) Positive control.

cytotoxicity and cathepsin B assays are closely related to the cancer process, it was noticed that the compounds with high cytotoxic activities in tumor cells lines (*Table 2*) exhibited low inhibitions or even did not inhibit the cathepsin B. These selected chalcones, *i.e.*, **4**, **6**, **13**, and **14**, with inhibitions higher than 80% the IC_{50} values were evaluated against cathepsin K (*Table 5*) and showed moderate activities.

Table 5. IC_{50} *Values of Chalcones* **4**, **6**, **13**, *and* **14** *against Cathepsin K*

Compound	IC_{50} Values [μM][a])
4	60.3 ± 1.3
6	109.9 ± 0.9
13	98.8 ± 0.9
14	42.5 ± 0.9

[a]) The values represent means of three replicates ± SD.

In summary, this study strongly suggests that, besides both assays being related to the cancer disease, based on our data we could not establish a correlation between the cytotoxic activity in tumor cell lines and the inhibition of cathepsin B. Although these findings are preliminary, further advanced studies should be conducted to understand this pathological process. This is the first time that the inhibition activities of these chalcone derivatives against cathepsin B and cathepsin K had been investigated.

Conclusions. – We synthesized a number of known chalcone derivatives with different aromatic substituents. Their structures were established by spectroscopic data which correspond to those in the literature, and the screening for cytotoxicity revealed that the compounds **12**, **13**, **14**, and **15** showed significant activities against MDA-MB-435, SF-295, and HCT-8 cell lines. Screening of chalcone derivatives against cathepsin K disclosed compounds **4**, **6**, **13**, and **14** to possess moderate potencies as enzyme inhibitors. However, no compound showed high activity against cathepsin B.

The authors gratefully acknowledge *CAPES*, *CNPq* and *FAPESP* for financial support and fellowship.

Experimental Part

General. All commercially available reagents were purchased from *Aldrich Chemical Co*. M.p.: *Micro Química MQAPF-301* apparatus. IR Spectra: *Bomem M102* spectrometer. ^{1}H-NMR spectra: *Bruker DRX-400* (400 MHz) instrument. MS: *Shimadzu GCMS-QP2000* instrument.

Chalcone Synthesis. Acidic Conditions. A soln. of the required acetophenone derivative (2.0 mmol) and 4-hydroxybenzaldehyde (2.0 mmol) in MeOH with a cat. amount of H_2SO_4 was heated at reflux for 24 h, and then it was neutralized with 15% NaOH. The org. phase was extracted with AcOEt and dried (Na_2SO_4) to yield compounds **1** and **2**.

Chalcone Synthesis. Basic Conditions. A soln. of acetophenone derivative (2 mmol) and an appropriate aldehyde (2 mmol) in MeOH (15 ml) with KOH 20% was stirred. The reaction time and the temp. dependend upon the substituents in the aromatic ring. The mixture was filtered, and then the precipitate was collected to yield compounds **3**–**15**.

Cytotoxicity Assay. Chalcones were tested for cytotoxicity against three tumor cell lines: MDA-MB-435 (melanoma), HCT-8 (colon cancer), and SF-295 (central nervous system). All cell lines were obtained from *National Cancer Institute* (*NCI*) and were cultivated in *RPMI 1640* medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37°, with 5% CO_2. The MTT assay was used for the cytotoxicity evaluation of chalcones. The cells were placed in 96-well plates (10^5 cells/well for adherent cells or 0.5×10^5 cells/well for those suspended in 100 μl of medium). Doxorubicin at 100 μg/ml and DMSO at 0.005% were used as positive and negative controls, resp. After 24 h, the chalcones were added into the wells to obtain the final concentration of 125 μg/ml. After 72 h, plates were centrifuged (5000 rpm, 10 min), and the supernatants were removed. An aliquot of 200 μl from the MTT soln. (0.5 mg/ml) was added into each well, and the plates were incubated for 3 h under the same conditions described above. Then, DMSO (150 μl) was poured to dissolve the precipitate, and the absorbance was measured at 550 nm. This experiment was run in three replicates, and all absorbance values were converted into a cell growth inhibition percentage (GI-%) by the following formula: $\text{GI-\%} = 100 - [(T/C) \times 100\%]$. C is the absorbance for the negative control, and T was the absorbance in the presence of the tested extract. Those compounds that presented more than 85% of activity were selected to be tested at concentrations varying from 0.078 to 5 μg/ml to determine IC_{50} values by nonlinear regression using GraphPad Prism 4.0 software.

Inhibition Assay of Cathepsin B and Cathepsin K. The protease inhibitor activity was carried out in triplicate in 96-well black plate by the method described by *Barret et al.* [37]. Test compounds were dissolved in DMSO, and then these inhibitors were screened against both the cathepsins B and K at initial concentration of 125 μM. The mixture contained 192 μl of a AcONa buffer (100 mM, 5 mM EDTA, 5 mM DTE, pH 5.5), 2 μl of 1 mM Z-Phe-Arg-MCA, 5 μl of sample, and 1 μl of cathepsin (32 nM). The enzyme was activated during 5 min with DTT at 27°, then the soln. was incubated during 5 min with the sample. The substrate was added to start the reaction, and the fluorescence of released 7-amino-4-methylcoumarin (AMC) was measured at λ_{ex} 355 and λ_{em} 460 nm. Control assays were performed with DMSO (negative control) and with E-64 (positive control). E-64 is an epoxide, 1-[*N*-[(L-3-*trans*-carboxyoxirane-2-carbonyl)-L-leucyl] amino]-4-guanidinobutane, which can irreversibly inhibit a wide range of cysteine peptidases and was first isolated and identified from *Aspergillus japonicus* in 1978 [38]. Those compounds that showed more than 80% of inhibition were selected for the test of IC_{50}. The values of IC_{50} were determined by rate measurements for at least seven inhibitor concentrations. Kinetics parameters were determined from collected data employing the *SigmaPlot* enzyme kinetics module.

REFERENCES

[1] WHO, World Health Organization, available in: http://www.who.int/mediacentre/factsheets/fs297/en/index.html; accessed August 6th, 2012.
[2] P. Cozzi, *Farmaco* **2003**, *58*, 213.
[3] M. Cabrera, M. Simoens, G. Falchi, M. L. Lavaggi, O. E. Piro, E. E. Castellano, A. Vidal, A. Azqueta, A. Monge, A. L. de Ceráin, G. Sagrera, G. Seoane, H. Cerecetto, M. González, *Bioorg. Med. Chem*. **2007**, *15*, 3356.

[4] L. Juvale, V. F. S. Pape, M. Wiese, *Bioorg. Med. Chem.* **2012**, *20*, 346.
[5] C. M. O. Simões, E. P. Schenkel, G. Gosmann, J. C. P. Mello, L. A. Mentz, P. R. Petrovick, 'Farmacognosia: da Planta ao Medicamento', Ed. UFRGS, Florianópolis, 2007.
[6] A. L. Kiselyov, K. V. Balakin, S. E. Tkachenko, N. Savchuk, A. V. Ivachtchenko, *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 189.
[7] C. Echeverria, J. F. Santibañez, O. Donoso-Tauda, C. A. Escobar, R. Ramirez-Tagle, *Int. J. Mol. Sci*. **2009**, *10*, 221.
[8] Z. Nowakowska, *Eur. J. Med. Chem*. **2007**, *42*, 125.
[9] Y. Yasuda, J. Kaletab, D. Bromme, *Adv. Drug Delivery Rev.* **2005**, *57*, 973.
[10] M. Liu, P. Wilairat, M.-L. Go, *J. Med. Chem*. **2001**, *44*, 4443.
[11] S. F. Nielsen, M. Chen, T. G. Theander, A. Kharazmi, S. B. Christensen, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 449.
[12] S. Vogel, M. Barbic, G. Jürgenliemk, J. Heilmann, *Eur. J. Med. Chem*. **2010**, *45*, 2206.
[13] J. C. Trivedi, J. B. Bariwal, K. D. Upadhyay, Y. T. Naliapara, S. K. Joshi, C. C. Pannecouque, E. De Clercqd, A. K. Shah, *Tetrahedron Lett*. **2007**, *48*, 8472.
[14] G. S. B. Viana, M. A. M. Bandeira, F. J. A. Matos, *Phytomedicine* **2003**,*10*, 189.
[15] R. De Vincezo, C. Ferlini, M. Distefano, C. Gaggini, A. Riva, E. Bombardelli, P. Morazzoni, P. Valenti, F. Belluti, F. O. Ranelletti, S. Mancuso, G. Scambia, *Cancer Chemother. Pharmacol*. **2000**, *46*, 305.
[16] M. L. Go, X. Wu, X. L. Liu, *Curr. Med. Chem*. **2005**, *12*, 483.
[17] H. N. Pati, H. L. Holt Jr., R. LeBlanc, J. Dickson, M. Stewart, T. Brown, M. Lee, *Med. Chem. Res.* **2005**, *14*, 19.
[18] H. O. Saxena, U. Faridi, J. K. Kumar, S. Luqmand, M. P. Darokar, K. Shanker, C. S. Chanotiya, M. M. Gupta, A. S. Negi, *Steroids* **2007**, *72*, 892.
[19] H. N. Pati, U. Das, H. Sakagami, M. Kawase, Q. Chu, Q Wang, J. P. Stables, J. R. Dimmock, *Arch. Pharm.* **2010**, *343*, 535.
[20] O. Vasiljeva, B. Turk, *Biochimie* **2008**, *90*, 380.
[21] J. Reiser, B. Adair, T. Reinheckel, *J. Clin. Invest*. **2010**, *10*, 3421.
[22] G.-Z. Zeng, X.-L. Pan, N.-H. Tan, J. Xiong, Y.-M. Zhang, *Eur. J. Med. Chem*. **2006**, *41*, 1247.
[23] V. Turk, J. Kos, B. Turk, *Cancer Cell* **2004**, *5*, 409.
[24] D. Brömme, F. Lecaille, *Expert Opin. Invest. Drugs* **2009**, *18*, 585.
[25] D. G. Powers, D. S. Casebier, D. Fokas, W. J. Ryan, J. R. Troth, D. L. Coffen, *Tetrahedron* **1998**, *54*, 4085.
[26] W. D. Seo, J. H. Kim, J. E. Kang, H. W. Ryu, M. J. Curtis-Long, H. S. Lee, M. S. Yang, K. H. Park, *Med. Chem. Lett*. **2005**, *15*, 5514.
[27] K. N. Gusak, N. G. Kozlov, *Russ. J. Gen. Chem.* **2003**, *73*, 964.
[28] S. Iwata, T. Nishino, N. Nagata, Y. Satomi, H. Nishino, S. Shibata, *Biol. Pharm. Bull.* **1995**, *18*, 1710.
[29] M. V. B. Reddy, C.-R. Su, W.-F. Chiou, Y.-N. Liu, R. Y.-H. Chen, K. F. Bastow, K.-H. Lee, T.-S. Wu, *Bioorg. Med. Chem.* **2008**, *16*, 7358.
[30] S. Ducki, D. Rennison, M. Woo, A. Kendall, J. F. D. Chabert, A. T. McGown, N. J. Lawrence, *Bioorg. Med. Chem*. **2009**, *17*, 7698.
[31] K. Henmi,Y. Hiwatashi, E. Hikita, N. Toyama, T. Hirano, *Biol. Pharm. Bull*. **2009**, *32*, 1109.
[32] P. C. Jimenez, S. C. Fortier, T. M. C. Lotufo, C. Pessoa, M. E. A. Moraes, M. O. de Moraes, L. V. Costa-Lotufo, *J. Exp. Mar. Biol. Ecol*. **2003**, *287*, 93.
[33] B. F. Sloane, J. Rozhin, K. Johnson, H. Taylor, J. D. Crissman, K. V. Honn, *Proc. Natl. Acad. Sci. U.S.A*. **1986**, *83*, 2483.
[34] B. Turk, D. Turk, V. Turk, *Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol.* **2000**, *1477*, 98.
[35] A. M. Eiján, E. O. Sandes, M. D. Riveros, S. Thompson, L. Pasik, H. Mallagrino, F. Celeste, A. R. Casabé, *Cancer* **2003**, *98*, 262.
[36] D. Kuester, H. Lippert, A. Roessner, S. Krueger, *Pathol. Res. Pract*. **2008**, *204*, 491.

[37] A. J. Barrett, A. A. Kembhavi, M. A. Brown, H. Kirschke, C. G. Knight, M. Tamai, K. Hanada, *Biochem. J.* **1982**, *201*, 189.
[38] K. Hanada, M. Tamai, M. Yamagishi, S. Ohmura, J. Sawada, I. Tanaka, *Agric. Biol. Chem.* **1978**, *42*, 523.

Received October 10, 2012